600 MEMBRANE STRUCTURE AND TRANSPORT. S. J. Singer, Department of Biology, University of California at San Diego, La Jolla, California 92093

The mechanisms whereby small polar molecules or ions and large soluble proteins are transported across membranes are subject to thermodynamic constraints. In the case of small molecules or ions, for which specific carrier proteins in the membrane have been implicated, the formerly popular rotating carrier mechanism for the translocation of the ions is now generally recognized to be thermodynamically unfeasible and has been experimentally ruled out (1). An alternative model, the subunit rearrangement mechanism, was proposed by Jardetzky (2) and Singer (3, 4). In this model, each carrier protein is an aggregate of two or more identical or similar polypeptide chains which span the membrane in the same orientation, and form a water-filled channel through the membrane. The active site(s) of the carrier are located in this channel. A relatively small input of free energy is adequate to drive a quaternary rearrangement of the subunits which translocates the active site from one surface of the membrane to the other. The Na-K ATPase has molecular properties which are consistent with this model (5).

In the case of protein transport across membranes, an endocytotic mechanism is widely used. The mechanism appears to involve the following sequence: 1) the binding of the protein to some specific receptor in the cell membrane; 2) the clustering of the bound receptors in the plane of the fluid membrane; 3) the activation of a contractile protein system on the cytoplasmic surface of the membrane opposite to the receptor clusters; 4) the endocytosis of the clustered regions of the membrane; and 5) the fusion of the endocytotic vesicles with other membrane elements (lysosomes) in the cell, which then results in some action on the transported protein. Experiments on the uptake of proteins by lymphocytes will be described that are consistent with the first four steps of this sequence.

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601 LEUCINE BINDING PROTEIN AND REGULATION OF TRANSPORT IN *E. coli*, Dale L. Oxender, James J. Anderson, Mary M. Mayo and Steven C. Quay, Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109.

Leucine is transported into *E. coli* by high affinity systems in which the leucine-binding protein (LIV-BP) and leucine specific-BP play a role and by a low affinity system (LIV-II) that appears to be membrane bound (1,2). Genetic loci that have been recently identified for these transport systems are *liv J*, *liv K* (identified with the LIV and leucine specific binding proteins, respectively), *liv H* (specifying a nonbinding protein component of the high affinity systems) and *liv P*, which specifies a component required for both high and low affinity leucine uptake. These loci appear to form a cluster of genes cotransducible with *malT* at minute 74 on the *E. coli* chromosome. A CRM^+ leucine specific binding protein mutant has been isolated (3) which maps in this region, suggesting that the structural gene is located here. The search for missense and deletion mutations in the binding protein structural genes has been facilitated by SDS-PAGE autoradiography of immunoaffinity-purified ^{35}sulfur-labeled proteins from shock fluids of mutant strains. Previous studies have shown that the LIV-binding protein and therefore leucine transport, is negatively regulated by a product of the *liv R* genetic locus (4). Leucyl-tRNA plays a role in leucine transport regulation (5, 6). The measurement of leucine transport regulation in *hisT* and *SuA* mutants of *E. coli* suggests further that a modified leucyl-tRNA and the *rho* factor are involved, respectively. These data suggest an attenuation site may be operative in the regulation of the leucine binding proteins and, therefore, leucine transport (7). Finally, we have determined that the leucine sensitivity of *E. coli* strains can be greatly enhanced through isoleucine limitations by transport-coupled exchange of intracellular isoleucine for extracelluar leucine (8).

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602 THE L-ARABINOSE BINDING PROTEIN OF ESCHERICHIA COLI B/r, Robert W. HOGG, Dept. of Microbiology, Case Western Reserve University, Cleveland, OH 44106; Mark A. Hermodson, Dept. of Medical Genetics, University of Washington, Seattle, WA 98195; F. A. Quiocho, G. N. Phillips, Jr., G. L. Gilliland, M. E. Newcomer, Dept. of Biochemistry, Rice University, Houston, TX 77001.

The accumulation of L-arabinose across the cytoplasmic membrane of E. coli is an inducible process facilitated by two known transport systems. One, designated araE, was first described by Novotny (1). The second transport system contains as a functional component the periplasmic L-arabinose binding protein (ABP) (2, 3). This system has a high affinity for the substrate($K_d \approx 10^{-7}$), is designated araF, (tentatively 45 min) and is functionally destroyed by osmotic shock. The ABP has been purified to homogeneity and crystallized (4). Functionally, the sole cysteine is required for binding as evidenced by mercurial inactivation and reversal by reducing reagents, and one or more tryptophanes are involved in or near the active site as evidenced by fluorescence spectroscopy and N-bromosuccinimide oxidation. The complete amino acid sequence has been determined and found to contain 306 residues, m.w. 33,200.

The three-dimensional structure of L-arabinose-binding protein has been determined at 3.5 Å resolution and a preliminary 2.8 Å electron density map calculated. These structural analyses show that the protein is ellipsoidal, with approximate dimensions 65 Å x 35 Å x 30 Å. The molecule consists of two globular domains (P and Q domains). The first two-thirds of the polypeptide chain makes up the two domains and the remainder provides most of the structural linkage between the two domains. The interior of each domain contains β-pleated sheet structure and both β-sheets are twisted or "fanned". The exterior of each domain contains four α-helices. The axes of all helices (9 of more than 2 turns) are roughly parallel to the long axis of the ellipsoidal molecule. Likewise, the chains comprising the β-sheet structures are more or less oriented along the long axis of the molecule. Between the two domains is a cleft.

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603 BINDING PROTEIN-INDEPENDENT SUBSTRATE TRANSLOCATION BY THE METHYL-GALACTOSIDE TRANSPORT SYSTEM OF ESCHERICHIA COLI. INDUCIBILITY OF mglB MUTANTS, Boris Rotman and April R. Robbins, Division of Biology and Medicine, Brown University, Providence, RI 02912

The transport of D-galactose and its analogs via the methylgalactoside permease depends on three genes, mglA, B, and C (1,2). Previous results indicate that the gene products of mglA and mglC mediate substrate translocation while the galactose-binding protein, the product of mglB, affects the affinity of the permease for extracellular substrate (3,4). The translocation step was shown to be rate limiting at maximal influx and to have the substrate specificity characteristic of the complete transport system (4).

Since it has been shown that induction of transport activity and synthesis of galactose-binding protein is coordinate (5), we examined the inducibility of translocation. Transport negative mutants derived from S185 (F⁻his, str, lac(Z,Y,A)del, ptsF, ara(C,O)del), an inducible mgl^+ strain, were characterized by both genetic complementation and in vitro measurements of galactose-binding protein. In each of 15 independent mglB mutants we determined the effect of D-fucose induction on three parameters: high affinity transport, translocation and specific activity of the galactose-binding protein. Four of these mutants after induction with 10^{-3}M D-fucose exhibited neither significant amounts of binding protein (ranging from 0.1 to 0.6% of the parental amount) nor high affinity transport (less than 0.2% of the parent). The level of translocation prior to induction was comparable to that of mglA and mglC mutants (110 CPM per 10^9 cells). Induction increased this level about 5-fold. We observed that the levels of translocation in all of the 15 mutants was increased by at least 3-fold following induction (range 3.6 to 9.5-fold).

These results indicate that at least one of the components responsible for binding protein-independent translocation is induced by D-fucose, a specific inducer of the methylgalactoside transport system.

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604 CHARACTERIZATION OF A PERIPLASMIC PROTEIN RELATED TO sn-GLYCEROL-3-PHOSPHATE OF ESCHERICHIA COLI, Winfried Boos, Fachbereich Biologie, University of Konstanz, 7750 Konstanz, West Germany

The cold osmotic shock procedure releases a protein (GLPT) from the cell envelope of Escherichia coli that is related to the transport of sn-glycerol-3-phosphate in this organism. The evidence for this correlation is as follows: i) GLPT is under the regulatory control of the glpR gene as well as under respiratory control. ii) some glpT mutants that were isolated as phosphonomycin resistant clones and defective in transport of sn-glycerol-3-phosphate do not synthesize GLPT. Revertants of these mutants (growth on sn-glycerol-3-phosphate) again synthesize GLPT. iii) amber mutations in glpT reduce the amount of GLPT while suppressed strains produce normal amounts.

GLPT is a soluble protein of molecular weight 160 000 and is composed of four identical subunits. The 160 000 molecular weight complex is stable in 1% sodiumdodecyl sulfate at room temperature. In this solution it dissociates in subunits upon boiling. 8M urea at room temperature also dissociates the complex in subunits. The protein is acidic in nature (isoelectric point is 4.4). In contrast to the typical transport related periplasmic binding proteins, no conditions could be found where pure GLPT exhibited binding activity toward its supposed substrate, sn-glycerol-3-phosphate.

Transport of sn-glycerol-3-phosphate in whole cells is very sensitive to the cold osmotic shock procedure, indicating the participation of an essential periplasmic component. However, isolated membrane vesicles that are devoid of periplasmic components, including GLPT, are fully active in sn-glycerol-3-phosphate transport. Therefore, we conclude that GLPT is essential in overcoming a diffusion barrier for sn-glycerol-3-phosphate established by the outer membrane. Possibly the charged nature of the substrate plays a role in its difficulty to pass the outer membrane. Attempts to isolate mutants that are transport negative in whole cells due to a defect in GLPT but are active in isolated membrane vesicles, have failed so far. All glpT mutants tested, whether or not they synthesize GLPT, are not active in isolated membrane vesicles.

605 INTERACTION OF BRUSH-BORDER HYDROLASES WITH LECITHIN MEMBRANES, Hans Wacker, Helmut Hauser, Joseph Brunner and Giorgio Semenza, Laboratorium für Biochemie der ETH, Universitätstrasse 16, CH-8092-Zurich, Switzerland.

Sucrase-isomaltase and aminopeptidase, when in their integral form, interact with lecithins to form stable lipoprotein complexes. The reconstituted system is a single-bilayer vesicle of approximately 300 Å diameter. The hydrophobic part of the amphipathic hydrolase is incorporated in the lecithin bilayer, while the portion carrying the enzymatic activity is oriented towards the external aqueous phase. This part, as in the "native" system, can be detached by proteases.

Vesicles containing one, two or more hydrolase molecules, respectively, are distinguished in density gradient centrifugation and in the analytical ultracentrifuge (1).

In the case of sucrase-isomaltase the hydrophobic part incorporated into the lipid bilayer belongs to the isomaltase subunit. The bulk of the protein is not in contact with the lipid surface. It sits on a stalk of a minimum length of approximately 10 Å.

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641 THE FOLATE AND THIAMINE TRANSPORT PROTEINS OF *Lactobacillus Casei*, Gary B. Henderson, Edward M. Zevely, Robert J. Kadner and F.M. Huennekens, Scripps Clinic and Research Foundation, LaJolla, California 92037

Two separate binding proteins, one specific for folate and the other for thiamine, have been isolated from membrane preparations of *L. casei*. Purification of the proteins to homogeneity was accomplished by fractionation of Triton X-100-solubilized preparations with microgranular silica and Sephadex G-150. Amino acid analyses revealed that the folate and thiamine binders have unusually low polarity constants, 0.32 and 0.26, respectively. Evidence obtained with intact cells has established a direct role for these binding proteins in transport of the corresponding vitamins. Pertinent findings are as follows: (A) In each case, the processes of binding and transport showed similar pH profiles, substrate affinities, and repression by excess vitamin in the growth medium. (B) Inhibitor studies employing amethopterin, CHO-FH_4, and CH_3-FH_4 (for folate) and thiamine-P and thiamine-P2 (for thiamine) have shown that the ability of these compounds to inhibit the transport of the corresponding vitamins is paralleled by their ability to inhibit binding. (C) A number of mutants have been isolated which contain a defective folate-transport system. In each case, impairment of folate transport is accompanied by a corresponding impairment in folate binding. In one of these mutants, folate transport and folate binding were each enhanced by the addition of thiols. The role of these binding proteins in transport of the vitamins will be discussed.

642 The Role of the mgl Operon Gene Products in the Entry and Exit Reactions of the ß Methyl Galactoside Transport System. David B. Wilson, Cornell Univ., Ithaca, N.Y.

The steady state level of ß methyl galactoside transport by the ß methyl galactoside transport system in E. coli results from a dynamic equilibrium between the entry reaction and an exit reaction. The exit reaction is first order at the highest substrate concentration tested (3×10^{-2}M). Surprisingly exit is stimulated 7 fold by the presence of an energy source in the medium and the stimulation requires the synthesis of ATP or a related compound. Mutants in all three cistrons (A, B, C) show normal exit and the exit is the same in cells that are induced or repressed in the synthesis of the mgl operon. High salt which inhibits the entry reaction has no effect on the exit reaction. These results indicate either that the exit reaction utilizes a different carrier than the entry reaction or that the carrier for both reactions is not encoded in the mgl operon.

643 3-O-METHYL GLUCOSE TRANSPORT IN NON-ENERGIZED INTESTINAL CELLS: A ROLE FOR MEMBRANE POTENTIAL, Christin Carter-Su and George Kimmich, The University of Rochester, Rochester, N.Y. 14642.

The role of membrane potential as a driving force for Na^+-dependent 3-O-methyl-glucose (3-OMG) transport was studied in energy-depleted isolated chicken intestinal cells. Rotenone or dinitrophenol treatment was used to decrease cellular ATP levels by over 90% and produce a cell population in which no steady state accumulation of 3-OMG against a concentration gradient was observed. Membrane potential in these cells was qualitatively manipulated by imposing various ion gradients to create induced diffusion potentials, and with the use of specific ionophores. A transient concentrative uptake of 3-OMG was observed for those conditions in which an inside negative membrane potential should be established, even in the absence of a trans-membrane Na^+ gradient. If a Na^+ gradient was also imposed, a greater and more prolonged "overshoot" was observed. The degree of "overshoot" and initial influx rates could be correlated with anion permeability for a given Na-anion imposed gradient ($SCN^- > Cl^- > SO_4^=$, isethionate$^-$). Concentrative 3-OMG entry could also be demonstrated by enhancing membrane K^+ permeability in the presence of a K^+ gradient ($K^+_{in} > K^+_{out}$). Again, the "overshoot" occurred in the presence or absence of an imposed Na^+ gradient, although it was greater in the former case. The valinomycin-induced uptake was greater when the Na^+ salt was $SO_4^=$ rather than Cl^- or SCN^-, as expected for a situation in which the K^+ diffusion potential was a greater fraction of the total imposed potential. These data all imply a role for the membrane potential as an important component of the total driving force for Na^+-dependent 3-OMG uptake.

644 LOCALIZATION OF β-GALACTOSIDASE AND LACTOSE PERMEASE PROTEIN(S) IN THE E. COLI ENVELOPE, Merna Villarejo, Department of Biochemistry and Biophysics, University of California, Davis, CA 95616

Several methods have been used to identify and localize membrane-bound lactose operon proteins in Escherichia coli. The appearance of the "M" protein, thought to be the y gene product, was detected through double amino acid labeling techniques and by Coomassie blue staining of analytical SDS polyacrylamide slab gels. Another polypeptide of MW ~20,000 was associated with the membrane after lac operon induction. In addition, as much as 10% of the cellular β-galactosidase activity fractionates with the envelope structure.

The inner and outer membrane layers of the cell envelope were separated by sucrose density gradient centrifugation or, alternatively, by selective solubilization of the inner membranes with the detergent Sarkosyl. The "M" protein was associated with the outer membrane while the β-galactosidase was preferentially bound to the inner membrane. The 20,000 dalton polypeptide was found in both inner and outer membrane fractions. Experiments to define the role of this protein in transport are in progress. The membrane alterations accompanying lac operon induction are more complex than anticipated and suggest a role for both outer and inner membranes in transport.

645 PHOSPHORYLATION OF PARTIALLY PURIFIED BAND 3 OF HUMAN ERYTHROCYTE MEMBRANES, M. Marlene Hosey and Mariano Tao, Univ. of Illinois Medical Center, Chicago, Ill.

Several studies have implicated band 3 protein(s) of the human erythrocyte membrane in the transport of glucose, anions, and water across the red cell membrane. Previous results from our laboratory have shown that at least two proteins migrating in the stained area of band 3 in intact human and rabbit erythrocyte ghosts are substrates for membrane-bound and soluble cyclic AMP-dependent and -independent erythrocyte protein kinases. The purpose of the present studies was to characterize the phosphorylation of partially purified band 3 proteins, i.e., in ghosts extracted with NaI or dimethyl maleic anhydride (DMMA), preparations known to retain functional glucose transport (binding) activity. NaI-extracted ghosts autophosphorylated a band 3 protein with ATP but not with GTP. DMMA-extracted ghosts, which contained less Coomassie-blue staining bands, were devoid of autophosphorylating activity. Both preparations contained at least two distinct proteins in the stained area of band 3 which were differentially phosphorylated by various cyclic AMP-dependent and -independent cytoplasmic and solubilized membrane protein kinases. One phosphoprotein was coincident with the slower migrating, diffusely stained tail of band 3 (2.9) and another was coincident with the leading edge of the stained band 3. The phosphorylation of these proteins differed according to the kinase and the phosphoryl donor (ATP or GTP) used. The results are consistent with those obtained with intact ghosts and suggest that more than one protein is contained in the area stained as band 3, that these proteins remain associated during NaI or DMMA extraction, and that they are capable of being phosphorylated differentially. (Supported by American Cancer Society Grant #BC-65C).

646 CROSS-LINKING OF INNER MITOCHONDRIAL MEMBRANE COMPONENTS, David D. Hackney, Carla Stayboldt, Michael Stevens and Paul D. Boyer, Molecular Biology Institute, Univ. of Calif., Los Angeles, California 90024.

Bilirubin is taken up by biological membranes and catalyzes the photodynamic peroxidation of unsaturated lipids. With red blood cell ghosts this reaction is accompanied by cross-linking of the membrane proteins either directly or via intervening lipid molecules [Girotti, Biochem. 14, 3377 (1975)]. The proteins of the submitochondrial particle can also be cross-linked by this procedure and exhibit interesting differences in their susceptibility The polypeptide pattern obtained by SDS polyacrylamide electrophoresis changes markedly with the rapid loss of many polypeptides and the appearance of new material at very high molecular weight. In general polypeptides which are easily stripped from the membrane by chaotropic agents are not readily cross-linked, while intrinsic membrane proteins are lost much more rapidly. This is the pattern expected if cross-linking is due to reactions occurring in the hydrophobic lipid regions of the membrane with little generation of reactive species which can diffuse to peripheral regions. The ATPase, F_1, is only loosely attached to the membrane and its α subunit is very resistant to cross-linking. The β subunit, however, is a striking exception. It is one of the most readily lost polypeptides. Soluble F_1 can be mixed with submitochondrial particles as a control, the mixture exposed to bilirubin plus light and then centrifuged. The soluble F_1 remains unchanged while the β subunit of the membrane bound enzyme rapidly disappears from the SDS gel. These results open the possibility that the β subunit has effective exposure to the hydrophobic regions of the membrane.

647 SUBUNIT FUNCTION IN NATIVE AND DIGITONIN-TREATED Na^+,K^+-ATP-ASE, Charles G. Winter, Shu-Mei Liang and Jimmy R. Lea, Dept Biochem, U Ark Col Med, Little Rock, AR. 72201

Digitonin treatment abolishes purified canine kidney Na^+,K^+-ATPase activity with survival of its ouabain-sensitive ADP-ATP exchange and acetylphosphatase activities. Cupric phenanthroline-catalyzed crosslinking studies reveal: 1) Native enzyme rapidly yields $\alpha\alpha$ dimers, then high polymers accumulate on the SDS gels. Below the membrane transition temperature (20^o), dimerization occurs but polymerization does not. 2) Digitonin treatment abolishes $\alpha\alpha$ dimerization and polymerization at all temperatures. Instead $\alpha\beta$ dimers are formed, based on apparent molecular weight, glycoprotein staining and two-dimensional SDS-gel electrophoresis. The α subunit mobility also shifts under these conditions, possibly because of intrachain disulfide formation. These effects occur above and below the membrane transition. Thus $\alpha\alpha$ and $\alpha\beta$ dimerization are intramolecular events. Cation activation studies of "partial" activities show altered $S_{0.5}$ values with unchanged Hill coefficient. These results suggest that $\alpha\beta$ dimers catalyze "partial" activities but not ATPase function and retain cation site cooperativity. Tryptic inactivation studies of native ATPase and acetylphosphatase yield results similar to Jorgensen's(Biochim.Biophys.Acta(1975) 401,399-415), with evidence for K^+- and Na^+-stabilized conformations interconvertible on ATP or MgATP addition. Loss of exchange activity shows less Na^+-K^+ discrimination. With K^+, ATP completely protects against trypsin and surprisingly induces loss of ouabain-sensitivity. With Na^+, a ouabain-stimulated exchange develops with time, suggesting loss of ouabain inhibition accompanied by ouabain stabilization of pump fragments. These results relate to α chain fragments produced, as shown on SDS-gels. (Supported in part by NIH grant AM16483).

648 SYNTHESIS AND CHARACTERIZATION OF PHOTOACTIVATED PROBES FOR HIGH RESOLUTION MAPPING OF MEMBRANE PROTEINS, Kenneth K. Iwata†, Carol M. Manweiller*, and Bernadine J. Wisnieski§, Dept. of Bact. and Mol. Biol. Inst., UCLA, Los Angeles, CA 90024

Recently, Chakrabarti and Khorana (1) attached azide moieties to the alkyl chains of fatty acids and produced phospholipids bearing these photoreactive functions. We have begun to explore the usefulness of related photoactivatible probes for resolving the vertical position of membrane proteins. The concept of a radioactive "depth charge" searching for proteins in zones of defined depth within the membrane has evolved from our recent work with ESR spin labels (2,3). In particular, we developed a spin label which allows us to monitor, with high specificity, the physical state of the surface monolayer of membrane systems. Use of this ESR probe in conjunction with RSO, ISO, and disrupted membrane preparations from animal cells showed that the inner and outer monolayers of the plasma membrane display unique, and mutually independent physical properties. We have synthesized several azide-labeled fatty acids, and derivatives, of high specific radioactivity which are the counterparts of the ESR spin labels. Thus, we feel confident about any predictions we make about their activity, location, and mobility in a given membrane system. We have initiated studies with simple model membrane systems to determine the validity of our approach. Results of our preliminary studies will be described.

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649 E. COLI PYRIDINE NUCLEOTIDE TRANSHYDROGENASE; PURIFICATION AND REGULATION, Ronald L. Hanson and Barbara Gerolimatos, Dept. of Biochem. Columbia University, New York, N.Y. 10032

The reduction of NADP by NADH catalyzed by the cytoplasmic membrane of E. coli can be driven by ATP hydrolysis or electron transport, and is sensitive to uncouplers of oxidative phosphorylation. This transhydrogenase has been extracted from the membrane with triton X-100 and purified 40-fold by chromatography on DEAE cellulose and sepharose 4B in buffers containing detergent. At this stage, the remaining proteins aggregate with the enzyme and further purification is not possible. The enzyme can be disaggregated without loss of activity by brief treatment with SDS followed by addition of excess non-ionic detergent (triton X-100 or brij 35). The disaggregated enzyme elutes (with an additional 3-fold purification) from sepharose 4B at 1.8 V_o, which is slightly smaller than the enzyme before aggregation (1.7 V_o) and slightly larger than the triton micelle (2.2 V_o).

Transhydrogenase activity is repressed by growth in media containing 2mM leucine as is leu transport. In mutants with leu transport not repressible by leu, transhydrogenase is also not repressed by leu.

650 PURIFICATION AND AMINO ACID SEQUENCE STUDIES OF A 23000 DALTON FRAGMENT OF BAND 3; A MEMBRANE SPANNING PROTEIN OF THE HUMAN ERYTHROCYTE. M.K. Singh, T.L. Steck and H. Köhler. The University of Chicago, Chicago, Illinois.

Band 3 is the predominant polypeptide of the human erythrocyte membrane. It spans the membrane asymetrically, the outer surface region bearing carbohydrate and the cytoplasmic end providing attachment sites for the glycolytic enzymes, glyceraldehyde 3-phosphate dehydrogenase and aldolase. This protein has been implicated in the transport of anions in this membrane.

The digestion of band 3 with 2-nitro-5-thiocyanobenzoate (NTCB) releases a 23000 dalton (23 K) fragment from its cytoplasmic pole (Steck, Ramos and Strapazon, Biochemistry 15, 1154, 1976). In the present study, this 23K fragment was generated by digestion of 0.1N NaOH-stripped ghosts with 1.0 mM NTCB in SDS at 50°C for 8 hours. The digest shows no residual band 3; the 23K fragment was the predominant stained band and was recovered in nearly stochiometric yield. The digest was precipitated with isopropanol and the fragment solubilized and partially purified by aqueous extraction of the pellet. Purification to homogeneity was achieved by gel filtration in the presence of SDS. The tryptic fingerprint of the 23K fragment showed at least 15 well-resolved spots, consistent with its compositional analysis (6 lysine and 11 arginine residues). The acid soluble portion of the tryptic digest contained 12-13 peptides. The amino acid sequence of six fragments has been analyzed as follows: (1) free arginine; (2) Ala-Leu-Leu-Leu-Lys; (3) Met-Glu-Ala-Ala-Arg; (4) Glu-Glu-Leu-Leu-Arg; (5) Phe-Phe-(Thr, Ala, Val)-Lys; (6) Glu-Ser-Glu-Ala-Gly-Leu-Leu-X-Ala-Ala-Leu-Gly-Val-Val-Pro-Lys-(Thr, Gly, Ala, His)-Arg.

651 THE MOLECULAR MECHANISMS OF DICARBOXYLIC ACIDS TRANSPORT IN ESCHERICHIA COLI, Theodore C.Y. Lo, Dept. of Biochemistry, University of Western Ontario, London, Ontario, Canada. Previous findings from this laboratory indicate that at least two membrane transport proteins (SBP 1 and SBP 2), and one periplasmic binding protein (PBP) are involved in dicarboxylate transport. We have been able to isolate biologically active SBP 1, SBP 2, and PBP through the use of aspartate-coupled Sepherose 4B. These isolated components have similar substrate specificity and affinity as the transport system. Genetic analyses of various transport mutants indicate that at least two genes (dct A and dct B) are responsible for the membrane bound transport components and at least one gene (cbt) is responsible for PBP. In the present study, we are able to demonstrate dct A mutants are defective in the SBP 2 protein, and dct B mutants are defective in the SBP 1 protein. This provides additional evidence that both SBP 1 and SBP 2 are indeed involved in the transport process. Binding studies with membrane vesicles, indicate that the substrate recognition site of SBP 2 is exposed to the outer surface of the cytoplasmic membrane. Experiments carried out with various non-penetrating covalent labelling reagents and cross-linking reagents indicate that both membrane transport components are integral proteins transversing the whole thickness of the membrane, and that they are lying adjacent to each other. These findings suggest the formation of a transport channel by SBP 1 and SBP 2. Therefore the above information may be taken as an indication that dicarboxylic acids are transported across the membrane via a "multimeric transport channel", instead of a "mobile carrier system".

652 THE SIALOGLYCOPROTEIN SUBUNITS OF HUMAN PLACENTAL BRUSH BORDER MEMBRANES, CHARACTERIZED BY TWO-DIMENSIONAL ELECTROPHORESIS, H. Garrett Wada, Stella Gornicki and Howard Sussman, Dept. of Pathology, Stanford University School of Medicine, Stanford CA94305

A brush border membrane enriched fraction was isolated from human, full term placenta. This membrane fraction exhibited large membrane fragments with microvilli projecting from the basal membrane in electron micrographs and was enriched ten-fold in alkaline phosphatase, a brush border enzyme marker (J. Biol. Chem. 251:4139 [1976]). The sialoglycoproteins associated with this membrane fraction were tritiated by mild periodate oxidation of sialic acid and reduction with tritiated $NaBH_4$. The membranes were solubilized in 8M Urea, 2% Triton X-100, and the tritiated glycoprotein subunits were reduced with B-mercaptoethanol and characterized by two-dimensional polyacrylamide gel electrophoresis using a method similar to that described by O'Farrell and by Bhakdi, Knüferman, and Wallach. The tritiated subunits were detected in the gels by autofluorography according to Bonner and Lasky. The two-dimensional subunit "maps" resolved at least 17 major sialoglycoprotein subunits whereas only ten major Periodate-Schiff Reagent staining components were resolved by one-dimensional SDS polyacrylamide gel electrophoresis. Placental alkaline phosphatase (PAP) was identified on the subunit maps by inclusion of ^{32}P-labeled PAP in the tritiated membrane sample. The ^{32}P-labeled PAP corresponded to a major tritiated sialoglycoprotein subunit, which was heterogeneous with respect to charge -- demonstrated by three closely running spots of the same molecular weight. Using this mapping technique, studies are being conducted to identify specific sialoglycoproteins which may participate in transport processes.

653 PHOTOAFFINITY LABELING OF ADIPOCYTE PLASMA MEMBRANE WITH [2-^{3}H]-8-AZIDO-ADENOSINE, Paul D. Rosenblit and Daniel Levy, Univ. of Southern California, School of Medicine, Department of Biochemistry, Los Angeles, CA 90033

Adenosine interacts with the plasma membrane of a variety of cells. In addition to a transport system for this nucleoside, there appear to be other sites of interaction on the cytoplasmic and exterior membrane surfaces. A photoaffinity derivative of adenosine has been used to probe the structure of adipocyte plasma membranes. [2-^{3}H]-8-azido-adenosine is synthesized by bromination of [2-^{3}H]-adenosine followed by treatment with sodium azide. 8-azido adenosine has been shown to competitively inhibit the uptake of ^{3}H-adenosine by intact adipocytes. The inhibition is quite specific in that the uptake of ^{3}H-leucine and ^{3}H-3-O-methyl-glucose is unaffected by the presence of 8-azido-adenosine. The uptake of [2-^{3}H]-8-azido-adenosine is also competitively inhibited by adenosine as well as by other inhibitors of adenosine transport. Equilibration of [2-^{3}H]-8-azido-adenosine with adipocyte plasma membranes followed by irradiation with light greater than 300 nm results in the incorporation of radioactivity into several membrane components. Photolysis of [2-^{3}H]-8-azido-adenosine in the presence of a large excess of adenosine appears to significantly inhibit the labeling of most of these membrane components. The uptake and inhibition studies suggest that one or more of these components may be functionally involved in the adenosine transport system. (Supported by Grants from the National Institutes of Health (CA 14089, CA 05297) and the Juvenile Diabetes Foundation)

654 PURIFICATION OF OVERPRODUCED PHOSPHATIDYLSERINE SYNTHETASE FROM ESCHERICHIA COLI, T. J. Larson and W. Dowhan, Department of Biochemistry and Molecular Biology, University of Texas Medical School, Houston, TX 77025

The enzymes responsible for synthesizing the phospholipid portion of the membrane are each present in small amounts (approximately 1000 copies/cell) in Escherichia coli. Hence, it has been difficult to obtain large enough quantities of purified enzymes in order to study their physical, chemical and enzymological properties which may be related to the control of membrane biogenesis. Phosphatidylserine (PS) synthetase is a key enzyme involved in the biogenesis of the cell membrane of E. coli. The synthetase has been purified 5500-fold to near homogeneity by an affinity chromatographic technique. The large-fold purification required has made it difficult to obtain sufficient amounts of pure enzyme for study (yield was 2.5 mg per 1b of cells). When ColE1 hybrid plasmid pLC34-44 (Clarke and Carbon, Cell 9, 91, 1976) is carried in a strain of E. coli, it corrects the temperature sensitivity of a PS synthetase mutant and causes a 15-fold increase in the specific activity of the PS synthetase in crude extracts. The enzyme required only a 300-fold purification to homogeneity from such a plasmid carrying strain and yielded 26 mg per 1b of cells. The overproduced synthetase is identical to that from strains not carrying hybrid plasmids in that 1) the enzymes are found associated with the ribosomal fraction in crude extracts; 2) the specific activities of the purified enzymes are the same; and 3) the molecular weights as determined by SDS gel electrophoresis are the same. These data show that PS synthetase is overproduced (not activated) in the strains carrying the hybrid plasmid. These strains will be valuble in obtaining larger amounts of purified PS synthetase. (Supported by Robert A. Welch Foundation Grant AU-599)

655 INHIBITION OF GLUCOSE TRANSPORT IN HUMAN RED CELLS AND RED CELL GHOSTS BY A SYNTHETIC PHLORETIN ANALOG, Franklin F. Fannin and Donald F. Diedrich, Dept. of Pharmacology, University of Kentucky, Lexington, Kentucky, 40506.

Phloretin and a synthetic analog (phloretin α-benzylamine; PBA) were compared as inhibitors of glucose entry in human red cells and red cell ghosts at pH 7.4. PBA, and to a lesser degree phloretin, were extensively bound to both intact cells and ghosts. Estimation of inhibitory potency could, therefore, not be made on the basis of the amount of agent added to the system. Free inhibitor concentrations had to be measured to determine Ki values, in which case PBA and phloretin were approximately equipotent (Ki≈2-3μM). Failure to determine free concentrations of phloretin and phloretin-like inhibitors under conditions normally used to measure sugar transport will lead to underestimation of inhibitory potency and may explain, in part, the wide range of Ki values for phloretin which have been reported in the literature. Binding of PBA and phloretin to red cells, red cell hemolysates (i.e., hemoglobin) and white erythrocyte ghosts was compared at pH 7.4. The results indicate that binding to hemoglobin can account for most of the red cell binding. Similar findings have been reported for phloretin binding at pH 6 (M.L. Jennings and A.K. Solomon, J. Gen. Physiol. 67 (1976) 381-397). Kinetic data obtained from uptake studies indicated that the synthetic analog is, like phloretin, a competitive inhibitor of glucose transport in intact red cells and in ghosts. It appears that the benzylamine group of PBA does not interfere with the binding of the phloretin moiety to the sugar transport system in the human erythrocyte membrane. The potential for use of the agent in affinity labeling of the carrier appears promising.

656 MEMBRANE POTENTIAL DEPENDENT BINDING OF SCORPION TOXIN TO ACTION POTENTIAL Na^+ CHANNELS IN ELECTRICALLY EXCITABLE NEUROBLASTOMA CELLS. William A. Catterall and Radharaman Ray, Laboratory of Biochemical Genetics, NHLBI, NIH, Bethesda, Md., 20014.

Venom of the scorpion Leiurus quinquestriatus specifically inhibits Na^+ channel inactivation in voltage clamp experiments. The venom and a polypeptide toxin purified 100 fold from the venom act cooperatively with the alkaloid neurotoxins veratridine, batrachotoxin, aconitine, and grayanotoxin to activate Na^+ channels in electrically excitable neuroblastoma cells. The drugs and toxins have no effect on variant neuroblastoma clones specifically lacking Na^+ channels.

The concentration of scorpion toxin required for 50% activation of Na^+ channels increases from 1 nM to 70 nM on depolarization of the cells. ^{125}I-labelled scorpion toxin binds to a class of saturable binding sites (K_D = 2 nM) in electrically excitable neuroblastoma cells but not in variant clones. The K_D for binding to these sites increases to 100 nM on depolarization of the cells. The results suggest that scorpion toxin binds specifically to a component of the Na^+ channel that undergoes a membrane potential dependent conformational change. Comparison of Na^+ permeability measurements and scorpion toxin binding shows that 1 x 10^8 Na^+ ions/min are transported per scorpion toxin binding site (2 pmho/site).

Starting from the Monod-Wyman-Changeux model, an allosteric model has been formulated which quantitatively accounts for both the ligand-induced and membrane potential-induced conformational changes caused by these drugs and toxins.

657 ATPASE IN THE PHLOEM OF PISUM SATIVUM AND ITS RELATION TO THE FUNCTION OF TRANSFER CELLS, Barbara J. Bentwood and James Cronshaw, Department of Biological Sciences, University of California, Santa Barbara, California 93106.

The cytochemical localization of ATPase in mature and differentiating phloem cells of Pisum sativum has been studied using a lead precipitation technique. Mature phloem transfer cells having elaborate cell wall ingrowths showed ATPase activity associated with the plasma membranes and nuclear envelopes. In mature sieve elements, reaction product was found in association with the parietal and stacked systems of endoplasmic reticulum, but not with either the crystalline or dispersed forms of P-protein. Transfer cells at early stages of differentiation exhibited strong enzyme activity in the endoplasmic reticulum with higher deposition of reaction product on plasma membranes and vacuolar membranes. As the transfer cells mature and develop their characteristic wall ingrowths, enzyme activity becomes strong in association with the plasma membranes and nuclear envelopes. In developing sieve elements there was little or no deposition of reaction product in the cells. Localization in the endoplasmic reticulum was apparent only after the cells were fully mature. The intense localization of reaction product on the plasma membranes of transfer cells may be evidence for a transport system possibly involved in the active movement of photosynthetic products into sieve elements for long distance transport. (Supported by NSF Grant # BMS 7422778).

658 DIFFUSIONAL TRANSPORT AND PARTITIONING OF CHLORINATED HYDROCARBONS AND METHYLMERCURIC CHLORIDE IN MEMBRANES, AS STUDIED BY FLUORESCENCE QUENCHING OF CARBAZOLE LABELED PHOSPHOLIPIDS, Joseph R. Lakowicz, Delman Hogen, Geneva Omann and Steven Rakow, The Freshwater Biological Institute, Box 100, Navarre, MN 55392

Dynamic quenching of fluorescence provides a measure of the collisional frequency between flurophore and quencher. Chlorinated hydrocarbons, such as DDT, lindane, mirex, CCl_4, DDE and endrin; halogen containing organophosphorous pesticides, such as dibrom, gardona and methylmercuric chloride were all found to be efficient dynamic quenchers of carbazole fluorescence. We have synthesized three phospholipids containing carbazole: N-(9-carbazolepropionyl)-phosphatidyl ethanolamine, dipalmitoyl, (CPA-PE); α-palmitoyl, β-(11-(9-carbazole)-undecanoic acid)-L-α-lecithin (CUA-LYSO); and α-palmitoyl, β-(9-carbazolepropionyl)-L-α-phosphatidyl choline (CPA-LYSO). We also synthesized α-palmitoyl, β-(1-pyrenebutyryl)-L-α-lecithin (PBA-LYSO) to provide a methylmercuric chloride (MMC) probe with a long fluorescence lifetime. Using these probes we have determined that lipid bilayers of dipalmitoyl lecithin have an MMC permeability equivalent to greater than 40% of the permeability of an equivalent thickness of organic solvent in the acyl side chain region, and greater than 20% in the glycerol backbone region. MMC does not partition ($P<10$) strongly into lipid bilayers. The carbazole probes have also been used to measure the uptake rates of DDE, lindane, and gardona from particulates and into membranes. We regard this latter process as a fundamental step in the process of bioaccumulation. In contrast to MMC, lindane partitions strongly into lipid bilayer ($P\approx3000$). In conclusion, the quenching method provides a novel method for investigation of the chemodynamics of toxic molecules in cell membranes.

659 ON THE STRUCTURE AND ACTION OF MEMBRANE PROTEINS, A. Keith Dunker, Program in Biochemistry and Biophysics, Washington State University, Pullman, WA., 99164.

Studies on the structure of filamentous bacteriophages (the phages are basically bundles of α-helices surrounding the viral DNA) and studies on the mechanism of phage assembly (the lipid-free phages result from a membrane-mediated assembly process; in essence, the viral DNA is transported across the cell membrane without cell lysis) have led to the following hypothesis: (1) transmembrane α-helices may aggregate together into bundles via knobs-into-holes packing between the α-helices; (2) within the membrane, the driving force for aggregation may be hydrogen bonds between the side chains; (3) a set of such hydrogen-bonded side chains could provide a natural pathway for proton transport via a proton-jump mechanism; and (4) the structure of such bundles of α-helices dictates a traveling wave mechanism* by which proton or ion gradients could be coupled to active transport.

Highly detailed packing equations derived from the knobs-into-holes packing hypothesis account exactly for several structural features of the protein from the purple membrane of Halobacterium Halobium.

The traveling wave transport hypothesis accounts for the specificity, directionality, and coupling observed in many transport systems, with no net motion and only very small excursions of the proteins involved. Tritium exchange experiments (by others), our studies on model membrane systems, and order of magnitude energy calculations together suggest that previous criticisms made on energetic grounds are insufficient for ruling out the traveling wave hypothesis.

*Dunker, Marvin, Zaleske and Jones (1976), Biophys. J. 16, 102a.

660 LATERAL PHASE SEPARATIONS AND CHLORTETRACYCLINE TRANSPORT IN BACILLUS MEGATERIUM, James A. Magnuson, Michael E. Dockter, and William R. Trumble, Washington State University, Pullman, WA. 99164

Fluorescence of tetracycline antibiotics can be used to follow their active accumulation. Breaks in Arrhenius plots of transport rates are observed for several bacteria. With Bacillus megaterium the Arrhenius plot is triphasic with breaks near 10° and 20°. Fluorescence polarization studies show a large change in antibiotic mobility at 20°. The lower characteristic temperature is not detectable by this method. N-phenylnapthylamine (NPN) fluorescence has been used to detect the 10° characteristic temperature. NPN is known to partition into the lipid regions of the membrane. The electron spin resonance probe 2,2-dimethyl,4-butyl,4-pentyl,N-oxyloxazolidine has been used to detect both the 10° and 20° characteristic temperature. Obviously some probes will indicate both characteristic temperatures while others respond more significantly to only one. We believe that the lower temperature corresponds to changes mostly in lipid domains while the upper corresponds to changes near the transport protein.

661 CHARACTERIZATION OF MEMBRANE PROTEINS SYNTHESIZED BY RABBIT RETICULOCYTES. Philip A. Knauf and Pamela J. Marchant, Research Institute, Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8.

Rabbit reticulocytes induced by repeated bleeding selectively synthesize certain membrane proteins. Since in this system the membranes are not affected by phenylhydrazine-catalyzed oxidation, it has been possible to characterize the proteins by selective extraction techniques. Most of the synthesized proteins are extracted at least in part at low ionic strength in the presence of EDTA. All of the synthesized proteins can be eluted by protein perturbants, such as high pH, 2,3-dimethyl-maleic anhydride (DMMA), or p-chloromercuri-phenyl sulfonic acid (PCMBS). Triton X-100 extracts all of the synthesized proteins, but is less effective in removing the synthesized protein of 37,000 daltons molecular weight. This protein is also more resistant to extraction by high pH and DMMA, but is more easily extracted by PCMBS. Although it has been suggested that this protein might be a subunit of the membrane bound enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), it was possible to elute more than 80% of the GAPDH activity at high ionic strength without removing this synthesized protein. None of the synthesized proteins is hydrolyzed by externally added trypsin or chymotrypsin. Although some of the synthesized bands co-migrate on SDS-polyacrylamide gels with proteins which are labelled by externally added pyridoxal phosphate or NAP-taurine, the labelled components do not co-migrate with the synthesized bands after proteolysis, or else do not co-elute with them after various extraction techniques. These results suggest that the synthesized proteins are peripheral membrane components exposed at the cytoplasmic surface. (Supported by Medical Research Council (Canada) Grant MA-5149.)

662 DIAMIDE INHIBITION OF ERYTHROCYTE MEMBRANE PHOSPHORYLATION, David A. Plut, M. Marlene Hosey, and Mariano Tao, Univ. of Illinois Medical Center, Chicago, Ill. 60612

Diamide, $(CH_3)_2NCON=NCON(CH_3)_2$, was initially introduced as a specific glutathione oxidizing agent. Subsequent work has also shown diamide to be a reversible inhibitor of amino acid and D-methyl glucoside transport in rat kidney cortex. This effect has been correlated with an inhibition of cyclic AMP-dependent protein kinase activity. This study reports the induction of cross-linking of membrane proteins and the inhibiton of certain aspects of membrane phosphorylation be diamide. Human and rabbit erythrocyte membranes were phosphorylated in the presence of [γ-^{32}P]ATP or [γ-^{32}P]GTP. Phosphorylation was analyzed by measuring the radioactivity in trichloroacetic acid precipitable protein or by SDS gel electrophoresis and radioautography. The autophosphorylation of membranes in the presence of GTP was completely and rapidly inhibited by 1-2 mM diamide whereas that in the presence of ATP was only partially inhibited. Phosphorylation of casein by solubilized erythrocyte membrane protein kinases was not affected by high concentrations of diamide. Analysis with SDS gel electrophoresis and radioautography indicated that the inhibition of phosphorylation was specific for certain membrane proteins. Diamide concentrations capable of inhibiting phosphorylation were shown to produce cross-links of membrane proteins similar to those obtained with *o*-phenanthroline-Cu^{++}-complex. The data suggests that the differential inhibition of erythrocyte membrane phosphorylation and possibly of the transport processes previously reported was produced, at least in part, by the cross-linking of membrane components by diamide. Supported by American Cancer Society Grant #BC-65C.

663 PROPERTIES OF THE HCO_3^--STIMULATED Mg^{2+}-ATPase ACTIVITY IN RED CELL MEMBRANES, Eileen L. Watson, Kenneth T. Izutsu and Ivens A. Siegel, Depts. of Pharmacology and Oral Biology, University of Washington, Seattle, WA 98195.

The HCO_3^--stimulated Mg^{2+}-ATPase activity in red cell ghost fragments was investigated. Heretofore, the validity of various proposed plasmalemmal HCO_3^--ATPases has been questioned on the basis of mitochondrial contamination. Red cell ghosts were prepared from New Zealand male rabbits according to the method of Quist and Roufagalis (FEBS Letters 50:135, 1975). Basic ATPase (Mg^{2+}-ATPase) was measured in a solution containing 5 mM $MgSO_4$, 50 mM Tris-H_2SO_4 (pH 7.5), 10^{-4} M ouabain, 5 mM Na_2ATP, and 0.4 mg red cell fragments; HCO_3^- + Mg^{2+}-ATPase activities contained varying amounts of $NaHCO_3$ (or $KHCO_3$), in addition. Assays were performed at 37°C. Succinate dehydrogenase activity, which was measured to check against the possibility of contamination by white blood cells during the preparation procedure, was not detectable. Basic ATPase activity was enhanced by increasing the HCO_3^- concentration in the incubation medium; maximal activity averaged 1.38 ± 0.30 µmoles of inorganic phosphate liberated per mg protein per hr. $NaHCO_3$ appeared to be more effective in this regard than $KHCO_3$. The ATPase activities were slightly stimulated by increases in ionic strength, and ITP was utilized almost as effectively as ATP. A Mg/ATP ratio of 1.0 and pH of 7.6 yielded maximal activity. The preceding properties of the HCO_3^- + Mg^{2+}-ATPase are of interest since the present enzyme is the only unquestionable instance where a HCO_3^--ATPase is located in the surface membrane of a cell, and its properties are different from those reported for the supposed transport enzymes in other tissues. The physiological importance of the present enzyme will not be known until any possible transport capacilities are evaluated.

606 GROWTH AND METABOLIC CAPACITIES OF BACTERIA AT ZERO PROTON-MOTIVE FORCE, Franklin M. Harold and Jennifer Van Brunt, Division of Molecular and Cellular Biology, National Jewish Hospital and Research Center, Denver, Co. 80206.

It is now widely accepted that metabolizing bacteria are traversed by a strong and continuous current of protons. The current is initiated by vectorial metabolic reactions across the membrane (respiration, ATPase) and serves as driving force for various energy transductions including active transport of metabolites, oxidative phosphorylation and motility (1). We have asked whether the proton circulation is involved in laying down the fabric of bacterial cells, in DNA replication or cell division. The results suggest that at this level the proton circulation is dispensable: Streptococcus faecalis could be grown in a complex medium in presence of gramicidin under conditions such that the membrane is permeable to H^+, K^+ and Na^+ and that ΔpH, $\Delta\psi$ and pmf are all zero. The organisms grew exponentially with a generation time near 45 min (controls, 35 min) for at least 10 generations; cell division and morphology appeared normal. Control experiments showed that such cells generated no ΔpH and probably no $\Delta\psi$ and that transport systems thought to require the proton circulation (β-galactosides, threonine) were uncoupled. Though such organisms grow well under optimal conditions the range that they can tolerate is narrow: They require a rich medium, high concentrations of K^+, an alkaline pH and are inhibited by Na^+. Maintenance of the cytoplasmic pH and K^+ content appear to be critical functions of the proton circulation.

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607 THE EFFECT OF EXOGENOUS ATP ON THE PERMEABILITY PROPERTIES OF TRANSFORMED CULTURES OF MOUSE CELL LINES, Enrique Rozengurt, Leon A. Heppel and Ilan Friedberg, Imperial Cancer Research Fund, London.

Treatment for 3 minutes at 37° with only 0.2mM ATP causes a 20-30 fold increase in the rate of efflux of nucleotide pools of cultures, transformed mouse cells (3T3, SV3T3, PY3T3). Pools were labeled with [^{3}H] uridine, [^{3}H] adenosine or [^{3}H] deoxyglucose. The effect is very much less in the case of untransformed 3T3 cells and secondary cultures of mouse embryo fibroblasts. The ATP cannot be replaced by ADP, other nucleoside triphosphates, EDTA, EGTA, inorganic pyrophosphate or other chelating agents. The efflux occurs in Tris, Hepes or phosphate buffered saline mixtures and is much greater at pH 8 than at pH 7.

Efflux continues if, after several minutes, the medium is replaced by fresh buffer lacking ATP. By thus dividing the process into 2 stages we determine that "activation" by ATP is sharply temperature dependent, while efflux itself is as rapid at 20° as at 37°. The ATP induced efflux is greatly suppressed in Dulbecco's serum-free medium, due to its content of divalent cations, glucose, bicarbonate and its nearly neutral pH. The whole process is reversible in complete growth medium; pools are regenerated and ATP-induced efflux can be repeated. Growth and cell division are unimpaired. Other effects of external ATP will be discussed [as in (1), (2)] as well as other treatments for increasing permeability (3).

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608 THE USE OF CALCIUM TRANSPORT IN THE INVESTIGATION OF MG^{2+}-ATPase FUNCTION AND MEMBRANE ORIENTATION IN VESICLES OF ESCHERICHIA COLI. Barry P. Rosen, Department of Biological Chemistry, University of Maryland School of Medicine, Baltimore,MD 21201.

One class of Mg^{2+}-ATPase (BF_oF_1) mutants of E. coli is unable to couple respiratory energy to active transport of many small molecules (1). Lack of the BF_1 portion of the complex has been shown to result in permeability to protons (2), leading to uncoupling of oxidation from active transport. To show the relationship of the BF_1 to proton permeability and to active transport, it was necessary to show that rebinding of the BF_1 of a wild-type strain to the membrane of a BF_1-deficient mutant restored coupling. Because the binding site of the BF_1 is on the inner surface of the inner membrane in vivo, it was necessary to use everted membrane vesicles for these experiments, since such vesicles have the BF_1-binding site on the outside. Methods were then developed for the measurement of active transport in everted vesicles.

Intact cells of E. coli do not accumulate calcium ion, but, instead, appear to actively extrude that ion (3). Everted membrane vesicles accumulate calcium actively when energy is supplied by oxidation of NADH, D-lactate, reduced PMS or succinate by the electron transport chain or by hydrolysis of ATP by the BF_oF_1(4). Everted vesicles prepared from a BF_1-deficient strain accumulate little calcium. Binding of purified BF_1 restores calcium transport activity when energy is derived either from respiration or ATP hydrolysis, demonstrating the relatedness of the presence of the BF_1 to coupling of respiration to energy-linked functions (5).

Membrane vesicles prepared by osmotic lysis of lysozyme-EDTA spheroplasts (6) do not accumulate calcium when energy is supplied in the form of D-lactate or reduced PMS (4). However, such vesicles do accumulate calcium when either NADH or ATP is supplied (7). Proline transport in those vesicles can be energized with D-lactate or reduced PMS but not with NADH or ATP. Neither calcium nor proline transport occurs when NADH or ATP are present simultaneously with D-lactate or reduced PMS. The results suggest that a completed proton circuit is established, leading to uncoupling of both transport systems. The results further suggest that vesicles prepared by the method of Kaback are functionally mosaic rather than strictly right-side-out.

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609 ENERGY SOURCE FOR AMINO ACID TRANSPORT IN ANIMAL CELLS, E. Heinz, P. Geck, C. Pietrzyk and G. Burckhardt, ZBC, J.W.Goethe-Universität, 6 Frankfurt/Main 70, Germany

There is increasing evidence that the active transport of most neutral amino acids into many animal cells is secondary, i.e. it is immediately and exclusively driven by the electrochemical potential gradient of Na ions. Even under conditions that this gradient is seemingly inadequate energetically, e.g. in cells depleted of K^+ and enriched with Na^+, the sodium-motive force appears still to be sufficient owing to an increment in electrical pd. produced by a K^+-stimulated electrogenic Na^+ pump (1). This could be demonstrated by two independent methods, the one based on the distribution of the lipophylic cation tetraphenylphosphonium (TPP) and the other based on a change in fluorescence of cyanine dyes, provided that the interfering influence of mitochondrial potentials could be sufficiently eliminated. The electrogenic effect of the Na^+ pump has previously been overlooked because it is not adequately reflected by the Cl^- distribution. There is evidence that this distribution is primarily controlled by a difference in pH, since Cl ions appear to exchange rapidly with OH^-, whose cellular concentration appears to rise under the influence of a proton pump stimulated by an increase in extracellular K^+ (2). Accordingly, Cl ions may be moved into the cell even though the electrical pd. should drive them outward. In a Na-amino acid cotransport system, amino acids should behave like Na^+ specific ionophores (3, 4, 5). Hence, the addition of amino acids should depress the electrical pd. to a quantitatively predictable extent, thereby decreasing the Na^+-dependent transport of other amino acids present in the system. This inhibition only affects the K_M of amino acid transport concerned, as could be explained in terms of a neutral transport carrier which becomes positive upon forming the ternary complex with amino acid and Na^+ (6). Hence competitive inhibition is simulated between amino acids which are transported by different systems, provided both depend on Na^+. These predictions could be verified experimentally.

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610 MOLECULAR BIOLOGY AND ENERGETICS OF ACTIVE TRANSPORT, H. R. Kaback, Department of Biochemistry, Roche Institute of Molecular Biology, Nutley, N.J. 07110.

Bacterial membrane vesicles retain the same sidedness as the membrane in the intact cell and catalyze active transport of many solutes by a respiration-dependent mechanism that does not involve the generation or utilization of ATP or other high-energy phosphate compounds. In E. coli vesicles, most of these transport systems are coupled to an electrochemical gradient of protons ($\Delta\bar{\mu}_{H^+}$, interior negative and alkaline) generated primarily by the oxidation of D-lactate or reduced phenazine methosulfate via a membrane-bound respiratory chain. Oxygen or, under appropriate conditions, fumarate or nitrate can function as terminal electron acceptors, and the site at which $\Delta\bar{\mu}_{H^+}$ is generated is located before cytochrome b_1 in the respiratory chain.

Addition of lactose or glucose-6-P to membrane vesicles containing the appropriate transport systems results in partial collapse of ΔpH, providing direct evidence for the proposition that respiratory energy can drive active transport via the pH gradient across the membrane. Titration studies with valinomycin and nigericin lead to the conclusion that at pH 5.5, there are two general classes of transport systems: Those that are coupled primarily to $\Delta\bar{\mu}_{H^+}$ (lactose, proline, serine, glycine, tyrosine, glutamate, leucine, lysine, cysteine, and succinate) and those that are coupled primarily to ΔpH (glucose-6-P, lactate, glucuronate, and gluconate). Strikingly, however, it is eminently clear that at pH 7.5, all of the transport systems are driven by the electrical potential ($\Delta\Psi$) which comprises the only component of $\Delta\bar{\mu}_{H^+}$ at this external pH. In addition, when the effect of external pH on the steady-state level of accumulation of various transport substrates is examined, none of the pH profiles corresponds to those observed for $\Delta\bar{\mu}_{H^+}$, $\Delta\Psi$, or ΔpH, and at external pH values exceeding 6.0-6.5, $\Delta\bar{\mu}_{H^+}$ is insufficient to account for the concentration gradients observed for most of the substrates. This finding and the observation that the accumulation of organic acids is coupled to $\Delta\Psi$ at relatively high external pH values indicate that the stoichiometry between protons and transport substrates may vary as a function of external pH.

Certain (N-dansyl)aminoalkyl-β-D-galactopyranosides (Dns-gal) and N(2-nitro-4-azidophenyl)-aminoalkyl 1-thio-β-D-galactopyranosides (APG) are competitive inhibitors of lactose transport but are not transported themselves. Various fluorescence techniques, direct binding assays, and photoinactivation studies demonstrate that the great bulk of the lac carrier protein (ca. 95%) does not bind ligand in the absence of energy-coupling. Upon generation of a membrane potential (interior negative), binding of Dns-gal and APG-dependent photoinactivation are observed. The data indicate that energy is coupled to the initial step in the transport process, and suggest that the lac carrier protein may be negatively charged.

611 TRANSPORT IN HALOBACTERIUM HALOBIUM: LIGHT-INDUCED CATION-GRADIENTS, AMINO ACID UPTAKE KINETICS AND RECONSTITUTION, Janos K. Lanyi, NASA-Ames Research Center, Moffett Field, CA 94035

Cell envelope vesicles prepared from H. halobium are largely right-side-out and contain bacteriorhodopsin. Upon illumination protons are ejected and ΔpH (interior alkaline) and $\Delta\Psi$ (interior negative) develops. Coupled to this process is the efflux of Na^+. Measurements of ^{22}Na flux, exterior pH change and $\Delta\Psi$ (with the dye, di-O-C_5) indicate that the means of Na-transport is exchange of H^+ for Na^+ across the membrane. The kinetics of the pH changes and other indirect evidence suggest that the H^+/Na^+ antiport is electrogenic ($H^+/Na^+ > 1$). The resulting large gradient for Na^+ ($Na^+_{out} \gg Na^+_{in}$), as well as the membrane potential, will drive the active transport of eighteen amino acids. The nineteenth, glutamate, is unique in that its accumulation is indifferent to $\Delta\Psi$: this amino acid is transported only when a Na^+-gradient is present. Thus, when more and more NaCl is included in the vesicles glutamate transport proceeds with longer and longer lags. Transport is unidirectional and very large glutamate gradients are produced. After illumination the gradient of H^+ collapses within 1 min, while the large Na^+-gradient and glutamate transporting activity persist for 10-15 mins, indicating that protonmotive force is not necessary for transport. ATP synthesis under these conditions is apparently absent in these vesicles. A Na^+-gradient, arranged by suspending vesicles loaded with KCl in NaCl, drives glutamate transport in the dark without other sources of energy, with V_{max} comparable to light-induced transport. A K_m of 1.3×10^{-7} M for glutamate is obtained in both cases. These and other lines of evidence suggest that the transport of glutamate is facilitated by symport with Na^+, in an electrically neutral fashion so that only the chemical component of the Na^+-gradient is a driving force.

A protein which binds glutamate has been found in cholate-solubilized H. halobium membranes, with an apparent molecular weight of 50,000. The dissociation constant for the amino acid is 6×10^{-6}M. When this fraction (but not others from the solubilized membrane) is reconstituted with soybean lipids, facilitated glutamate transport is obtained. The transport exhibits a K_m of 1.7×10^{-7} M glutamate. Neither binding nor reconstituted transport depend on the presence of Na^+. No transport for aspartate is observed. The evidence suggests that the protein involved may be a component of the glutamate transport system.

612 THE Na^+ -STIMULATED ACTIVE TRANSPORT OF NEUTRAL AMINO ACIDS IN PLASMA MEMBRANE VESICLES ISOLATED FROM MOUSE FIBROBLASTS, Julia E. Lever, Department of Cell Regulation, Imperial Cancer Research Fund Laboratories, P. O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, England.

Vesicles isolated from the surface membrane of untransformed and Simian virus 40- transformed mouse fibroblasts catalyzed carrier-mediated active transport of several neutral amino acids (1,2). Transport was stimulated as a function of an electrochemical Na^+ gradient artificially imposed across the membrane and was decreased when the Na^+ gradient was dissipated by ionophores or Na^+ preincubation. Transport in vesicles occurred dissociated from intracellular metabolism and endogenous plasma membrane ($Na^+ + K^+$)ATPase activity. Competitive interactions among neutral amino acids, pH profiles and apparent Km values for transport into vesicles stimulated by a Na^+ gradient were similar to those described for their uptake by Ehrlich cells (3).

Amino acid accumulation in vesicles in the presence of artificially-imposed differences in membrane potential, estimated by the Nernst equation from the equilibrium distributions of the permeant cation triphenylmethylphosphonium, indicated that transport in the presence of Na^+ occurs by an electrogenic process stimulated by an interior-negative membrane potential. Gradients of K^+ or H^+ or Na^+/K^+ antiport are not directly coupled to this process. These observations provide support for a mechanism of transport via the 'A System' (3) of the plasma membrane in which amino acid accumulation is chemiosmotically coupled to an interior-negative membrane potential by means of an electrochemical Na^+ gradient (reviewed in ref. 4).

Alterations in neutral amino acid uptake by mouse fibroblasts which accompany cell-density inhibition or Simian virus 40 transformation (5) were expressed in their isolated membrane vesicles as changes both in the maximal velocity of influx and also in the steady-state accumulation driven by a standard Na^+ gradient (1,6). This suggests that multiple types of control of transport may operate at the level of the surface membrane which affect both the number or mobility of amino acid carriers as well as membrane permeability to ions.

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613 TRANSPORT IN PLASMA MEMBRANE VESICLES OF EPITHELIAL CELLS, Ulrich Hopfer, Developmental Biology Center and Department of Anatomy, School of Medicine, Case Western Reserve University, Cleveland, OH 44106.

Epithelial cells are specialized in transcellular transport. To understand the basis of this vectorial transport, enterocytes have been fractionated and the luminal (brush border) membrane isolated separately from the contraluminal (basolateral) plasma membrane. The two regions of the surface membrane are distinctly different in their enzymatic composition (1). For example, various disaccharidases, alkaline phosphatase and leucine amino peptidase are localized in the brush border. In contrast, the basolateral membrane contains Na^+,K^+-ATPase, adenyl cyclase and 5'nucleotidase. Under appropriate homogenization conditions, the isolated membranes form vesicles so that transport of small solutes can be studied under defined conditions of medium composition and transmembrane gradients. Transport mechanisms for monosaccharides, neutral amino acids, sodium and anions have been investigated (2-5). For glucose and neutral amino acids, the existence of distinctly different types of transport in brush border and basolateral plasma membrane have been established (6,7). Only the transport systems of the brush border appear to be able to utilize the energy of an electrochemical sodium gradient to move sugars and amino acids against their concentration gradient. In other words, the brush border transport systems catalyze an electrogenic co-transport of non-electrolytes and sodium. In contrast, transport systems of the basolateral regions appear to mediate "facilitated diffusion" of their substrates. Characteristics of the non-electrolyte transport systems and the interrelationship of non-electrolyte and electrolyte transport will be discussed.

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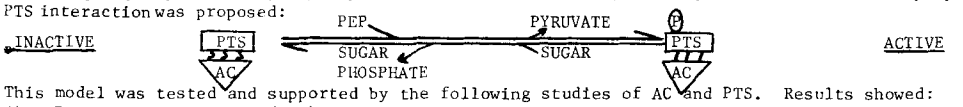
664 REGULATION OF E. COLI ADENYLATE CYCLASE (AC) ACTIVITY BY PHOSPHORYLATION STATE OF THE PHOSHOENOLPYRUVATE (PEP): SUGAR PHOSPHOTRANSFERASE (PTS). Jose E. Gonzalez and Alan Peterkofsky, NIH, Bethesda, Md. 20014

We previously reported (Peterkofsky and Gazdar, PNAS 72, 2920 (1975) that E. coli AC activity in toluene-treated cells (TTC) is stimulated by PEP and inhibited by PTS sugar substrates. A phosphorylation-dephosphorylation model (shown below) for regulation of AC activity by PTS interaction was proposed:



This model was tested and supported by the following studies of AC and PTS. Results showed: 1) PEP and 2-deoxyglucose (DOG), a glucose analogue, are non-competitive for PTS. This is consistent with the established multi-enzyme pathway for sugar transport. However, PEP and DOG are competitive for AC, suggesting regulatory interaction of one PTS protein with AC. 2) In presence of PEP, Km for DOG for PTS is identical to Ki for DOG for AC (330 μM). 3) In absence of added PEP, maximum AC inhibition is achieved at [DOG] (40 nmoles DOG/mg cell protein) comparable to [PTS complex]. This supports the idea that PTS phosphoproteins activate AC and can be stoichimoetrically dephosphorylated by DOG to inactivate AC. 4) At saturating [DOG], extent of AC inhibition is inversely proportional to [PEP], suggesting that [PTS] in phosphorylated form determines AC activity. 5)Pyridine (100 mM) which inhibits PTS by dephosphorylation of phosphohistidine proteins, inhibits AC activity 90% in TTC but not AC in broken cell extracts where AC is insensitive to sugar inhibition and therefore presumably dissociated from PTS.

665 PHOSPHATE AND ‹-AMINOISOBUTYRIC ACID TRANSPORT BY MEMBRANE VESICLES ISOLATED FROM FIBROBLASTS, Marit Nilsen-Hamilton and Richard Hamilton, The Salk Institute, Box 1809, San Diego, CA 92112

In the absence of added sodium ions, membrane vesicles derived principally from the plasma membrane and endoplasmic reticulum of mouse embryo fibroblasts take up phosphate by a mediated process. Although a Kt_{app} of 0.8 mM can be derived, uptake appears not to be saturable. The Kt_{app} of 0.8 mM phosphate measured with the vesicles in the absence of NaCl is higher than that for SV3T3, benzpyrene-transformed 3T3 (BP3T3), and 3T3 cells, which is 0.3 mM. The addition of 50 mM NaCl to the uptake reaction mixture increased the initial rate of phosphate uptake by the membranes and the maximal amount taken up by approximately 2-fold. In the presence of 50 mM NaCl, a Kt_{app} of 0.3 mM was obtained. The stimulation of uptake was specific for Na^+. This close correspondence between Kt_{app} values obtained for membrane vesicles and cells is evidence that the Na^+-dependent Pi transport system of the whole cell has been retained intact in the membrane vesicles.

We have shown that fibroblast growth factor (FGF) stimulates phosphate uptake by quiescent BP3T3 and 3T3 cells, and uptake by membrane vesicles isolated from quiescent BP3T3 cells. We have obtained similar results with ‹-aminoisobutyric acid uptake by the membranes, including a specific dependence upon sodium ions and stimulation of uptake into membranes by FGF.

666 RECONSTITUTION OF PROLINE TRANSPORT IN DETERGENT EXTRACTED MEMBRANE VISICLES FROM MYCOBACTERIUM PHLIE, Soon-Ho Lee, Natalie S. Cohen, Aaron J. Jacobs, and Arnold F. Brodie, University of Southern California, School of Medicine, Los Angeles, California 90033

Membrane visicles form M. phlei contain carrier proteins for proline, glutamine, and glutamic acid. The transport of these amino acids is Na^+-dependent and requires substrate oxidation. A proline carrier protein was solubilized from the vesicles by treatment with cholate and Triton X-100. The detergent extracted proteins were purified by means of sucrose density gradient centrifugation, followed by gel filtration and isoelectric focusing.

When the isolated carrier protein was incubated with the detergent-treated visicles, it restored the substrate-dependent uptake of proline. Studies of the reconstitution of proline transport in phospholipid visicles will be discussed.

667 ENERGETICS OF GALACTOSE TRANSPORT IN A CYTOCHROME-DEFICIENT MUTANT OF SALMONELLA TYPHIMURIUM. A.P. Singh and P.D. Bragg, University of British Columbia, Vancouver, B.C., Canada V6T 1W5.

S. typhimurium SASY28 does not form cytochromes unless supplemented with 5-aminolevulinic acid. Uptake of [^{14}C]galactose and [^{14}C]β-methyl galactoside into cytochrome-deficient cells of this organism was energized by fructose, but not by oxidation of D-lactate. In contrast, uptake of galactose and β-methyl galactoside, and also of proline and glutamine, into cytochrome-containing cells of this strain was energized by endogenous substrates, fructose, and D-lactate. Uptake of galactose and β-methyl galactoside into cytochrome-deficient cells of this mutant was inhibited by sodium azide and N,N'-dicyclohexylcarbodiimide, inhibitors of the Ca^{2+}, Mg^{2+}-activated adenosine triphosphatase. Arsenate, which decreases the ATP pool formed during glycolysis, and the uncoupling agents 2,4-dinitrophenol and carbonyl cyanide m-chlorophenylhydrazone also inhibited the uptake of these compounds. In contrast to the findings of Wilson (J. Bacteriol. 120, 866-871 (1974)) for the β-methyl galactoside and galactose-specific transport systems of Escherichia coli, our results are consistent with the hypothesis that an electrochemical gradient of protons formed either by substrate oxidation or by ATP hydrolysis through the Ca^{2+}, Mg^{2+}-activated ATPase is the driving force for galactose and β-methyl galactoside transport in S. typhimurium SASY28.

668 Na^+-DEPENDENT PANTOTHENATE TRANSPORT IN SALMONELLA TYPHIMURIUM. Stanley D. Dunn and Esmond E. Snell, The University of California, Berkeley, California 94720 and the University of Texas at Austin, Austin, Texas 78712.

The pantothenate transport system of Salmonella typhimurium LT2 was investigated using a mutant, panKl, with a thermolabile pantothenate kinase to avoid metabolism. Accumulation of ^{14}C-pantothenate by panKl at 40°, the nonpermissive temperature, pH 7.0, is linear for 1-2 minutes and reaches a plateau within 8-10 minutes. Concentrative uptake is dependent upon Na^+. At 0.6 μM extracellular pantothenate, the intracellular plateau concentration ranges from approximately the extracellular level in the absence of Na^+ to 20x higher in the presence of 10 mM Na^+. The apparent K_m for pantothenate ranges from 0.3 μM in the presence of 10 mM Na^+ to 2 μM in the presence of 0.2 mM Na^+, while V_{max} is constant at 40 pmol/min/mg (dry weight) of cells. If metabolism of pantothenate is permitted by using either LT2 at 40° or panKl at 30°, radioactivity is accumulated in the absence of Na^+, indicating that the transport system can function as a facilitated diffusion system in the absence of Na^+. These results suggest the possibility that accumulation of pantothenate is coupled to a Na^+ gradient of opposite polarity through the K_m effect. The existence of such a gradient was demonstrated with ^{22}Na. Cells of panKl energized with glucose in buffer containing 1 mM Na^+ maintain an internal concentration of about 50 μM Na^+, while in starved cells the level approaches equilibrium. Addition of glucose to the starved cells results in rapid efflux of Na^+ to the level observed in energized cells. Consistent with the hypothesis, imposition of a Na^+ gradient by addition of NaCl to a suspension of cells in Na^+-free buffer containing CCCP results in a transient accumulation of pantothenate to 2-3x its external concentration.

669 THE INHIBITORY EFFECT OF THE ARTIFICIAL ELECTRON DONOR SYSTEM, PHENAZINEMETHOSULFATE-ASCORBATE ON BACTERIAL ACTIVE TRANSPORT MECHANISMS, R. G. Eagon, B. D. Gitter and J. J. Rowe, Dept. Microbiol. University of Georgia, Athens, GA 30602

When attempting to apply the experimental procedure of Berger and Heppel (J. Biol. Chem. 249: 7747 [1974])to Pseudomonas aeruginosa, we noted that active transport of a variety of substrates was inhibited by the artificial electron donor system, phenazinemethosulfate (PMS)-ascorbate, irrespective of whether the cells were normal or starved and irrespective of whether the active transport systems were shock-sensitive or shock-resistant. This was curious because PMS-ascorbate energizes active transport by membrane vesicles prepared from P. aeruginosa and from a wide variety of other bacterial species as well; and because PMS-ascorbate energizes active transport systems in starved cells of Escherichia coli. PMS-ascorbate also inhibited active transport of proline by cells of a wide variety of bacterial species except Escherichia coli. In the case of the latter, PMS-ascorbate stimulated proline transport. N,N,N',N',-Tetramethyl-p-phenylenediamine dihydrochloride could substitute for PMS but a higher concentration was required for inhibition. The apparent K_m of glucose active transport of untreated cells of P. aeruginosa was 40 μM while the K_m of cells incubated with PMS-ascorbate was 25 mM; and PMS-ascorbate had no effect on efflux of accumulated glucose. These latter data indicate that PMS-ascorbate converted the active transport system of glucose to a facilitated diffusion system. The data also point out that there are fundamental differences between the response of intact cells and of membrane vesicles to exogenous electron donors even though the mechanism of PMS-ascorbate inhibition remains unclear.

670 PROTON FLUXES AND THEIR RELATION TO BACTERIAL MEMBRANE ENERGIZATION, J. Michael Gould and W. A. Cramer,Dept. Biol. Sci., Purdue Univ., W. Lafayette, IN 47907.

A small pulse of O_2 added to an anaerobic log-phase E. coli suspension induces a transient energization of the membrane accompanied by a slow acidification of the medium ($t_{\frac{1}{2}}$≈10s) which is only very slowly reversed ($t_{\frac{1}{2}}$>several min.). The slow kinetics for H^+ efflux and the small extent (H^+/O≈0.5) cannot be explained by respiratory control since a) little respiratory control could be detected, and b) the H^+/O is independent of both the amount of O_2 in the pulse and the cell concentration, even at levels of O_2/cell too small to maximally energize the membrane. In cells treated with the permeant anion SCN^-, or with colicin E1 (which increases membrane permeability to K^+), the rates of H^+ efflux and subsequent H^+ influx after an O_2 pulse are very fast ($t_{\frac{1}{2}}$≤1s and ≈10-20s, respectively), and the H^+/O is >2.0, implying that the membrane can no longer be polarized by H^+ transfer. In untreated cells, a small O_2 pulse induces a rapid ($t_{\frac{1}{2}}$≤0.5s) decrease in the fluorescence intensity ($\Delta F\downarrow$) of the probe N-phenyl-1-napthylamine (PhNap) followed by a slower ($t_{\frac{1}{2}}$≈40s) return of the fluorescence to the original level ($\Delta F\uparrow$). The extent of $\Delta F\downarrow$ is proportional to the amount of O_2 added, although the half-time for $\Delta F\uparrow$ is independent of the amount of O_2 added. Colicin E1 (plus K^+) and the uncoupler FCCP greatly decrease the half-time of $\Delta F\uparrow$, while only slightly affecting the extent of $\Delta F\downarrow$, indicating that $\Delta F\downarrow$ is reporting the energization of the membrane while $\Delta F\uparrow$ is reporting the subsequent deenergization resulting from counterion redistributions. The fact that the efflux of H^+ into the medium after an O_2 pulse is small and much slower ($t_{\frac{1}{2}}$≈10s) than the actual energization of the membrane ($t_{\frac{1}{2}}$<0.5s) suggests that the current of respiratory H^+ involved in membrane energization is within the bacterial membrane.

671 DISSIPATION OF THE PROTONMOTIVE FORCE BY K^+ AND NA^+ TRANSPORT IN FERMENTING S. LACTIS CELLS. E.R. Kashket and S.L. Barker, Boston University School of Medicine, Boston, Mass. 02118

Bacteria conserve and transduce energy at the plasma membrane in the form of an electrochemical gradient of hydrogen ions (Δp). Energized cells of Streptococcus lactis presumably extrude sodium ions by a Na^+/H^+ antiporter and accumulate K^+ in exchange for H^+. We reasoned that if the rate of H^+ extrusion by the membrane ATPase is rate limiting and not well regulated, then an increase in energy-consuming cation transport processes should result in a lowered steady-state Δp. We determined the electrical gradient ($\Delta\psi$) from the fluorescence of a membrane potential-sensitive cyanine dye, and the chemical H^+ gradient (ΔpH) from the distribution of benzoic acid in glycolyzing cells incubated at pH 5. Addition of NaCl (0-200 mM) dissipated the Δp (from 155 mV to 90 mV) by decreasing $\Delta\psi$; the ΔpH was constant at about 0.8. KCl (0-100 mM) dissipated the Δp very little, but lowered the $\Delta\psi$ while increasing the ΔpH from 1 to 2 units. These effects were shown not to be due to swelling or shrinkage of the cells. Thus in this system energy is utilized for active transport of Na^+ and K^+ cannot be compensated for by increases in the rate of glycolysis or H^+ expulsion. Supported by a grant from the National Science Foundation.

672 ISOLATION OF MEMBRANE VESICLES WITH INVERTED TOPOLOGY BY OSMOTIC LYSIS OF AZOTOBACTER VINELANDII SPHEROPLASTS. Eugene M. Barnes, Jr. and Pinakilal Bhattacharyya, Baylor Coll. of Med., Houston, TX 77030.

Spheroplasts derived by lysozyme-EDTA treatment of Azotobacter vinelandii strain O cells were lysed in 50 mM Tris-acetate buffer, pH 7.8. Closed membrane vesicles, 0.1-0.8 μM in diameter were isolated from the lysate. These "Tris" vesicles contain a trypsin-activated, DCCD-sensitive Mg-ATPase (130 nmol Pi released/min/mg). The vesicles have an active transport system for calcium (Km = 48 μM); calcium accumulation is coupled to the oxidation of D-lactate or to ATP hydrolysis after trypsin treatment. In contrast, vesicles isolated from spheroplast lysates in potassium phosphate buffer (pH 7.0) have much lower ATPase activity (19 nmol/min/mg) and transport calcium at less than 10% of the rates observed for "Tris" vesicles either in the presence of ATP or D-lactate. The formation of a pH gradient across the membrane of the "Tris" vesicles was examined by quinacrine fluorescence. The fluorescence intensity of quinacrine was reduced 25% by lactate oxidation and 17% by ATP hydrolysis and these effects were blocked by 1 μM CCP. This indicates that a pH gradient (inside-acid) is established across the membrane under energized conditions. The data support the view that the "Tris" preparations consist primarily of vesicles with a topology which is inverted with respect to the intact cell. (Supported by NIH Grant GM-18962 and NSF Grant PCM 75-13591).

673 CALCIUM-DEPENDENT INTERACTION BETWEEN CYTOPLASMIC (Ca-Mg)ATPase ACTIVATOR AND THE ERYTHROCYTE MEMBRANE. Frank F. Vincenzi and Martha L. Farrance, Department of Pharmacology, University of Washington, Seattle WA 98195.

Membranes from human erythrocytes (RBC) hemolyzed in 310 imOsm (isosmotic) imidazole buffer (I310), pH 7.4, show enhanced (Ca-Mg)ATPase activity (3.25 ± .07 μmole P_i mg protein^{-1}hr^{-1}) compared to low activity (0.76 ± .04 μmole P_i mg protein^{-1}hr^{-1}) membranes from RBC hemolyzed in 20 imOsm imidazole buffer (I20), pH 7.4. Inclusion of 5 mM EGTA in any of the hemolysis or washing buffers yields membranes with low (Ca-Mg)ATPase activity. I20 membranes exposed to the supernate of RBC hemolysis in I310 demonstrate enhanced (Ca-Mg)ATPase activity. This is prevented by EGTA. A cytoplasmic protein (Ca-Mg)ATPase activator which can be partially purified from the supernate of RBC hemolysis with an ion-exchange column (Luthra et al., Biochem. Biophys. Acta, 419: 164, 1976) is responsible for these observations. I20 membranes exposed to partially purified activator in the presence of I310 show enhanced (Ca-Mg)ATPase activity. This is prevented by EGTA. I20 or I310 membranes treated with EGTA prior to activator in I310 show no enhancement of (Ca-Mg)ATPase activity unless Ca (10^{-5} M) is added. (Ca-Mg)ATPase activity of I20 and EGTA treated membranes can also be enhanced during ATPase assay incubation if both activator and Ca are present. Preparations with high (Ca-Mg)ATPase activity have activator associated with the membrane and low activity membranes have no activator. Activator can be removed from membranes by treatment with EGTA. It is suggested that Ca-dependent association of cytoplasmic activator with the membrane may regulate the Ca-pump and that a useful purification step for activator could be based on its membrane-binding properties. Supported by USPHS AM-16436 and GM 07270.

674 A PROTONMOTIVE FORCE DRIVES THE FLAGELLAR MOTOR OF BACTERIA, Michael D. Manson, Pat Tedesco, and Howard C. Berg, Dept. of Molecular, Cellular, and Developmental Biology, Univ. of Colorado, Boulder, Colorado 80309, and Franklin M. Harold, Div. of Research, National Jewish Hospital and Research Center, Denver, Colorado, 80206

In the presence of glucose a motile strain of Streptococcus, V4051, exhibits the same swimming behavior of alternating runs and twiddles as does Escherichia coli. When deprived of a carbon source, V4051 cells quickly deplete their ATP reserves and become immotile. An inwardly directed protonmotive force (pmf) can be induced in these starved cells in two ways. A K^+ diffusion potential (interior negative) is established by adding valinomycin to cells suspended in low K^+ medium. This treatment restores motility, although exclusively in the twiddle mode. The response lasts for about 1 minute as the membrane potential decreases and a pH gradient (cell interior acid) develops. Alternatively, a pH gradient (cell interior alkaline) can be created by acidifying the external medium. This causes the cells to twiddle for more than 5 minutes. The motility induced in starved cells is independent of ATP since cells respond in arsenate buffers in which basal ATP levels are very low and in which ATP synthesis is negligible. No cations other than H^+ seem to be necessary for motility. If known chemoattractants are added together with valinomycin the cells run rather than twiddle; if such attractants are added as the external medium is acidified the cells twiddle, as before.

675 MONOSACCHARIDE TRANSPORT IN PROTEIN-DEPLETED VESICLES FROM ERYTHROCYTE MEMBRANES, Gustav E. Lienhard, Frank R. Gorga and Michael A. Zoccoli, Dept. Biochem., Dartmouth Med. Sch., Hanover, N.H. 03755

Steck and Yu [J. Supramol. Struct. 1, 220 (1973)] found that reaction of human erythrocyte membranes with dimethylmaleic anhydride releases the polypeptides of bands 1,2, 2.1, 4.1, 4.2, 5 and 6 and leaves membrane fragments in which the only major polypeptides are those of bands 3, 4.5, 7 and the glycoproteins PAS 1-3 (nomenclature of Steck and Yu). We have isolated sealed vesicles from these protein-depleted membrane fragments by density gradient centrifugation on Dextran T70 gradients. On the basis of the activity of the enzyme acetylcholinesterase measured in the absence and presence of Triton X-100, the membranes of about 90% of the vesicles are oriented inside-out relative to intact cells. The vesicles take up L-sorbose; and the finding that D-glucose, cytochalasin B, and phloretin inhibit the uptake shows that transport occurs by the erythrocyte monosaccharide transport system. The activity of the transport system in the vesicles, based on the initial rates of sorbose uptake and expressed per mg membrane protein, is about 95% of that in erythrocytes. Consequently, the eluted polypeptides do not have an obligatory role in sugar transport. Preliminary experiments indicate that inter-chain disulfide formation between band 3 polypeptides brought about by treatment of the vesicles with cupric ion - o-phenanthroline complex does not alter the rate of sorbose entry. Thus, band 3 may also not participate in sugar transport. Supported by grant 1 R01 GM22996 from the National Institutes of Health.

676 EFFECTS OF CYSTEINE ON METHIONINE TRANSPORT IN HALOBACTERIUM HALOBIUM VESICLES, S. L. Helgerson and J. K. Lanyi, NASA-Ames Research Center, Moffett Field, CA 94035

Illumination of H. halobium cell envelope vesicles containing bacteriorhodopsin results in the development of a transmembrane protonmotive force. Methionine transport can be driven either by light, primarily in response to the electrical potential, or by a high outside/inside Na^+ gradient in the dark. Cysteine inhibition of methionine transport occurs without dissipation of the driving force and can be completely reversed by washing. The K_m for methionine transport (5.7 μM) is unchanged in the presence of 30 μM cysteine (5.0 μM) indicating noncompetitive inhibition. The K_i of cysteine inhibition of both light- and Na^+ gradient-induced transport is 55 μM. Glutathione causes similar inhibition while cystine has no effect on transport. Dithioerythritol and mercaptoethanol (1 mM) inhibit transport by only 15-20%. The mechanism by which cysteine causes the inhibition has not been defined: mercurials cause only slight inhibition; agents known to interact with thiol groups (iodoacetamide, sulfite, cadmium, arsenite) do not inhibit; treatment with a reducing agent (dithionite) or bubbling air through the reaction mixture has no effect on the extent of cysteine inhibition. Addition of an excess of unlabeled methionine to vesicles suspended in high external Na^+ and loaded with labeled methionine by light-induced transport causes efflux of labeled methionine. The halftime of efflux is stimulated by the presence of Na^+ inside the vesicles (30 min in K^+-loaded vesicles; 10 min in Na^+-loaded vesicles). Addition of cysteine instead of methionine causes identical efflux kinetics. This suggests that (a) the efflux of methionine is dependent on Na^+ as is the uptake process and (b) cysteine inhibits only the inward transport process.

677 TRANS-MEMBRANE ION TRANSPORT IN MITOGENIC STIMULATION, John Bramhall and Jim Morgan, Dept. Bacteriol. and Molec. Biol. Inst., UCLA, Los Angeles, California 90024; and Friedrich-Miescher Laboratorium der Max-Planck-Gesellschaft, 74 Tübingen, W. Germany.

There are a number of mitogenic agents, including hormones, which have a requirement for calcium ions in the extracellular milieu if they are to exert their mitogenic potential, and calcium itself is mitogenic at elevated concentrations. A modification of the cellular ion balance is one of the primary responses following cell-mitogen interaction. Mitogens also promote changes in the intracellular cyclic nucleotide concentrations, and we have established that cyclic nucleotides themselves can exert a mitogenic effect in thymocyte populations. However, we demonstrate that the cyclic nucleotides do not immediately affect trans-membrane potential and/or ion permeability, as assessed by fluorescent probe and radio-tracer studies, although they do induce changes in the membrane sensitivity to selected ions. The magnitude of the cellular response to those mitogens which exert their effect through an alteration in cellular ion balance may be augmented by changes in the cyclic nucleotide balance within the cell. The sequence of events initiated by calcium-dependent mitogens has been partially dissected using steroids which modify responses of the plasma membrane to calcium and other mitogens. Results show that there is a trans-membrane ion flux, triggered by the action of calcium on the membrane, and that one of the earliest responses to mitogenic stimulation is a membrane hyperpolarisation which appears to be highly calcium-sensitive. The subsequent redistribution of ions across the membrane may be a sufficient stimulus for the completion of the mitogenic response to be effected.

678 CHARACTERISTICS OF CYCLIC-GMP BINDING TO THE ISOLATED RAT INTESTINAL BRUSH BORDER MEMBRANE, Peter H. Burrill and Kenneth K. Tsuboi, Dept. of Pediatrics, Stanford Univ. Med. Ctr., Stanford, CA 94305

Isolated rat intestinal brush border membranes were found to bind cyclic-GMP with less than 3% non-specific binding at 50 nM cyclic-GMP. A time course of the binding at 0° C indicated that equilibrium was established within 60 min and was stable for several hours. At 25° C, however, maximum binding was observed within the first 5 min followed by rapid loss of label. A pH curve of cGMP binding at a concentration of 50 nM over a pH range of 4-10 pH units showed a maximum binding at pH 5 followed by a sharp decline and plateau from pH 7-10. Binding at pH 5 was about twice that observed at pH 7. The addition of 0.1% Triton X-100 did not appreciably alter the pH curve. Separation of the Triton-extracted material, however, from the insoluble portion resulted in a loss of the maximum binding at pH 5 in both fractions which was not restored by adding the two fractions together. A Scatchard plot of cyclic-GMP binding at pH 5 resulted in a double-limbed curve with estimated K_d values of 2 and 20 nM for the upper and lower limbs, respectively. Analysis of the concentration dependent binding data obtained at pH 7 suggested negative cooperativity among the sites under these conditions. In view of the regulatory nature of cyclic nucleotides, these data allow for the speculation that cyclic-GMP may be involved in the regulation of some aspect of brush border function, such as the transport of nutrients. Preincubation of membrane vesicles with cyclic-GMP and ATP, however, did not effect D-glucose uptake in the presence or absence of Na^+. (Supported in part by USPHS. NIH Grant # HD 00049, HD 02147 and HD 00391).

679 ENERGY FLUX ASSOCIATED WITH ACTIVE TRANSPORT IN ESCHERICHIA COLI, William G. Martin, Richard A. Long and Henry Schneider, Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada.

Changes in energy flux in E. coli ML308-225 associated with active transport of the non-metabolisable substrate methylthio-β-D-galactoside were evaluated by calorimetry. Such changes were found to be a function of the amount of uptake. At high levels a major effect was stimulation of the rate of heat production to a high and essentially constant level. However, at lower levels the initial increase in heat production was followed by a decrease to the basal level observed before the addition of substrate. In both instances, flux continued and the substrate gradient remained constant during the calorimetric experiment. It appeared from the measurements that for low levels of uptake, maintenance of the gradient did not require much energy and exchange diffusion could have occurred. Higher levels on the other hand required significant energy flux which increased in a non-linear way with internal substrate concentration. Also in general CO_2 production and respiration followed the pattern of heat effects. The magnitude of the thermal effects could lead to identifying the energy coupling processes specifically linked to the uptake of β-galactosides.

680 EFFECT OF IONS ON HEXOSE PHOSPHATE UPTAKE BY ESCHERICHIA COLI. Richard C. Essenberg and Donald J. Taylor. Oklahoma State University, Stillwater, Oklahoma 74074.

Hexose phosphate uptake by E. coli proceeds faster in the presence of salts. For chloride salts of monovalent cations, there is a broad optimum between 150 and 500 mM. Cs^+, Rb^+ and K^+ are about equally effective, with NH_4^+, Na^+, Li^+ less so. Tetramethyl ammonium, choline and tris-(hydroxymethyl) amino methane are ineffective. In a series of methylammonium salts, di-, tri- and tetramethyl showed no effect and monomethyl stimulated, though not as well as ammonium. Changing the anion in a series of K^+ salts shows little effect except that F^- and HCO_3^- are inhibitory. Used alone, $MgCl_2$ stimulated, but with a lower maximal activity and at a lower concentration. If added at concentrations less than 10 mM to solutions 140 mM in KCl, most divalent cations had no effect. Hg^{++}, Cu^{++}, Fe^{++} and Sn^{++} were inhibitory. The inhibition by Hg^{++} and Cu^{++} is not reversible, and is complete at very low concentrations of the free ion. La^{+++} causes a small stimulation. (Supported by the Oklahoma Agricultural Experiment Station and NSF grant BMS 74-17347)

681 CALCIUM EXTRUSION FROM SQUID AXONS: UPHILL TRANSPORT POWERED BY THE SODIUM ELECTROCHEMICAL GRADIENT. M.P. BLAUSTEIN, Washington Univ. Sch.of Med. St.Louis,MO. 63110.

Calcium-45 efflux was measured in squid axons whose internal solute concentration was controlled by means of internal dialysis. Most of the Ca efflux requires either external Na (=Na-Ca exchange) or external Ca plus an alkali metal ion (=Ca-Ca exchange; cf. Blaustein & Russell, J.Membrane Biol. 22:285,1975). Both Na-Ca and Ca-Ca exchange are apparently mediated by a single mechanism because both are inhibited by Sr and Mn, and because addition of Na to an external medium optimal for Ca-Ca exchange inhibits Ca efflux (="occlusion"). The transport involves simultaneous (as opposed to sequential) ion counterflow because the fractional saturation by internal Ca (Ca_i) does not affect the external Na (Na_o) activation kinetics; also, Na_o promotes Ca efflux whether or not an alkali metal ion is present inside, whereas Ca-Ca exchange requires alkali metal ions both internally and externally (i.e., internal and external sites must be appropriately loaded simultaneously). ATP increases the affinity of the transport mechanism for both Ca_i and Na_o, but it does not affect the maximal transport rate at saturating [Ca_i] and [Na_o]; this suggests that ATP may be acting as a catalyst and not as an energy source. Hill plots of the Na_o activation data yield slopes ≈ 3 for both ATP-depleted and ATP-fueled axons - compatible with a 3 Na^+-for-1 Ca^{2+} exchange. Na influx and Ca efflux, measured under comparable conditions, also indicate a 3-for-1 stoichiometry. With this stoichiometry, the Na electrochemical gradient, alone could provide sufficient energy to maintain ionized [Ca_i^{2+}] in the physiological range (about 10^{-7}M).

682 IRON TRANSPORT BY RETICULOCYTE GHOSTS, George W. Bates, and Erwin Workman, Jr., Dept. Biochemistry and Biophysics, Texas A&M Univ., College Station, TX 77843

The assimilation of iron by the developing erythrocyte is an important aspect of cellular maturation which is coordianted with heme and globin synthesis. Receptor sites associated with the plasma membrane bind circulating Fe^{3+}-transferrin-CO_3^{2-}, disrupt the very stable iron-protein complex and release apotransferrin back to the circulation. The subsequent transmembrane transport of iron and donation to cytoplasmic transport agents is the subject of this poster session. We have prepared specifically labelled ^{59}Fe-ghosts from whole reticulocytes previously incubated with ^{59}Fe-transferrin-CO_3^{2-}. The mobilization of ^{59}Fe by unlabeled reticulocyte lysate, erythrocyte lysate, and chelating agents has been examined. The time course of mobilization exhibits five apparently zero order phases. The rates and extents of the phases are linearly dependent on the ^{59}Fe-ghost concentration, yet, exhibit saturation kinetics with regard to lysate concentration. Rather surprisingly, the mobilization of ^{59}Fe by erythrocyte lysate and by chelating agents follows the same chronological order of the phases. The thrust of this research is threefold: (1) establishing the validity of the ^{59}Fe-ghost transport system; (2) the kinetic corroboration of a multicomponent membrane iron transport system; and (3) identification of the plasma membrane as an important regulatory organelle in the iron metabolism of the developing erythrocyte.

683 THE UPTAKE KINETICS OF SIMPLE SUGARS INTO RENAL BRUSH BORDER VESICLES. R.J. Turner and M. Silverman. Univ. of Toronto, Toronto, Ontario.

Previous in vivo studies have demonstrated the existence of 3 distinct sugar transport systems localized in the brush border membrane (BBM) of the canine proximal tubule (Am. J. Physiol. 218(1970)743 and B.B.A. 332(1974)248). The present work extends these studies to an in vitro membrane preparation. A fraction enriched in BBM and containing osmotically active closed vesicles was obtained from dog kidney cortex by a modification of the method of Schmitz et al (B.B.A. 323(1973)98). The uptake of a series of radiolabelled sugars was measured relative to L-glucose. Experiments were carried out at 25°C in a 1mM Tris-HEPES buffer containing 100 mM mannitol. The uptakes of D-glucose, α-methyl-D-glucose (αMG) and D-galactose were strongly Na^+-dependent and sensitive to inhibition by 1-10 μM phlorizin. In contrast, D-mannose, 2-deoxy-D-glucose and 3-O-methyl-D-glucose uptakes showed minimal Na^+-dependence and little phlorizin sensitivity. This data is consistent with the existence of a D-glucose transporter in the BBM shared by αMG and D-galactose. A Scatchard plot of the initial uptake of D-glucose indicated the presence of two Na^+-dependent phlorizin sensitive transporters with approximate K_m's of 0.3 and 2.0 mM. In a similar plot for αMG only the lower affinity site was seen. In addition, the V_{max} of the high affinity site was an order of magnitude below that expected from in vivo studies. From this and other evidence related to the specificity of inhibition by homologous sugar substrates we conclude that the lower affinity site represents the physiologic D-glucose transporter.

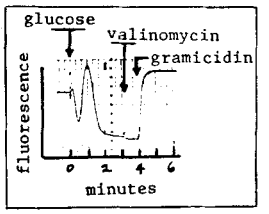
684 AMINO ACID UPTAKE BY HUMAN PLACENTAL MICROVILLUS MEMBRANE VESICLES, Carl H. Smith and Stephen M. Ruzycki, Washington University School of Medicine, St. Louis, MO 63110

The recent characterization of a vesicular microvillus plasma membrane preparation from human placenta has made possible study of amino acid uptake by the isolated membrane. Variation of medium osmolarity demonstrated that α-aminoisobutyric acid (AIB) is taken up virtually entirely into a vesicular space. To permit calculation of intravesicular concentration the vesicular volume was determined from equilibrium uptake of 3 dissimilar non-amino acid substrates all of which gave a common value. By several criteria amino acid uptake by the membrane vesicles resembles that by whole villus tissue. The vesicles take up AIB, glycine, and serine by a temperature dependent saturable process. The diffusion constant and K_m of AIB uptake were very close to our earlier determinations in whole tissue. Competitive inhibition demonstrated that the specificity of AIB uptake by the vesicles was virtually the same as in whole tissue.

AIB uptake was stimulated by an inward sodium gradient and unaffected by an outward potassium gradient. The sodium stimulation was specific for this ion. It persisted up to 2 hours after the gradient was established and this could be correlated with the slow equilibration of sodium across the membrane. AIB loaded membrane vesicles were found to bind tightly to cellulose ester filters. Transfer of these filters to a large volume of fresh medium permitted study of exodus with minimal problems of substrate recapture. The exodus process was saturable with a K_m considerably greater than that for entry. This increased K_m may play a role in maintenance of a concentration gradient by the membrane *in utero*.

685 KINETICS OF MEMBRANE POTENTIAL CHANGES IN *S. LACTIS* CELLS FOLLOWING ADDITION OF GLUCOSE. A. S. Waggoner and D. P. Carbone, Department of Chemistry, Amherst College, Amherst, Mass. 01002

Changes in the transmembrane electrical potential of energy-starved *S. lactis* cells following glucose addition were measured by the use of two fluorescent dyes, merocyanine 540-C_3 and diS-C_2-(5). The fluorescence response for a suspension of cells in Na phosphate buffer, pH 7.5, containing diS-C_2-(5) is shown in the figure. The "rebound" following glucose addition is also reflected in the intracellular ATP concentration (fire-fly luciferase assay) which is linked energetically to the membrane potential and pH gradient by a membrane bound (H^+)-ATPase. The fluorescence rebound is absent if the suspension medium pH is lowered to 5.5 or if choline is used in place of Na or K in the suspension medium. The kinetics of intracellular NADH concentration and pH changes were also monitored after glucose addition. The relative merits of several possible mechanisms for the membrane potential rebound (including competition for ATP between the early "feedback" steps of glycolysis and the (H^+)-ATPase, and allosteric switching of permeases by nucleotide phosphates and pH changes) will be discussed.



686 SUFFICIENCY OF THE SODIUM GRADIENT AS THE ENERGY SOURCE FOR AMINO ACID TRANSPORT IN EHRLICH ASCITES TUMOR CELLS, Thomas C. Smith and Ramona Adams, Dept. Physiology, The University of Texas Health Sciences Center, San Antonio, Tx 78284

The accumulation of amino acids in Ehrlich ascites cells is closely linked to Na^+ movements and the Na^+ electrochemical potential gradient (X_{Na}). Whether the X_{Na} and the coupling efficiency between Na^+ and amino acid movements are sufficient to account for amino acid transport is unresolved. Resolution requires accurate measurement of Na^+ and amino acid concentrations within the transport compartment. We have recently demonstrated that exposure of the cells to La^{+3} permits analysis of intracellular compartmentation (J. Membrane Biol., in press). In the present studies, we have applied the technique to analyze intracellular cation and α-amino-isobutyric acid (AIB) distributions, cation leak fluxes and net AIB fluxes in cells inhibited by ouabain. In steady state cells, Na^+ is sequestered in the nucleus. Exposure to ouabain permits Na^+ accumulation, also preferentially sequestered in the nucleus. The rate coefficient (k) for the Na^+ leak flux (J_{Na}) is .0038 min^{-1}. Addition of AIB (1 mM) increases k to .0052. Net 1 min AIB flux (J_{AIB}) decreases with increased time of exposure to ouabain. Comparison of the increase in J_{Na} in the presence of AIB to J_{AIB} gives $\partial J_{Na}/\partial J_{AIB}$ = 0.36. Incubation of cells in AIB plus ouabain shows AIB preferentially sequestered in the nucleus. [AIB] in the cytoplasm decreases with time in ouabain. At constant [AIB], the unidirectional flux ratio, J_{AIB}/J_{Na} = 2.5. These values of $\partial J_{Na}/\partial J_{AIB}$ and J_{AIB}/J_{Na} yield a maximum efficiency of energy transfer = 69%. Comparison of X_{Na} needed to maintain constant [AIB] in the cytoplasm is consistent with a coupling efficiency of 69%. We conclude that in these experiments, X_{Na} alone is sufficient to account for AIB transport. (Supported by research grant BMS 75-13489 from NSF.)

687 ASSOCIATION OF ACTIVE CALCIUM TRANSPORT AND (Mg+Ca)-ATPase ACTIVITY IN VESICLES OF HUMAN ERYTHROCYTE MEMBRANES. Basil D. Roufogalis and Eugene E. Quist, Lab. Mol. Pharmacol. Fac. Pharm. Sci. Univ. of B.C. Vancouver, B.C. Canada V6T 1W5.

Human erythrocyte membranes prepared by stepwise hemolysis exhibit (Mg+Ca)-ATPase activities with either high, low or both high and low Ca^{2+} affinities, depending on the method of preparation. We have attempted to determine which of these (Mg+Ca)-ATPase activities are associated with active Ca^{2+} transport. "Inside-out" membrane vesicles prepared from ghosts treated with 0.5 mM NaCl at 37° for 30 min had a (Mg+Ca)-ATPase characteristic of the "low Ca^{2+} affinity" activity. In these vesicles Ca^{2+} uptake and the ATPase activity showed a parallel Ca^{2+} dependence. Addition of a soluble protein fraction previously extracted from the membranes by the low ionic strength treatment at 37° doubled the (Mg+Ca)-ATPase activity and restored its "high Ca^{2+} affinity", but had no effect on the velocity of Ca^{2+} transport. It is concluded that the (Mg+Ca)-ATPase with Ca^{2+} affinity in the range 0.3 to 330 μM supports active Ca^{2+} transport in human erythrocytes (supported by the MRC of Canada).

614 RECONSTITUTION AND PURIFICATION OF THE GLUCOSE TRANSPORT PROTEIN FROM HUMAN ERYTHROCYTES

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A method was developed for incorporation of the D-glucose transport protein solubilized from ghosts with Triton X-100 into liposomes which then show specific D-glucose permeability. Triton X-100 was removed from the extract by incubation with Biobeads SM-2 or Amberlite XAD-2. The original reconstitution method (1) was modified by including a freeze-thaw step after addition of the protein to liposomes and by sonicating the thawed mixture for only 20 seconds.

The proteins solubilized by Triton (bands 3, 4.1, 4.2, 4.5, 7, PAS1-3) were chromatographed on a DEAE-cellulose column equilibrated with 50 mM Tris HCl, pH 7.4, containing 0.5% Triton X-100. As described previously (2) the glucose transport activity eluted from the column in the early fractions which contained a major protein (96%) with an apparent molecular weight by SDS-polyacrylamide gel electrophoresis of 55,000 and corresponded to a part of band 4.5. Band 7 was a minor contaminant and phospholipids were also present in the active fractions. Some of the properties of the 55,000 m.w. protein will be reported in addition to studies of glucose transport by the reconstituted system.

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615 ISOLATION OF ALANINE CARRIER FROM THERMOPHILIC BACTERIUM AND ITS RECONSTITUTION INTO VESICLES CAPABLE OF TRANSPORT

Hajime Hirata, Nobuhito Sone, Masasuke Yoshida and Yasuo Kagawa, Department of Biochemistry, Jichi Medical School, Tochigi-ken, Japan 329-04

Isolated bacterial membranes are capable of transporting nutrients when appropriate energy is supplied (1-2). For more detailed studies on molecular mechanisms of transport a much purer system is required. To date no transport carrier has been isolated from bacterial membranes in a physiologically active form. This might be due to its unstable nature when solubilized from membranes and lack of information on reconstitution of functional vesicles. Enzymes of thermophilic bacterium PS3 are stable against various physicochemical treatments (3), from its membranes a DCCD-sensitive ATPase complex has been successfully purified and reconstituted into proteoliposomes capable of energy transformation (4-5).

Recently we have also succeeded in solubilizing an alanine carrier from the same materials and reconstituting it into proteoliposomes with phospholipids (6). The transport carrier activity is defined as a protein(s) catalyzing active transport of alanine into reconstituted vesicles in response to an artificially imposed membrane potential. The carrier protein is solubilized from membranes by cholate-deoxycholate mixture and partially purified by DEAE cellulose column chromatography and gel filtration in the presence of Triton X-100. On Sepharose 6B in the presence of 0.5% Triton X-100 the activity appears in fractions between urease (MW 483,000) and yeast alcohol dehydrogenase (MW 150,000). The final preparation has an apparent Km for alanine transport of 10 μM which is exactly the same as that observed in original membranes. The reconstituted vesicles transport glycine as well as alanine. Glycine inhibits alanine transport competitively, indicating that the carrier has an affinity for both alanine and glycine. Among various phospholipids tested, only those derived from the original bacterium are able to form functional vesicles with the alanine carrier. Those phospholipids are found to have no unsaturation in their fatty acyl groups such as 1-heptadecanoyl-2-pentadecanoyl-phosphatidylethanolamine or 1,2-dipentadecanoyl-phosphatidylglycerol (7).

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616 RECONSTITUTION OF ß-D-GALACTOSIDE UPTAKE IN TRANSPORT-NEGATIVE MEMBRANE VESICLES FROM ESCHERICHIA COLI, Karlheinz Altendorf[+], Clemens R. Müller[++] and Heinrich Sandermann[++], [+]Universität Tübingen, Lehrstuhl Mikrobiologie II, 7400 Tübingen and [++]Universität Freiburg, Lehrstuhl Biochemie der Pflanzen, 7800 Freiburg, W.-Germany

The transport of ß-D-galactosides by E.coli is mediated by an integral membrane protein which is coded for by the lac-operon y gene (1). The translocation of one lactose molecule through the cytoplasmic membrane by the carrier protein is accompanied by the movement of one proton in the same direction (2). Since such a symport transport system has interesting mechanistic aspects, especially with regard to energy coupling (3), the extraction of the transport protein from the membrane, its purification and the reconstitution of transport activity in a defined system (liposomes) might assist in solving the still open questions. The solubilization of this protein by detergents and the determination of its molecular weight have been achieved (4,5). However, the ß-D-galactoside transport protein was completely inactivated at very low detergent concentrations (6).

Aprotic solvents have recently been found to be effective solubilizing agents for E.coli membrane proteins (7, 8). At relatively low concentrations (<3 M), the aprotic solvents dimethyl sulfoxide (DMSO), N-methylpyrrolidone (MP), tetramethylurea (TEMUR), and hexamethylphosphoric triamide (HMPT) inhibited ß-D-galactoside transport in whole cells of E.coli. Inhibition was readily reversed by simply removing the aprotic solvents (7). Similar results were obtained with membrane vesicles.

At high concentrations of aprotic solvents (90 % MP or HMPT), 50 - 80 % of the total membrane protein of transport-positive vesicles derived from E.coli ML 308-225 was solubilized. When these extracts were added, with sonication, to intact transport-negative vesicles from E.coli ML 35, lactose uptake was reconstituted (9). The criteria for the successful reconstitution will be presented.

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617 *RECONSTITUTION OF THE COUPLED TRANSPORTS OF Na^+ AND K^+ IN VESICLES CONTAINING THE PURIFIED NaK ATPase FROM THE RECTAL GLAND OF SQUALUS ACANTHIAS*, *Lowell E. Hokin, Dept. of Pharmacology, University of Wisconsin, Madison, WI 53706*

Vesicles prepared from shark rectal gland NaK ATPase (90-95% pure) by dialyzing for two days with egg lecithin, cholate, 20 mM NaCl and 50 mM KCl were capable of coupled transport of Na^+ and K^+ if MgATP was added to the outside of the vesicles (Na^+ was transported in and K^+ was transported out). Coupled Na^+-K^+ transport was blocked if ouabain was trapped inside the vesicles by adding the glycoside to the preparation prior to dialysis but transport was not blocked if ouabain was added to the outside after the vesicles were prepared; this demonstrates the correct asymmetry for the ouabain binding site in relation to the ATP site. Uphill transport of K^+ out of the vesicles was dependent on external Na^+; this provides evidence for the coupled nature of the transport. Concentration gradients approaching the physiological levels were established by the reconstituted pump. The ratio of Na^+ transported in to K^+ transported out was 2.8, which is very close to that previously reported for red cell and squid axon membranes. In the absence of K^+ an ATP-dependent Na^+-Na^+ exchange which was inhibited by ouabain could be demonstrated. In the absence of Na^+ K^+-K^+ exchange which was dependent on ATP + Pi and inhibited by ouabain could be demonstrated. If the endogenous phospholipids in the purified NaK ATPase were displaced by exogenous egg lecithin (1-2), ouabain inhibitable Na^+-K^+ transport was as great or greater than with the enzyme containing endogenous phospholipids. The above results establish that the shark rectal gland NaK ATPase, which is 90-95% pure, is the isolated pump for the coupled transports of Na^+ and K^+.

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618 ATPASE AND ENERGY TRANSDUCTION, Robert D. Simoni, Department of Biological Sciences, Stanford University, Stanford, CA 94305

The Ca^{++}, Mg^{++} ATPase complex plays a central role in energy transduction in mitochondria, chloroplasts and bacteria. Its role in oxidative phosphorylation and photophosphorylation is well established and thought to couple energy from electron flow to ATP synthesis by functioning as a reversible proton translocation. In microbial systems this reversibility is particularly important, since under anaerobic conditions ATP hydrolysis results in the generation of a proton gradient which is the source of energy for the transport of many solutes.

The structure of the ATPase complex is not fully understood but can be considered to exist in three parts: a head sector of 5 polypeptides, a stalk sector of 1 or 2 polypeptides, and a membrane sector of 3 to 5.

The discussion in this workshop will center around genetic and biochemical analysis of both structure and function of this important energy transducing complex.

619 ACTIVE TRANSPORT OF CA^{2+} DUE TO MOLECULAR COUPLING BETWEEN ATP HYDROLYTIC AND CA^{2+}-TRANSLOCATING (IONOPHORIC) SITES, Adil E. Shamoo, Dept. of Radiation Biology & Biophysics, University of Rochester School of Medicine & Dent., Rochester, N.Y. 14642.

Sarcoplasmic reticulum (SR) obtained from rabbit white skeletal muscle has been shown to have ATP-dependent pump for Ca^{2+}. Purified SR Ca^{2+} + Mg^{2+}-ATPase was reconstituted into vesicles and shown to contain the primary pump machine for Ca^{2+}. Intact Ca^{2+} + Mg^{2+}-ATPase has been shown to contain a Ca^{2+}-dependent and selective ionophoric activity in artificial lipid bilayers (1). In 1 M sucrose, trypsin cleaves first the 100K Ca^{2+} + Mg^{2+}-ATPase into 55K and 45K daltons where the hydrolytic and Ca^{2+}-transport activities remain at the level of undigested ATPase. Trypsin subsequently cleaves the 55K into 30K and 20K dalton fragments. The 55K and 20K dalton fragments have Ca^{2+}-ionophoric activity inhibited by ruthenium red and by mercuric chloride but not by methylmercury, an inhibitor of the hydrolytic site of the enzyme. The 55K and 30K dalton fragments have been shown to contain the ATP hydrolytic site as evidenced by the appearance of phosphorylation and H^3-NEM binding onto the two fragments (2). As the 55K is cleaved, Ca^{2+}-transport activity declines rapidly and is correlated with the disappearance of the 55K and the appearance of the 30K and 20K fragments derived from the 55K. Thus, Ca^{2+}-transport and ATPase activity are uncoupled at the point of cleavage of the 55K to 30K and 20K fragments, and hydrolysis remains intact while transport is abolished.

The 20K ionophoric activity has been shown to be the same in lipid bilayer conductometry using two bilayer-forming solutions; oxidized cholesterol and phosphatidylcholine: cholesterol (5:1 mg/ml) (3). The ionic dependency and permeability sequence of the 20K in PC:cholesterol membrane Ca^{2+}, Ba^{2+} > Sr^{2+}, Mn^{2+} > Mg^{2+} > Cd^{2+} Zn^{2+}

Subsequent cleavage of the 20K fragment with cyanogen bromide reveals that a fragment of about 8K contains the ionophoric site. Discussion will focus on the reconstitution of the 20K into vesicles, peptide mapping of the two sites and the energy linkage between the two sites.

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620 COATED-PIT COATED VESICLE MEDIATED SPECIFIC UPTAKE OF PROTEINS IN EUKARYOTIC CELLS, Thomas F. Roth, Michael P. Woodward, Stanley Yusko and John Woods, Department of Biological Sciences, University of Maryland Baltimore County, Catonsville, Maryland 21228.

Specialized regions of the plasma membrane of all eukaryotic cells appear to play a fundamental role in receptor mediated uptake of protein (1-2). These specialized regions of the plasma membrane, called coated pits, occur as invaginations with a 150Å thick lattice-like coating on the cytoplasmic surface. Receptor molecules, localized in these regions, face the extracellular milieu. Following invagination, coated pits pinch off the plasma membrane giving rise to coated vesicles (CV) which in turn lose their cytoplasmic coat and become naked protein filled vesicles. However, only in developing oocytes and LDL uptake by fibroblasts (3) is the identity of the proteins transported by this mechanism known.

Developing chicken oocytes selectively sequester immunoglobulin G(IgG) and phosvitin-lipovitellin (PvLv) (4-5). Ultrastructural studies on the developing chicken oocyte with ferritin conjugated IgG have shown that binding to the membrane occurs only in the coated pit regions (6). PvLv binds to the oocyte membrane with an apparent Kd of 1×10^{-6}M, has a pH optimum of 6 and shows no cooperativity. Only PvLv and Pv compete with ^{125}I-PvLv for binding, from which we infer that the Pv portion of PvLv is recognized by the receptor. Recent studies suggest that the receptor for PvLv is a protein.

Recently, in our lab and elsewhere (7), it has been possible to purify coated vesicles from a variety of tissues. SDS-gel profiles of purified coated vesicles from brain and developing chicken oocytes have three major bands with apparent molecular weights of 180K daltons, 120K daltons and 55K daltons. Many minor bands are also observed, but these appear to be tissue specific. Treatment of brain CV's with 2M urea, 0.25$MgCl_2$ or pH9 selectively solubilizes the 180,000 dalton protein with concommitant disappearance of the coat structure leaving naked vesicles. The precise relation of the supramolecular complex of the coat and membrane proteins to the overall transport process is as yet unclear. However, it seems likely that the receptor is locked into these regions and mediates the coated pit to coated vesicle transition.

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688 ASSEMBLY OF THE CATALYTIC UNIT OF THE E. COLI MEMBRANE ATPase IN VITRO REQUIRES THE γ SUBUNIT, Jeffrey B. Smith and Robert J. Larson, Cornell Univ., Ithaca, NY 14853

Freezing the purified F_1 portion of the E. coli membrane ATPase (EC-F_1) at high ionic strength with KNO_3 reversibly inactivated the enzyme and dissociated the two larger α and β subunits to monomers, in contrast to the incomplete dissociation obtained by Vogel and Steinhart (Biochemistry 15: 208, 1976) without KNO_3. After cold inactivation the EC-F_1 subunits were separated into two fractions by hydroxylapatite chromatography. One fraction contained chiefly the α and β subunits (αβ-fraction), while the other contained α, β and a three- or four-fold excess of γ and ε (γε-rich fraction), by comparison to the native enzyme. Dialysis against MgATP restored full ATPase activity to the γε-rich fraction but the αβ-fraction remained inactive. Combining the two fractions produced a synergistic restoration of ATPase activity. With an excess of the αβ-fraction, about 3-fold more activity was restored than was obtained with the γε-rich fraction alone. Purified δ and ε, alone or together, were ineffective in restoring activity to the αβ-fraction and had no effect on its reconstitution by the γε-rich fraction. Since the ATPase appears to be fully active without an intact γ subunit, (Nelson, N., et al, PNAS 71: 2720, 1974) it is probably required for the assembly of the catalytic unit after cold inactivation. Epsilon may or may not be needed along with γ to restore activity to α and β. Assembly was not catalyzed by γ but required a fixed amount relative to α and β. Surprisingly the αβ-fraction becomes highly viscous (about 50-fold greater than native EC-F_1) after dialysis against MgATP. This novel property of the EC-F_1 subunits will be described.

689 CHARACTERIZATION OF THE PURIFIED DELTA AND EPSILON SUBUNITS OF THE PROTON TRANSLOCATING ATPase OF E. COLI. Paul C. Sternweis and Jeffrey B. Smith, Cornell University, Ithaca, NY 14853

The active delta and epsilon subunits of the F_1 portion of the membrane ATPase from E. coli (EC-F_1) have been purified to homogeneity[1] (Smith and Sternweis, Biochem. in press). The delta subunit has a molecular weight of about 18,000 as measured by SDS-electrophoresis or by sedimentation equilibrium either with or without 6 M guanidine hydrochloride. The active subunit therefore exists as a monomer and the apparent high molecular weight of about 33,000 obtained from molecular sieve chromatography suggests that delta is probably an elongated molecule with a calculated f/f_o of 1.3. Circular dichroism spectra indicate that the subunit has a high degree of secondary structure with an α-helix content of about 55-70%. Amino acid analysis of delta reveals the presence of tryptophan and cysteine which contrasts with the lack of these in the delta subunits of the mitochondrial and chloroplast ATPase. Reconstitution experiments with δ and enzyme lacking δ indicate that there is probably only one δ in the active coupling factor (EC-F_1). Epsilon inhibits the hydrolytic activity of five subunit EC-F_1 and enzyme lacking δ but not EC-F_1 from which γ, δ, and ε have been removed by digestion with trypsin (J. Supramol. Struc. 3: 248, 1975). The enzyme containing only α and β subunits was made sensitive to epsilon inhibition by treatment with a γ-rich fraction obtained from hydroxylapatite fractionation of EC-F_1 dissociated by cold inactivation. We conclude that the ATPase inhibiting activity of ε is mediated by the γ subunit.

690 TOTAL RESTORATION OF ATPase ACTIVITY TO AN INACTIVE FORM OF MITOCHONDRIAL F_1 BY SUBMICROMOLAR LEVELS OF UNCOUPLERS AND BY OTHER MANIPULATIONS. A. Stephen Dahms, R. S. Iyengar, T. K. Wong and R. E. Krawczyk. San Diego State University, San Diego, CA 92182

Beef heart mitochondrial ATPase (F_1) purified by conventional means or the solvent-release method of Beechy et al. (Biochem. J. 148, 533) has been converted to a totally inactive apparent allomorph (PF_1) in a light-dependent reaction. $S_{20,w}$ values for ^{125}I-labeled PF_1 and ^{125}I-labeled F_1 were 10.1 and 11.9 S, respectively. The tyrosine difference spectrum has a maximum at 290 nm and is consistent with the burying of 8-9 tyrosines with the $F_1 \rightarrow PF_1$ conversion. PF_1 exhibits a slightly lower mobility relative to F_1 on PAGE; SDS-PAGE of PF_1 results in a subunit pattern virtually indistinguishable from F_1. PF_1 does not bind ^{14}C-ADP whereas under the same conditions F_1 binds 0.50 mole ADP/mole. ATPase activity is fully restored by heating PF_1 for 1' at 60°C either in the presence of 2 mM ATP-EDTA or 5 mM $MgCl_2$; the reactivated enzyme binds 0.43 mole ADP/mole. Full reactivation is also achieved by a brief incubation of PF_1 at 4°C; subsequent to reactivation, the ATPase manifests cold lability with the same kinetics of inactivation as F_1. ATPase activity can also be fully restored by DNP, m-ClCCP and "S-13"; half-maximal activation occurs at 47.9, 0.562 and 0.0115 μM, respectively; the "S-13" titration curve demonstrates a stoichiometry of 0.94 mole "S-13" per mole F_1 at 95% activation (40.1 nM "S-13"). The data suggest that soluble PF_1 reflects the conformation of the ATP synthetase of the coupled inner mitochondrial membrane and, further, that uncouplers produce a conformational change in the latter resulting in unidirectional hydrolytic activity. PF_1 does not catalyze $P_i \rightleftharpoons$ ATP or ADP $\rightleftharpoons$ ATP exchanges.

691 THE USE OF PHOTO-SENSITIVE CROSSLINKER, Tae H. Ji and Douglas J. Kiehm, Div. of Biochemistry, University of Wyoming, Laramie, Wyoming 82071.

Several photo-sensitive crosslinking reagents were synthesized and the condition for their application in elucidating crosslinking membranous molecules were established. Crosslinking has been performed on the membranes of erythrocyte ghosts and sperm. The extent of crosslinking of protein molecules in the two membranes were remarkably different. The proteins in the erythrocyte ghosts were resistant to a very short length crosslinker (5 Å), whereas those in the sperm membrane were extensively crosslinked. The difference may be due to a distinctive dissimilarity in the molecular organization in those two types of membranes. (Supported by grant, NSF-BM75-09230. Wyoming Experiment Station SA-750.)

692 ROLE OF CALCIUM AND ANION CHANNEL ACTIVATION IN RELEASE OF EPINEPHRINE AND PROTEIN FROM ISOLATED SECRETORY VESICLES. Harvey B. Pollard, Christopher J. Pazoles, and Carl E. Creutz. The National Institutes of Health; Bethesda, Maryland 20014.

Isolated secretory vesicles from the adrenal medulla (chromaffin granules) release their contents when exposed to Ca^{++}, Mg^{2+}-ATP and high levels of chloride ion. The mechanism by which these reagents provoke release from vesicles has attracted our attention as a possible model for the exocytosis process. Chloride gradients occur across most secretory cell plasma membranes, and we therefore directed our attention towards the mechanism of the anion requirement. We have found that in the presence of Ca^{++} (0.1-1.0 μM), Mg^{2+}-ATP allowed permeant ions such as ^{36}Cl and ^{14}C-SCN to enter the isolated granule. Anion entry was found to be a consequence of Mg^{2++} ATP mediated changes in the granule transmembrane potential. At 37°C, this anion permeation step resulted in release of vesicle contents. The release mechanism appeared to involve recruitment of osmotically active particles from the vesicle core and subsequent osmotic shock. Specific reagents such as SITS, probenecid and pyridoxal phosphate, which block anion movement across RBC membranes, were found to block release from granules in a manner competitive with external chloride and with Ki values similar to those reported for the RBC anion channel system. We concluded that release from isolated secretory vesicles occurred as a consequence of exposure to Ca^{++}, followed by Mg^{2+}-ATP evoked anion entry through an anion channel in the vesicle membrane. We suggest that this process, resulting in release by osmotic shock, may also prove to be of fundamental importance for the process of exocytosis from cells.

693 THE UPTAKE OF RECEPTOR-BOUND LOW DENSITY LIPOPROTEIN BY COATED ENDOCYTIC VESICLES IN HUMAN FIBROBLASTS, R.G.W. Anderson, J.L. Goldstein, and M.S. Brown, Depts. of Cell Biol. & Internal Med., Univ. of Texas Health Sci. Ctr., Dallas, TX 75235

Ferritin-labelled low density lipoprotein (ferritin-LDL) was used as a visual probe to study the distribution of LDL receptors on the cell surface and to examine the mechanism of endocytosis of this lipoprotein in cultured human fibroblasts. The uptake of this macromolecular complex was compared with the mechanism of uptake of the electron dense probe horseradish peroxidase. When cells were exposed to ferritin-LDL (44 μg/ml of LDL) for 2 hr at 4°C, we determined that 60-70% of the label was localized over short, coated segments of the plasma membrane that accounted for no more than 2% of the total cell surface. Following the exposure at 4°C, cells were warmed to 37°C for various times. Within 10 min, almost 90% of the bound LDL-ferritin was removed from the cell surface and internalized exclusively by the formation of coated endocytic vesicles from the coated segments of surface membrane. When examined at various times after warming to 37°C, these coated vesicles containing ferritin-LDL were observed to migrate into the cytoplasm (1 min), to lose their cytoplasmic coat (2 min), and to fuse with either primary or secondary lysosomes (6 min). Fibroblasts exposed to horseradish peroxidase (5 mg/ml) for 60 min at 37°C had reaction product in both coated and noncoated endocytic vesicles. The current data indicate that although molecules may enter the cell by either coated or uncoated endocytic vesicles, the coated regions of plasma membrane are specialized for transporting receptor-bound LDL, and perhaps other receptor-bound molecules, into the cell.

694 PHOSPHOLIPID SUBSTITUTION OF THE PURPLE MEMBRANE AND ITS INFLUENCE ON THE FUNCTION OF BACTERIORHODOPSIN, Evert P. Bakker*, Department of Membrane Research, The Weizmann Institute of Science, Rehovot, Israel

The purple membrane of H. halobium consists of 25% of lipids (mainly diphytanoylether analogs of phospho- and glycolipids) and 75% of bacteriorhodopsin, which protein functions as a light-driven proton pump. To study the role of lipids in the function of bacteriorhodopsin, the natural lipids were replaced by various phosphatidylcholine species as described by Warren (1). The resulting complexes had the same protein to lipid ratio as the natural membrane and were in the form of bent open sheets. The extent of substitution increased with the fatty acid chain length of the phosphatidylcholine used, indicating that bacteriorhodopsin requires long chain fatty acids in its environment. Substitution of 95% of the lipid does not affect the spectral properties of bacteriorhodopsin, except that it slows down the photocycle 10-20 times. Due to this slow rate, we were able to measure in the light the ratio between net proton release and accumulation of the last intermediate in the photocycle, the unprotonated M_{412}. Depending on pH this ratio varied between 1.6 and 0. If bleaching to M_{412} involved only deprotonation of the Schiff-base between retinal and lysine, this ratio should have a constant value of 1.0. The variable ratio indicates that light-induced conformational changes occur in the non-chromophore part of the protein, which shift the pK_a of unidentified groups so as to cause binding or release of additional protons. These changes in pK_a may have a function in the transfer of protons through the protein.

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695 RECONSTITUTION AND PARTIAL PURIFICATION OF NEUTRAL AMINO ACID TRANSPORT COMPONENTS FROM EHRLICH ASCITES TUMOR CELLS, Gary Cecchini, Gregory S. Payne, and Dale L. Oxender, Department of Biological Chemistry, University of Michigan, Ann Arbor, 48109

Plasma membranes enriched 25 fold based upon $Na^+ + K^+$ ATPase activity have been prepared from Ehrlich ascites tumor cells. Solubilized protein fractions have been obtained from the plasma membranes either by extraction with 0.5% Triton X-100 or by extraction with 2% cholate. Partial purification of the solubilized protein fraction has been obtained by utilizing a combination of ammonium sulfate precipitations and column chromatography. Leucine binding activity has been detected in the Trition X-100 solubilized plasma membrane fraction. The leucine binding activity was measured by equilibrium dialysis and is saturable with high levels of leucine or phenylalanine and is only partially saturable by alanine. These properties are similar to those previously identified as System L. The cholate extracted plasma membrane fraction was incorporated into liposomes by addition to sonicated liposomes or by dialysis against potassium phosphate buffer in the presence of phospholipids. Phospholipids composed of a mixture of phosphatidylcholine and phosphatidylethanolamine or a mixture of soybean phospholipids were used for formation of the liposomes. Time dependent transport for alanine or leucine could be demonstrated in the liposomes when energy was supplied as a membrane potential produced by valinomycin mediated K^+-diffusion.

(Supported by National Institutes of Health Grant GM 20737).

696 RECONSTITUTION OF D-GLUCOSE TRANSPORT IN VESICLES COMPOSED OF LIPIDS AND INTRINSIC PROTEIN (ZONE 4.5) OF THE HUMAN ERYTHROCYTE MEMBRANE, Arthur Kahlenberg and Cedric A. Zala, Laboratory of Membrane Biochemistry, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Quebec, Canada H3T 1E2

The stereospecific efflux of D-versus L-glucose from liposomes formed upon sonication of erythrocyte lipid and various fractions of membrane protein was measured in order to assess their monosaccharide transport activity. Extrinsic membrane proteins solubilized by extraction of ghosts with 2,3-dimethylmaleic anhydride did not support D-glucose transport. However, reconstitution of D-glucose transport was catalyzed by the residual intrinsic membrane proteins and by a Triton X-100 extract of these proteins. The D-glucose transport protein was further fractionated by ion exchange chromatography of the Triton X-100 solubilized proteins on aminoethyl cellulose. Reconstitution of D-glucose transport was associated with an early column eluate (μ = 0.100) which, when analyzed by SDS-polyacrylamide gel electrophoresis, consisted solely of the proteins of Zone 4.5 (enumerated as per Steck, T.L., (1974) J. Cell Biol. 62, 1-19). The remaining solubilized membrane proteins, e.g., Band 3, the sialoglycoproteins (PAS 1-3), etc., which were eluted with Na phosphate buffers of increasing ionic strength, were all devoid of D-glucose transport activity. Further purification of the D-glucose transport protein is in progress.

(C.A.Z. is a Post-doctoral Fellow of the MRC. Supported by MRC grant MT-3120).

697 POTASSIUM TRANSPORT IN 3T3 AND VIRUS-TRANSFORMED 3T3 CELLS: EFFECTS OF CELL POPULATION DENSITY, Kenneth D. Brown, The Salk Institute, Box 1809, San Diego, CA. 92112

Untransformed 3T3 cells showed a marked reduction (3- to 5-fold) in the rate of uptake of K^+ with increasing cell population density. In contrast, the virus-transformed SV3T3 and Py3T3 cells did not exhibit a density-dependent reduction in K^+ transport. The reduction in the rate of K^+ uptake in 3T3 cells showed the following characteristics:

1) Began at low cell densities and preceded the cessation of cell growth.
2) Was due to a decrease in "Na-pump" activity with no change in the passive permeability of the cell membrane to K^+.
3) Was attributable to a decrease in the V_{max} with no change in the K_m of the transport system.

The reduction in Na-K exchange was accompanied by a fall in the intracellular K^+ concentration and a rise in the intracellular Na^+ concentration. It is possible that these changes in monovalent cation transport and concentrations are involved in the regulation of cell growth.

698 THE BRUSH-BORDER INTESTINAL AMINOPEPTIDASE AND AMPHIPATHIC TRANSMEMBRANE PROTEIN. D. Louvard*, S. Maroux, M. Semeriva, P. Desnuelle, CNRS, Centre de Biochimie et de Biologie Moleculaire, 13009 Marseille. *Present Address: Department of Biology, University of California at San Diego, La Jolla, California 92093.

It has been postulated that the brush-border intestinal aminopeptidase played a role in the intestinal transport of amino acids from peptides. This protein is an amphipathic molecule composed of two distinct domains. An hydrophilic part is located at the outer surface of the membrane and bears the total sugar content of the molecule as well as the enzymatic activity (M.W. 280,000 daltons). A smaller hydrophobic part (M.W. 8,500-9,000 daltons) anchors the enzyme tightly to the brush-border membrane. The hydrophilic part can be completely removed from closed right-side-out membrane vesicles by a papain treatment to give the so-called "papain-form". On the other hand, the integral molecule of aminopeptidase can be obtained by a detergent extraction of the membrane. When hydrolyzed by trypsin this generates the hydrophobic and hydrophilic parts. By using a specific antibody directed against the "papain form" almost all the antigenic determinants present on the hydrophilic domain could be detected on the membrane-bound enzyme. The hydrophobic domain of the molecule could be labelled from the inside of vesicles using a photogenerated macromolecular reagent, 4-fluoro 3-nitrophenyl azide covalently attached to the Fab fragment of a human myeloma protein.

These results lead to the conclusion that the aminopeptidase is a transmembrane protein oriented such that the NH_2 terminus is facing the cytoplasmic side of the membrane. These structural features support the idea that the mode of integration of this enzyme into the membrane may be related to its physiological function.

699 CONTROL OF AMINO ACID TRANSPORT ON THE MAMMARY GLAND OF THE PREGNANT MOUSE. Margaret C. Neville and Cinda Lobitz. Univ. Colo. Med. Ctr., Denver, CO 80262.

Insulin stimulated epithelial cell proliferation in diced mammary gland from the pregnant mouse in vitro. An early effect of insulin on this system is an increase in transport of α-aminoisobutyric Acid (AIB) transport (Friedberg et al., PNAS 67:1493, 1970). During an investigation of the mechanism of this effect, we made the following observations: (1) A 2-3 hr treatment with insulin stimulated the incorporation of ^{14}C-tryptophane into protein. This response was blocked by cycloheximide and by actinomycin D, suggesting that insulin increases protein synthesis at the transcriptional level. (2) The 10 min uptake of AIB was increased after 3 hrs of incubation with insulin, the result of an increase in V_{max} with no change in K_m. (3) Cycloheximide, at concentrations sufficient to abolish 90% of ^{14}C-tryptophane incorporation into protein, depressed AIB uptake by 20-30% within 3 hrs. This effect was prevented by insulin suggesting a post-translational effect on amino acid transport. (4) Addition of cycloheximide to insulin-stimulated tissues decreased AIB uptake within 2 hrs, suggesting that insulin does not act by decreasing carrier turnover. (5) Insulin does not alter xylose transport in this system, ruling out a non-specific effect on membrane permeability.

These findings are consistent with the hypothesis that alterations in the rate of protein synthesis have an indirect effect on amino acid transport in pregnant mouse mammary gland, possibly acting through alterations in the size or turnover of the intracellular amino acid pools. (Supported by Grant #CA 19389 awarded by the National Cancer Inst., DHEW. MCN is the recipient of Research Career Development Award 5 K04 AM 00038.)

700 EFFECT OF TRITON WR-1339 ON THE ETHINYL ESTRADIOL (EE) INDUCED ABNORMALITIES IN HEPAITC BILE SECRETION. Manuel Gonzalez.

Bile acids (BA) are transported by the liver cell against a concentration gradient into the bile, by a saturable, energy and sodium dependent process. Administration of EE, a synthetic estrogen, to rats impairs this process as described by maximum capacity to excrete BA (T_m) and decreased bile flow (BF). These changes may be the result of abnormalities in liver surface membrane (LSM) lipid composition that reduce the ability of specific receptors to translocate BA. Previous studies have demonstrated that the number of BA receptors sites are unchanged by EE, but there are alterations in the LSM lipid composition. Since Triton WR-1339 has been shown to reverse the hepatic lipid changes, we examined the effect of this agent on BF and BA T_m in the EE treated animal.

	% of Control		
	EE	EE + WR	WR
BF	61	108	100
BA T_m	63	167	163

These results suggest that EE induces changes in LSM lipid composition that are responsible for the reduction in BF and BA T_m, and these changes can be reversed with Triton WR-1339.

701 MECHANISM OF ACTION OF MEMBRANE BOUND cAMP ACTIVATED PROTEIN KINASE, James R. Owens and Boyd E. Haley, University of Wyoming, Division of Biochemistry, Laramie, WY 82071.

Recent hypotheses propose that cAMP effects on the translocation of certain molecular species is mediated by a membrane bound, cAMP activated, protein kinase. Using a radioactively tagged, photoaffinity analog of cAMP, [^{32}P]-8-N_3cAMP, and [γ^{32}P]-ATP we have investigated the membrane binding properties of both the regulatory and catalytic subunits of the cAMP activated protein kinase of human erythrocytes. [^{32}P]-8-N_3cAMP was used to locate and measure regulatory subunit. Increased membrane protein phosphorylation by [γ^{32}P]-ATP was used to determine the presence of the catalytic subunit. Incorporation of ^{32}P onto specific membrane proteins was determined by autoradiography of SDS-polyacrylimide gels (on which the membrane proteins had been separated) and by liquid scintillation counting. The data support a mechanism which operates through a tightly membrane bound regulatory subunit and a catalytic subunit that is released from the membrane when cAMP is added.

702 IONIC INTERACTIONS BETWEEN THE MITOCHONDRIAL ATPASE AND PHOSPHOLIPIDS, Carol C. Cunningham and Govit Sinthusek, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103

In order to evaluate the importance of ionic interactions between the membrane associated mitochondrial ATPase and phospholipids, the relationship between coulombic charge on liposomes of phospholipids and their ability to activate the ATPase has been measured. Phosphatidyl glycerol (PG) and phosphatidyl glycerol lysine (PGL), purified from Staphylococcus aureus, were combined in various proportions and liposomes of the resulting mixtures were prepared by brief sonication in dilute Tris-SO_4, pH 7.7. The zeta potentials of the resulting liposomes were measured by microelectrophoresis and the activity of the ATPase ($CF_0 \cdot F_1$) in the presence of the liposome preparations was determined. The zeta potentials of PGL (80% pure) and PG (90-95% pure) were +34.8 mV and -84.5 mV, respectively. The ATPase activity increased linearly with an increase in the negative charge on the liposome. The small amount of activity with PGL was not oligomycin sensitive whereas the activities stimulated with liposomes containing PG were inhibited by the antibiotic. Similar studies were performed with liposomes of either phosphatidic acid or cardiolipin. The linear relationship between zeta potential and stimulatory activity with the PG-PGL mixtures accurately predicted the relationship with phosphatidic acid. The stimulatory activity of cardiolipin was greater than was predicted by its zeta potential. The variations in the concentrations of phospholipid which gave rise to one-half V_{max} were not related to zeta potential of the liposomes. This study demonstrates that ionic interactions between the ATPase complex and phospholipids are important in influencing the activity of the enzyme.

703 PLASMA MEMBRANE TURNOVER IN THE AVIAN SALT GLAND, F.E. Hossler and M.P. Sarras, Jr., Dept.Anat.,LSU,New Orleans, La. Functional integrity and volume of the plasma membrane (pl. memb.) are maintained despite processes such as exocytosis and pinocytosis which seem to add to and subtract from the pl. memb. substance. However, the mechanisms by which pl. memb. components are synthesized and inserted into the membrane are unknown. Several observations indicate that the salt gland of ducklings provides an ideal model system for the study of these phenomena. (1) Stressing the ducklings by feeding 1% NaCl induces a very rapid, massive proliferation of pl. memb. in the gland cells. This results in extensive infoldings of the basal and lateral, but not the apical pl. memb. of these cells. (2) Simultaneously, there is a 4-5 fold increase in the specific activity of the Na,K-ATPase (a pl. memb. associated enzyme) in the gland. Binding studies with ^{3}H-ouabain (an inhibitor which binds specifically to the Na,K-ATPase) show that the total number of Na,K-ATPase molecules also increases to a maximum after 7-9 days of stress. Constants calculated for ouabain binding and for Na,K-ATPase inhibition by ouabain are identical. Autoradiographs show that ^{3}H-ouabain binds only to secretory cells. (3) If stressed ducklings are fed fresh H_2O, the process reverses: pl. memb. infoldings decrease and Na,K-ATPase activity and ouabain binding decrease to the control levels in 7-9 days. During this "destressing" we also observe an increase in filamentous material and increases in acid phosphatase and protease activities within the cells. The relationship of these changes to pl. memb. turnover, and to the fate of the excess plasma membrane during destressing are under investigation. The salt gland thus provides a naturally controllable system for the study of pl. memb. turnover; and the Na,K-ATPase provides an excellent membrane protein marker for monitoring this phenomenon.

704 SIDEROPHORE RECEPTORS IN MEMBRANE VESICLES OF BACILLUS MEGATERIUM. B.R. Byers, J. E. Aswell, A.H. Haydon, H.R. Turner, C.A. Dawkins and J.E.L. Arceneaux, Dept. Microbiology, Univ. Mississippi Med. Ctr., Jackson, Miss. 39216.

Membrane vesicles of Bacillus megaterium showed energy-independent binding (without transport) of the ferric chelates of the siderophores (iron transport cofactors) ferrischizokinen and ferriferrioxamine B. Equilibrium was reached within 2-5 min. Ninety percent dissociation of bound [^{59}Fe]ferrischizokinen was achieved in 60 sec. by addition of unlabeled ferrischizokinen. Ferriferrioxamine B caused no detectable release of [^{59}Fe]ferrischizokinen from the membranes; of other siderophores tested only ferriferrichrome A caused low release (11%) of [^{59}Fe]ferrischizokinen. [^{59}Fe]ferriferrioxamine B was dissociated (90%) in 60 sec. by ferriferrioxamine B; its structural relative ferriA22765 produced 67% release, but ferrischizokinen gave only 6% dissociation. Binding affinity constants and maximal binding capacities were 1.4×10^{7} M^{-1} and 186 pmol/mg protein for ferrischizokinen and 1.1×10^{8} M^{-1} and 23 pmol/mg protein for ferriferrioxamine B. These data indicate separate, specific binding sites (receptors) for siderophores; these receptors may be components of independent transport systems for the two siderophores. Detergent treatment (SDS) of membrane vesicles prelabeled with either [^{59}Fe]ferrischizokinen or [^{59}Fe]ferriferrioxamine B released membrane components (MW about 40,000) still associated with the labeled chelates. These siderophore-binding molecules may represent parts of the respective receptor sites.

705 A SECOND ANDROGEN RECEPTOR OF THE PROSTATE: THE GLYCOPROTEIN UNIT OF MEMBRANE ATPASE. W.E. Farnsworth, Biochem. Res., VA Hospital, Buffalo, N.Y. 14215.

While the positive impact of androgen, via the cytosolic receptor, on prostatic nuclear replication and transcription and ultimate translation of new protein is well established, these processes poorly account for our finding that, during 30 min. incubation of rat or human prostate tissue, the presence of 10^{-7}M or 10^{-9}M testosterone (T) significantly increases respiration, citric acid synthesis and secretion and uptake of K^{+}, uridine and alpha-aminoisobutyric acid. Neither cytosolic nor nuclear mediation can be involved in the acceleration of (Na^{+} + K^{+})-dependent ATPase activity of rat and human microsomes or human prostatic membranes by T and dihydrotestosterone (DHT). The manyfold increase in the Km of the ATPase and the lowered energy of activation which result from the steroid's presence suggest increased accessibility of ATPase active sites to the substrate or cation. Brief pretreatment of microsomes or membranes with T or DHT specifically increases Na^{+}-dependent phosphorylation and, in the process, steroid is bound to the preparation. We now find, upon PAGE analysis of the treated preparation, that the glycoprotein subunit binds the steroid which facilitates the phosphorylation. We postulate that (a) the extranuclear actions of androgen may be accomplished through facilitation of transport coupled to oxidative phosphorylation and/or glycolysis and (b) this is done through the action (perhaps allosteric) of the steroid-loaded glycoprotein on the reactivity of the catalytic subunit. (Supported in part by VAH, Buffalo MRIS # 2746-01).

706 Conformational Changes and Location of Divalent Cation Binding Sites in the Na-K ATPase studied with a Specific Fluorescent Probe," E.G. Moczydlowski and P.A.G. Fortes., Biology Dept., University of California, San Diego, La Jolla, Ca. 92093

We have synthesized a fluorescent derivative of ouabain, anthroyl ouabain (AO), that binds specifically to the cardiac glycoside receptor of the Na-K ATPase (E) with increased quantum yield and characteristic spectral shifts. ATP, Pi, K, Na and inhibitors alter AO binding kinetics and equilibria. This allows direct measurements of ligand-induced conformational changes and determination of ligand binding constants. AO binding requires Mg^{++}, but Mn^{++} and Co^{++} can substitute for Mg^{++}. Since AO emission and Co^{++} absorption spectra overlap, Co^{++} should quench AO fluorescence by energy transfer if the binding sites are close enough and properly oriented. We found that Co^{++} bound to a high affinity site (K_D<20μM) promotes AO binding without quenching AO fluorescence, indicating that this site is either too far or not oriented to allow efficient energy transfer. A second type of Co^{++} site(s), of lower affinity (K_D~0.7mM), is closer to the cardiac glycoside receptor, since occupation by Co^{++} quenches AO fluorescence ~50% and shortens the AO lifetime. The low affinity Co^{++} site may be inhibitory since E activity decreases at Co^{++} concentrations that quench AO fluorescence. These results indicate that E has at least two divalent cation sites, with different function, located at different distances from the cardiac glycoside receptor. Knowledge of the relative orientations of AO and Co^{++} will allow accurate distance estimates between these sites. (Supported by USPHS grants RR-08135 and HL-20262, and a grant-in-aid from the American Heart Association).

707 SERINE TRANSPORT IN CULTURED TOBACCO CELLS, Ivan K. Smith, Ohio Univ. Athens, OHIO.

The rate of serine transport into tobacco cells increased with decreasing pH, in either citrate or bis-tris-propane buffer, at any particular pH rates were higher in BTP. Transport was stimulated by Ca throughout the pH range 4-7 using either buffer. The standard transport medium contained 1% sucrose, 5mM BTP pH 6.0 and 0.1mM C-14-serine. Preincubation of cells with 0.5mM Ca caused a time dependent stimulation of serine transport; 0,2,4,6hr of preincubation gave serine transport rates of 250, 1200, 2000 and 2500 nmoles/g. fr. wt./hr. 0.5mM Ca could be replaced by 5mM Mg, but La, K, and Na had no effect. Addition of Ca to cells growing in B5 medium had no effect on subsequent transport rates; i.e. harvested cells had low rates which could be stimulated by preincubation with Ca in transport medium. It was concluded that either a component of B5 medium inhibited Ca-stimulated serine uptake or the cells produced a compound which binds Ca. The plant growth regulator 2,4-D was the only B5 component to inhibit transport; e.g. 50, 70, and 98% inhibition at 1, 10, and 50 mg/l, respectively. However, 2,4-D inhibition was independent of the Ca effect, stimulation by Ca of a control from 200-1900 compared with stimulation in the presence of 2,4-D from 73 to 630. Cells incubated with ^{45}Ca immediately bound 4000 nmoles/g.fr.wt., no increase in cellular Ca occurred during the next 8hr. Suspension of these cells for 5min. in 5, 0.5mM Ca, 0.5mM Mg, mM K or water resulted in the loss of 90, 80, 66, 33, and 27%, respectively. Ca binding to cells was not affected by 2,4-D in the range 0.1-50 mg/l. Recent work indicates the presence in the growth medium of a cell product capable of binding Ca, this is presently being characterized. The effect of pH, Ca, and 2,4-D on serine transport will be discussed.

708 ACTIVATION MECHANISM OF THE ENERGY-TRANSDUCING LATENT ATPASE FROM MYCOBACTERIUM PHLEI, Carolyn J. Ritz, Cliff M. Gold, and Arnold P. Brodie, Dept. of Biochem., USC Sch. of Med., Los Angeles, CA 90033

The membrane-bound coupling factor-latent ATPase of Mycobacterium phlei can be solubilized from membrane vesicles. Trypsin treatment of the soluble enzyme unmasks the ATPase activity and inactivates the coupling factor activity. The latent enzyme comprises at least five subunits: A(molecular weight 64,000), B(53,000), C(33,000), D(14,000), and E(8,000) with a ratio of 3:3:1 for A:B:C. The fully activated enzyme consists of the same four smallest subunits with an A"(58,000) species in place of the A subunit and a ratio of 3:3:1 for A":B:C. Partially activated samples contain an additional intermediate species A'(61,000). The fully unmasked enzyme was incapable of rebinding to membrane vesicles depleted of the enzyme. Progressive unmasking of ATPase activity is accompanied by a sequential conversion of the A subunit to the A" species via the A' intermediate and by a gradual loss of coupling factor activity and ability to rebind to the membrane. ATPase activation, coupling factor inactivation, and loss of rebinding capacity each followed a unique time course, indicating that each function depends on a different structural arrangement of the A, A', and A" subunit forms. A Fortran program for correlation of the data with a stochastic model of the ATPase activation process will be presented, and implications for the molecular mechanism of energy transduction in Mycobacterium phlei will be discussed.

621 MEMBRANE TRANSPORT AND GROWTH CONTROL OF MAMMALIAN CELLS: INTRODUCTION, Arthur B. Pardee, Sidney Farber Cancer Institute and Harvard Medical School, Boston, MA 02115

An important role in the regulation of growth of mammalian cells by membranes and by transmembrane transport has been proposed for at least a dozen years (see 1). Fundamentally, growth is regulated by external factors such as nutrients and hormones; these must pass through or impinge on the membrane before influencing events within the cell. Derangements of the membrane with respect to its interaction with these factors could cause ineffectual growth control, as in cancer cells. In particular, it has been suggested that growth may be limited by transport of nutrients (2). An enormous amount of work has since been done on the properties of cell membranes as influenced by transformation, loss of growth control in vitro, or dependence on conditions that modify growth (3). Many altered membrane properties have been described (see 4). In general, correlations have been found, but no definite proof that any compound becomes growth limiting by virtue of its limited rate of transport. Recently some evidence against growth limitation by transport rates has appeared, for some compounds. Some of our data on amino acid and sugar transport in relation to growth will be presented (5). Dramatic changes in the rates of transport of metabolites have been discovered for animal cells, as had been previously found for bacteria. However, these conditions that alter transport do not necessarily also alter growth rates. Therefore, transport changes could be consequences rather than causes of cell growth variations.

Presentations in this Session will deal with aspects of transport and growth control. Dr. Christensen will discuss properties of mammalian transport systems. Dr. Amos will talk about changes in transport as influenced by serum, sugars, and other factors. Dr. Cunningham will present results in which growth and transport are compared under conditions where it should be possible to decide whether there is a cause-effect relationship.

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622 NADH BY SHUTTLE ENERGIZING AMINO ACID TRANSPORT IN HIGHER ANIMAL CELLS? H.N.Christensen, J. Garcia-Sancho & A. Sanchez, Dept Biol Chem, Univ of Mich, Ann Arbor MI 48109

In contrast to bacteria, many cells of higher animals appear not to generate large electric or H^+ gradients across the plasma membrane, so that active uptake of nutrients cannot be adequately explained by cotransport with H^+ all the way across the membrane. Although cotransport with Na^+ plays an important role in energization of amino acid uptake, this explanation appears inadequate unless unexpectedly large transmembrane potentials arise. Even that possibility does not assist the explanation for cases where Na^+-dependent uptake is unaccompanied by substantial Na^+ influx (1). Energization by ATP cleavage appears also to serve, even though amino acid stimulation of ATPase has unsuitable properties. Concentrative uptake can continue, however, when cellular ATP is largely depleted, even with unfavorable alkali-ion gradients (Eddy; Schafer & Heinz; Schafer & Williams). We find that after depletion of ATP in the Ehrlich cell with dinitrophenol and iodoacetate, concentrative uptake of model amino acids resumes on resupplying pyruvate, before either the ATP level or alkali-ion gradients recover. Sensitivity of this recovery to rotenone points to a mitochondrial energy source. If not necessarily as ATP, in what form could energy flow to the membrane? Older observations of effects of deficiency and repletion of Vitamin B_6 (Riggs & Walker) or feeding of B_6 analogs or glutamate could support a role for the malate-aspartate shuttle in supplying NADH to the plasma membrane. NADH dehydrogenase activity has been observed for the plasma membrane of red blood cells,lymphocytes hepatocytes and adipocytes (2-5) using various H-acceptors. This activity is characteristically sensitive to quinacrine (atebrin). NADH inhibits the adenylate cyclase of the adipocyte membrane, and hormones that stimulate the cyclase intensify the dehydrogenase activity at similar levels (4). We find that quinacrine suppresses MeAIB uptake more completely and quickly (in 30 sec) than other agents, before the ATP level is halved and before alkali-ion gradients begin to fall. The recovery stimulated by pyruvate is produced also by 20mM ascorbate and 0.1mM phenazine methosulfate. The latter stimulation is insensitive to rotenone, but as for the pyruvate effect, eliminated by quinacrine or gramicidin D. Significantly, restoration of transport by either pyruvate or phenazine-ascorbate is more sensitive to ouabain than alkali-ion transport itself. We propose then that amino acid uptake in cells of higher animals can be energized by shuttled passage of the reducing equivalents of NADH to an unidentified plasma membrane acceptor. Support is acknowledged from National Institutes of Health, Institute of Child Health & Human Development, HD01233, USPHS, and Juan March Foundation, Madrid, Spain.

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623 HEXOSE TRANSPORT SUBSTRATE AND MACROMOLECULAR FACTORS, Harold Amos, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Mass. 02115

The rate of transport of hexose into mammalian cells in culture is highly dependent upon the immediate prehistory of the cells (1, 2, 3). Entry is effected by carrier-mediated diffusion in all cells so far studied, which do not include intestinal epithelium, kidney tubular cells or erythrocytes. At least two carriers (4) have been identified: (a) a low affinity constitutive carrier (Km 1mM); and (b) a high affinity derepressible carrier (Km 0.05-0.02mM). Derepression of the high affinity carrier is sensitive to glucose, bicarbonate, pyruvate, lactate, ascorbate, insulin and a protein released by cells into the medium that is also extractable from a cell membrane fraction (GEM Factor).

We have coined the term "DEPRIVATION DEREPRESSION" to specify the growing class of transport processes derepressed when mammalian cells are starved for substrate. "DEPRIVATION DEREPRESSION" appears to apply to amino acids as well as sugars (5). Although deprival of a carbon source is a powerful derepressant for glucose transport, D-fructose substituted for glucose is equally effective as are the pentoses D-xylose and D-ribose and some derivatives of both (1). D-xylose and D-fructose alone support the growth of most cells with a generation time closely approaching that on glucose (6). The pattern of cell response to glucose, other hexoses, and pentoses suggests a role for catabolite repression in the phenomena observed. The membrane content of GEM factor does not appear to depend upon the state of derepression of the glucose transport system.

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624 NUTRIENT UPTAKE AND CONTROL OF ANIMAL CELL GROWTH, Dennis D. Cunningham, Gregory S. Barsh, Deborah B. Greenberg, and William P. Naiditch, Department of Medical Microbiology, College of Medicine, University of California, Irvine, CA 92717

Several findings about the uptake of phosphate ion (P_i) and glucose by cultured animal cells have supported hypotheses(1,2) that transport of certain nutrients and/or growth factors might participate in the control of cell growth. For example, initiation of division of quiescent cells by fresh serum brings about rapid increases in the uptake of P_i and glucose that can be detected within several minutes (3,4). Also, uptake of these nutrients decreases as cells grow to quiescence (3,4). In addition, lowering the concentration of P_i or glucose in the medium arrests growth of cells in the G_1 phase of the cell cycle; readdition of the limiting nutrient leads to initiation of DNA synthesis (5).

These considerations prompted us to determine if changes in P_i and glucose uptake controlled cell proliferation under usual culture conditions. Quiescent mouse 3T3, human diploid foreskin and secondary chick embryo cells were switched to fresh medium containing fresh serum and either the normal amount of P_i (or glucose) or a reduced level that kept the rate of P_i (or glucose) uptake below the rate characteristic of quiescent control cells. The subsequent increases in cell number were equal in both media. Thus, the increases in P_i and glucose uptake, commonly observed after serum stimulation of quiescent fibroblasts, are not required for initiation of cell division. We also switched nonconfluent growing cells to media containing low levels of P_i (or glucose) that kept the rate of uptake of these nutrients below levels characteristic of quiescent cells. This did not affect rates of DNA synthesis or cell division over a several day period. Thus, the decreases in P_i and glucose uptake, which usually parallel the decrease in DNA synthesis as cells grow to quiescence are not responsible for the decline in cell proliferation. Although these experiments have not revealed a causal link between nutrient uptake and control of growth, other studies have indicated that the cell surface might participate in the regulation of growth by controlling the binding (and perhaps uptake) of growth factors.

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625 WHAT CAN WE LEARN FROM RECONSTITUTIONS OF MEMBRANOUS TRANSPORT SYSTEMS?

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During the recent past, we have used five different methods by which membranous proteins involved in ion transport can be incorporated into artificial liposomes. Among the problems that can be studied by such reconstitution experiments are: Identification of components required for function, asymmetry of the membrane, role of phospholipid composition, orientation of proteins within the membrane and mechanism of action of the transport system. The mechanism of ATP-driven ion pumps will be the major topic of discussion.

709 INTERACTIONS OF PICORNAVIRUSES WITH SYNTHETIC LIPID VESICLES, Tazewell Wilson, Robert L. Taber and Dmitri Papahadjopoulos, Roswell Park Memorial Institute, Buffalo, NY 14263.

Picornaviruses which have been "encapsulated" by large unilamellar lipid vesicles show several altered biological properties: they are no longer sensitive to neutralizing antibody and they are able to infect cells which lack surface viral receptors and are therefore normally resistant. When virions are mixed with preformed vesicles and sedimented on a density gradient, a small fraction of the virus sediments with the lipid and appears to have the same biological properties as the encapsulated virus. We are studying this virus-lipid interaction by observing changes both in the properties of the lipid, particularly with regard to alterations in lipid fluidity and vesicle permeability, and changes in the viral particle mimicing those occurring in vivo. We hope to develop this system as a model for studying transport of non-enveloped viruses across the plasma membrane.

710 HORMONAL REGULATION OF MEMBRANE PHENOTYPE, Thomas D. Gelehrter and Sarah A. Carlson, Dept. of Human Genetics and Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan 48109.

Neoplastic transformation is associated with structural and functional alterations in the cell membrane which may have an important role in growth regulation. Hormones can also affect membrane phenotype in a manner similar or opposite to neoplastic transformation. In HTC cells, an established line of rat hepatoma cells in tissue culture, glucocorticoids reverse several membrane properties characteristic of transformed cells, but do not alter the growth rate of the cells. Dexamethasone (Dex), a synthetic glucocorticoid, (1), rapidly and reversibily inhibits the rate of influx of selected amino acids by a process requiring concomitant protein synthesis; (2) decreases the number of microvilli on the surface of HTC cells in suspension culture, as assessed by scanning electron microscopy; and (3) increases the adhesiveness of HTC cells. Dexamethasone also decreases the production of plasminogen activator, an intracellular protease which may significantly modulate various membrane properties. Using an agar-fibrin overlay technique to detect plasminogen activator production by individual colonies of HTC cells, we have selected lines of HTC cells resistant to the inhibitory effect of Dex. All variant lines tested show normal Dex induction of tyrosine aminotransferase, indicating intact glucocorticoid receptor function. One variant may be pleiotropic in that it is also resistant to the Dex inhibition of amino acid transport. Combined genetic and biochemical analysis of such Dex-resistant variants should facilitate the study of hormonal regulation of specific membrane phenotypes.

711 CULTURED MOUSE LYMPHOSARCOMA CELL VARIANTS DEFICIENT IN NUCLEOSIDE TRANSPORT, Amos Cohen, Buddy Ullman, and David W. Martin, Departments of Medicine and Biochemistry and Biophysics, School of Medicine, University of California, San Francisco, San Francisco, Ca. 94143

A clone (AE_1) of cells resistant to adenosine cytotoxicity was isolated from mutagenized S49 mouse lymphosarcoma cells in continuous culture. These variant cells have also an impaired ability to salvage thymidine. Transport studies reveal that the mutant cells are deficient in the transport of both purine nucleosides and pyrimidine nucleosides, but can transport purine bases at a rate comparable to the wild-type cells. In cell free extracts of the mutant cells the enzymes responsible for the phosphorylations and phosphorolysis of these nucleosides are present in normal quantities. Kinetic analyses of the transport of these nucleosides in the wild-type lymphosarcoma cells provides further evidence that purine and pyrimidine nucleosides are transported by a common transport system, likely a common membrane associated protein. Further studies are in process to identify and characterize this carrier molecule.

712 HORMONE RESPONSIVENESS OF AN ESTABLISHED BUT DIFFERENTIATED KIDNEY EPITHELIAL CELL LINE (MDCK), Michael J. Rindler, Lorraine M. Chuman and Milton H. Saier, Jr., University of California, San Diego, La Jolla, CA 92093

The MDCK dog kidney epithelial cell line has been shown to retain (1) the capacity for vectorial salt and fluid transport (2) sensitivity to growth regulation, and (3) the ability to regenerate kidney tubular-like structures when injected into athymic nude mice (Cancer Res. 36 1353-1360 (1976)). MDCK cells grown in tissue culture or in baby nude mice have the morphological properties of distal tubular cells, form tight and gap junctions, lack proximal tubular enzyme markers and possess high activities of the Na^+, K^+ ATPase, ecto-leucine aminopeptidase and ecto-alkaline phosphatase. Application of fluorescent antibody techniques indicated that leucine aminopeptidase is localized to the brush border membrane. Adenylate cyclase in intact cells was responsive to vasopressin, oxytosin, prostaglandins E_1 and E_2, and glucagon. Hormone responses were observed at physiological concentrations but exhibited the property of negative cooperativity. Desensitization was not marked. Hormone-stimulated adenylate cyclase activity in isolated membrane preparations was dependent on low concentrations of GTP. The results suggest that the MDCK cell line has retained the differentiated properties of the kidney epithelial cells of origin and that a clonally isolated cell possesses the receptor, transmission, and target enzyme systems necessary for the regulation of transcellular fluid transport. (Supported by ACS#BC-224 & NIH#1R01 CA16521).

713 POSSIBLE ROLE OF THE MEMBRANE POTENTIAL IN SERUM-STIMULATED UPTAKE OF AMINO ACID IN A DIPLOID HUMAN FIBROBLAST. Mitchel L. Villereal[1] and John S. Cook, University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences and Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.

The Na^+-dependent accumulation of α-aminoisobutyric acid (AIB), measured in normal growing and quiescent (serum-deprived) HSWP cells (human diploid fibroblast), was found to be 2-fold higher ($AIB_{in}/AIB_{out} \approx 20$-25) under normal growing conditions. Serum stimulation of quiescent cells increases their AIB concentrating capacity by approximately 70% within 1 hr. These observations suggest that the driving forces for AIB accumulation may be reversibly influenced by the serum concentration of the growth medium. Addition of valinomycin (Val) to cells pre-equilibrated with AIB causes an enhanced accumulation of AIB, suggesting that the membrane potential can serve as a driving force for AIB accumulation. After pre-equilibration with AIB in 6 mM K^+, transition to 94 mM K^+ with Val results in a marked and rapid net loss of AIB. The effect of Val on the accumulation of AIB is greatest in quiescent cells, with the intracellular AIB concentrations reaching those seen in both Val-stimulated, normal cells and Val-stimulated, serum-stimulated cells. In summary, these data suggest that the accumulation of AIB in HSWP cells may be influenced by changes in membrane potential and that a serum-associated membrane hyperpolarization could be partly responsible for the increased capacity for AIB accumulation in serum-stimulated cells. [Supported by NCI and ERDA under contract with Union Carbide Corporation. [1]Postdoctoral Investigator on Carcinogenesis Training Grant #CA 05296 from NCI.]

714 CATION TRANSPORT VARIANTS OF CHO CELLS WITH GREATER ABILITY TO PROLIFERATE IN LOW [K] MEDIUM. James S. Graves, Div. of Biol. Research, Ontario Cancer Inst., Toronto, Canada.

Reducing the [K] in the culture medium to levels below 1.0 mM slows the rate of proliferation of CHO cells, and prolonged exposure to 0.25 mM K medium causes appreciable cell death. From cultures exposed to 0.25 mM K medium for 4-6 d several clonal cell-lines have been isolated which are resistant to the cytotoxicity of low external [K]. In an attempt to determine the physiological alteration in one such variant, LK^RA5, I monitored the time-course of cell number, intracellular [Na] and [K], and the rate of protein synthesis during a 72 h exposure to 0.3 mM K medium in both A5 and the parental cell-type, WT. In these experiments A5 shows a moderate rate of proliferation after the first 24 h, while WT begins to proliferate slowly only after 48 h. In both cell-lines [K]i decreases initially to about 40 mmol/l·cell and then recovers to the control level of >90 mmol/l·cell by 72 h. The changes observed in [Na]i differ markedly between WT and A5. In WT [Na]i rises to greater than 100 mmol/l·cell by 24 h and is not reduced at 72 h. However, in A5 [Na]i rises to about 90 mmol/l·cell at 24 h and is reduced to 50 mmol/l·cell by 72 h. The rate of protein synthesis (i.e. ^3H-leu incorp.) transiently decreases with [K]i in both A5 and WT, and it appears unaffected by [Na]i. The failure of WT cells to extrude Na while reaccumulating K could result from uncoupling of the Na-K pump under these conditions, and the lower [Na]i maintained in A5 may be due to a more tightly coupled pump. Also, the data indicate that the long-term inhibition of proliferation by low [K] medium is not due to a permanent reduction in [K]i or in protein synthesis, but high [Na]i may be involved in this inhibition.

715 EFFECT OF INSULIN AND OF OXIDANTS ON THE RATE OF NUTRIENT TRANSPORT AND ON THE MORPHOLOGY OF CHICK EMBRYO FIBROBLASTS. Heidi Smith-Johannsen, Hannelore Sauerwein and James F. Perdue, Lady Davis Institute for Medical Research of the Jewish General Hospital, Montreal, Canada H3T 1E2.

The effect of insulin and of sulfhydryl oxidizing agents on the rate of nutrient transport was studied in serum-starved chick embryo fibroblasts (CEF). Exposure of cells to 5 μg/ml insulin for 10 min at 37° stimulates sugar, amino acid and nucleoside transport 20-40%, a response comparable to that induced by 10 min exposure of CEF to 4% serum. Exposure of CEF to the oxidants diamide, hydrogen peroxide or methylene blue stimulates nutrient transport in a time- and concentration-dependent manner. Control and treated cultures were examined by scanning electron microscopy. In general, although all cultures display pleomorphism, confluent and serum-starved CEF are very flat and exhibit few microvilli. Treatment of serum-starved CEF with insulin or chemical oxidants for 10 min does not significantly increase the number of microvilli on these cells. The mechanism by which insulin and oxidizing agents stimulate transport probably involves activation of transport sites preexisting in the membrane, rather than addition of membrane containing activated transport proteins.

716 REGULATION OF AMINO ACID TRANSPORT BY INSULIN AND GLUCAGON IN THE ISOLATED PERFUSED LIVER. M.S. Kilberg and O.W. Neuhaus, Section on Biochemistry, Division of Biochem/Phys/Pharm, Univ. So. Dak. Sch. Med., Vermillion, So. Dak. 57069.

Amino acid transport is stimulated when either glucagon or insulin is added to the perfusate of isolated rat livers. Although these two hormones are known to function antagonistically *in vivo*, it is not clear what effect this interaction has upon hepatic amino acid uptake. It is also not understood why both hormones exhibit a stimulatory effect on 2-aminoisobutyric acid (AIB) transport. We now report that insulin appears to play a dual role in regulating amino acid uptake into the liver cells. Subcutaneous injection of donor rats with glucagon (glucagon pretreatment) 1 hr prior to the removal of the liver increased the accumulation of AIB 2-fold during a 2 hr perfusion. After this perfusion time, 20.4% (expressed as % uptake/10 g liver/2 hr) of the total AIB accumulated in the normal livers while 41.4% was found in the livers of the pretreated rats. When livers from the glucagon pretreated animals were perfused with 1 μg of insulin, the AIB uptake was 32.0% of the total. On the other hand when 5 μg were used, the liver accumulated 50.7% of the amino acid. The addition of 1 or 5 μg of insulin to a liver from a non-pretreated rat resulted in AIB levels of 29.7% or 46.9%. These data show that insulin exerts a stimulatory action upon amino acid transport in the normal liver, but inhibits the uptake stimulated by glucagon. This inhibition may mean a lowering of hepatic cAMP levels. The importance of this interaction upon amino acid transport *in vivo* will be discussed. [Supported by a grant from the USPHS].

717 DEFECTIVE TRANSPORT OF THYMIDINE IN CULTURED CELLS RESISTANT TO 5-BROMODEOXYURIDINE. Thomas P. Lynch, Carol E. Cass, and Alan R.P. Paterson, Cancer Research Unit and Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

A line of HeLa cells resistant to 5-bromo-2-deoxyuridine (BUdR) was established by continuous culture in growth medium containing BUdR; during the selection period, BUdR concentrations, initially 15 μM, were gradually increased to 100 μM. A clone (HeLa/B5) was established from this line; these cells were cross-resistant to 5-fluoro-2'-deoxyuridine (FUdR), but not to the free base 5-fluorouracil. Although extracts of HeLa/B5 cells exhibited levels of thymidine kinase activity comparable to those of parental cells, the rates of uptake of BUdR, FUdR and thymidine into intact cells were greatly reduced. The kinetics of uptake of uridine and adenosine, nucleosides which each appear to be transported independently of thymidine in HeLa cells, were similar for HeLa/B5 and the parental line. Thymidine uptake by HeLa/B5 cells was relatively insensitive, when compared to uptake by the parental line, to nitrobenzylthioinosine (NBMPR), a potent, membrane-specific inhibitor of nucleoside transport in various types of animal cells. The altered kinetics of uptake of thymidine and the insensitivity of thymidine uptake in HeLa/B5 cells to NBMPR suggest that resistance to BUdR is due to an altered thymidine transport mechanism.

Supported by the National Cancer Institute of Canada and the Medical Research Council of Canada.

718 CHARACTERIZATION OF THE INSULIN-SENSITIVE D-GLUCOSE TRANSPORT SYSTEM IN PLASMA MEMBRANE VESICLES FROM RAT ADIPOCYTES. Michael F.Shanahan,Dennis J. Pillion, and Michael P. Czech,Brown University,Providence RI 02912.

Partially purified adipocyte plasma membrane vesicles(PM)are known to exhibit stereospecific facilitated diffusion of D-glucose. In addition,PM derived from adipocytes treated with insulin for 10 min.prior to disruption exhibit a significantly enhanced transport activity. In the present studies, low concentrations of cytochalasin B(CB)inhibited both control and insulin-activated transport activity and bound to PM with high affinity. The insulin-stimulated uptake was markedly inhibited with 2.5mM dithiothreitol while transport activity in control PM was essential unaltered. These data are consistant with the model (J.Biol.Chem.251: 1164,1976)which proposes that activation of transport by insulin involves sulfhydryl oxidation to the disulfide form in a key membrane protein . Extraction of the PM by dimethylmaleic anhydride resulted in selective release of up to 80% of the membrane protein. The residual material consisted almost entirely of 2 glycoprotein bands on SDS-gels, and still exhibited stereospecific D-glucose uptake which was sensitive to inhibition by CB. These results indicate that one or both of the glycoprotein fractions contain the glucose transport components of the membrane. Experiments to determine whether insulin-stimulated transport activity also survives this extraction procedure are in progress.

719 REGULATION OF AMINO ACID TRANSPORT DURING DIFFERENTIATION OF SKELETAL MUSCLE CELLS, Barbara Kay Grove and Frank E. Stockdale, Stanford University, Stanford, CA 94305

Nowhere do cellular membranes undergo more profound morphological and physiological changes than during skeletal muscle cell differentiation. As myoblasts differentiate to form mature myotubes, their membranes fuse, the membrane resting potential increases, and specialized muscle membrane components including acetylcholine receptors and acetylcholinesterase are elaborated. We have monitored differentiating muscle cells for alterations in membrane transport properties. During the transition from a myoblast (18 hours of incubation) to the well developed myotube (66 hours of incubation), there is an 8.1-fold increase in cellular protein, a 7.4-fold increase in surface area, and a 2.5-fold increase in nuclei --the myotubes having about 3 times as much protein and surface area per nucleus as myoblasts. We have found that there is a two-fold decrease in transport rates of α-aminoisobutyric acid (AIB) as myoblasts fuse into myotubes and that the decrease in AIB transport is concomitant with cell fusion. The V_{max} in myoblasts decreased from 23.7 to 12.9 nmoles/mg protein/min in myotubes, whereas there was no significant change in the K_m (1.73 vs 1.34 mM). The difference in V_{max} was shown to represent a decrease in transport sites rather than a difference in affinity or translocation. Thus, as cellular protein and surface area increase, the available membrane AIB transport sites do not increase proportionally. New membrane formation and accumulation appear not to determine the number of transport sites in these cells, nor does the rate of protein synthesis or accumulation appear correlated with transport site number. The regulation of the amino acid transport system appears quantitatively related to the nuclear complement of the cell.

720 LIPOSOME-MEDIATED TRANSFER OF 6-CARBOXYFLUORESCEIN INTO LYMPHOCYTES: KINETICS WITH AND WITHOUT METABOLIC INHIBITORS. R. Blumenthal (LTB), J.N. Weinstein (LTB), S.O. Sharrow (IB), and P. Henkart (IB), National Cancer Institute, NIH, Bethesda, MD. 20014.

To investigate the possible roles of endocytosis and membrane fusion in liposome-cell interaction, we have studied the transfer of the water-soluble fluorescent dye 6-carboxyfluorescein (6-CF) from sonicated dioleoyl lecithin (DOL) vesicles into human peripheral blood lymphocytes. Incorporation of dye into the cells was assessed by fluorescence microscopy, which showed quite uniform intracellular distribution of 6-CF, and by flow microfluorometry, which indicated that all of the cells took up dye to roughly the same extent (Weinstein, et al., Science, in press).

The amount of dye in the cells after 25 minutes of incubation with 6-CF vesicles at 37°C was decreased only 22% by inclusion of the metabolic inhibitors NaN_3 and 2-deoxyglucose, in combination. Thus, most of the incorporation appeared to be passive, probably by fusion of vesicles with the plasma membrane. Substantially the same results were obtained with vesicles of distearoyl lecithin and vesicles of DOL containing 10% (w/w) phosphatidyl serine.

A more detailed kinetic study of the vesicle-cell interaction showed the following major features: (i) in the absence of metabolic inhibitors, cell fluorescence leveled off after about one hour of incubation, at a steady state value determined by the balance between influx of vesicle dye and efflux of free dye from the cells; (ii) inclusion of NaN_3 and 2-deoxyglucose inhibited a major component of the efflux. As a result of (ii), the fluorescence of inhibitor-treated cells did not plateau, and the net effect for times of incubation greater than one to two hours was that the inhibitors appeared to enhance uptake.

721 ISOLATION AND CHARACTERIZATION OF THE ANION TRANSPORTER FROM HUMAN ERYTHROCYTES, H. Ginsburg, J.M. Wolosin and Z.I. Cabantchik, Biophysics Group, The Institute of Life Sciences, The Hebrew University of Jerusalem, Israel.

Successive steps of mild protein extraction resulted in vesicles containing predominately the 95,000 daltons polypeptide (Band 3) associated with the lipids of the original membrane. The freeze-fractured faces of the vesicular membrane has the particulate nature of the native membrane. Fluxes of sulfate and phosphate in the net and the exchange modes were measured at various pH and temperatures and under the influence of specific inhibitors. It was found that the anion transport function of the vesicles resembles the native system according to the following criteria: pH dependence and energy of activation of fluxes; the relative specificity towards phosphate and sulfate transport is maintained as in the native membrane; both net and exchange fluxes are fully inhibited by DIDS, a specific inhibitor which acts only from the outer side. Thus, the original orientation of the transporter in the membrane is maintained. Based on these and other criteria, the functional properties of isolated Band 3-vesicles could be equated to those of the intact cell system. Direct support for the putative role of the 95,000 daltons polypeptide in anion transport is provided.

722 EFFECTS OF TEMPERATURE ON THE TRANSPORT OF GALACTOSE IN HUMAN ERYTHROCYTES, Hagai Ginsburg, Department of Biochemistry, School of Medicine, University of Virginia, Charlottesville, Va. 22901.

Analysis of the kinetic properties of galactose transport in human erythrocytes is resolved in a mechanism which involves two asymmetric carriers operating in antiparallel fashion. The effects of temperature on this mediated transport in the range of 0°-25°C indicate that the Michaelis constants for zero-trans influx and efflux and equilibrium exchange efflux are invariable with temperature. Arrhenius plots of the maximal velocities show a break between 10° and 15°C with mean activation energy of 53 ± 7 Kcal mol^{-1} below this break and 22 ± 2 Kcal mol^{-1} above it. The relative contribution of the two types of carriers to the total transport rate is not affected by temperature. The kinetic properties of the prevalent type of carriers are analyzed in terms of the simple carrier model as formulated by Lieb and Stein (Biochim. Biophys. Acta 373 (1974) 178). This analysis indicates the following features: the relative concentration of the carrier-substrate complex at the outer interface of the membrane, increases upon cooling; the rate constant of dissociation of the carrier-substrate complex on the inner interface is much smaller than that on the outer interface at the lower temperature range; the free energy of translocation of the unloaded carrier and the entropy change involved in this step, are significantly larger in the lower temperature range (0°-5°C) than in the higher one (15°-25°C). On the basis of these results it is suggested that the membrane protein involved in the transport of galactose interacts strongly with its lipid environment and that the inner monolayer of the erythrocyte membrane undergoes a phase separation or a phase transition at 10°-15°C.

723 KINETIC ANALYSIS OF THYMIDINE TRANSPORT IN NOVIKOFF RAT HEPATOMA CELLS: INHIBITION BY OTHER NUCLEOSIDES AND BY PERSANTIN AND CYTOCHALASIN B, Richard Marz, Robert M. Wohlhueter and Peter G.W. Plagemann, Dept. Microbiol., Univ. Minnesota, Minneapolis, MN 55455

Thymidine (dThd) transport was studied in a subline of cultured Novikoff rat hepatoma cells which are deficient in dThd kinase. Initial rates were estimated by a rapid kinetic technique (fast mixing and centrifugation through oil layers at 1.5 sec intervals). Zero-trans and equilibrium-exchange measurements of dThd transport gave maximal initial rates V^{zt}_{max} = 14 and V^{ee}_{max} = 50 pmoles/µl cell H_2O·sec; K^{zt}_m = 80 and K^{ee}_m = 225-325 µM respectively. Competitive inhibition of dThd transport was observed with uridine, inosine, deoxyuridine, 5'-bromodeoxyuridine, and deoxyinosine with K^{zt}_i values of 30-80 µM. Persantin ($K^{zt}_{i,\ slope}$ = 0.1, $K^{zt}_{i,\ intercept}$ = 0.7 µM) and Cytochalasin B ($K^{zt}_{i,\ slope}$ = 7, $K^{zt}_{i,\ intercept}$ = 43 µM) both raised the K^{zt}_m and lowered the V^{zt}_{max} of dThd transport and thus displayed neither simple competitive nor simple noncompetitive inhibition. These results fit a model for nucleoside transport in which the loaded carrier "moves" faster than the unloaded one, and in which other nucleosides inhibit dThd transport by competing for the substrate binding site, while Persantin and Cytochalasin B inhibit the "movement" of the carrier. Supported by NIH Research Grant CA 16228, Training Grant CA 09138 and postdoctoral fellowship CA 00800 (R.M.).

724 OSCILLATIONS OF MEMBRANE POTENTIAL IN CULTURED NON-EXCITABLE CELLS. Guy Roy. Yasanobu Okada, Wako Tsuchiya, Yukio Doida and Akira Inouye. Dept. of Physiology, Kyoto University, Kyoto, Japan.

The membrane potential of L cells in culture was found to oscillate continously between -15mV and -40mV at a slow frequency of 3 to 4 cycles per minute. Simultaneously, the membrane resistance was found to oscillate between 37 Mohms and 22 Mohms. These oscillations are always observed in large cells, but only occasionnaly in small cells. Upon application of hyperpolarizing or depolarizing steady currents, oscillations continue with an amplitude depending on the value of the membrane potential, but the membrane resistance does not show any modification. It means that these oscillations are produced by a periodically changing membrane resistance, but this resistance change is not dependent on membrane potential. During application of constant currents, it was possible to determine a reversal potential for these oscillations. It is very close to the K^+ equilibrium potential. If the K^+ content of the external medium is changed, the reversal potential follows the Nernst equation for K^+. From these results, it is concluded that oscillations are produced by a specific change of the membrane conductance to K^+ ions, and this K^+ conductance is voltage independent. In order to identify the origin of these membrane K^+ conductance oscillations, metabolic inhibitors and cooling were applied. Both blocked oscillations. Ouabain was also applied, but no effect was observed. Removing external Ca^{++} reversibly blocked oscillations. The origin of these oscillations is not yet determined but they are apparently related to some membrane function requiring the entry of Ca^{++} inside the cells.

725 $^{67}Cu^{2+}$-UPTAKE BY HUMAN DIPLOID FIBROBLASTS. John R. Riordan, Manuel Buchwald, and Andrew Sass-Kortsak, Research Institute, Hospital for Sick Children, Toronto, Canada.

Skin biopsies were obtained from the forearms of two patients with Wilson's disease and two matched undiseased individuals and grown to confluence on the bottoms of glass liquid scintillation vials. The association of $^{67}Cu^{2+}$ with the cell monolayers increased with time and concentration of the metal. The kinetics of association or uptake were biphasic. There was an early rapid phase, maximal at 30 minutes followed by a decline to a minimum at 1 hour. Between one and four hours there was a gradual further increase in cell associated copper. During the latter phase there was a greater uptake of $^{67}Cu^{2+}$ by the cells from the patients with Wilson's disease than the controls. $^{67}Cu^{2+}$ uptake was enhanced by the ionophore A23187 in the case of cells of both origins, consistent with an increased binding capacity rather than an altered permeability of the cells from the individuals with Wilson's disease. (Supported by the Medical Research Council of Canada.)

726 MEMBRANE EFFECTS OF PHLORIZIN ON p-AMINOHIPPURIC ACID TRANSPORT IN PROXIMAL TUBULES, Richard P. Wedeen, Jersey City Medical Center, Jersey City, NJ 07304

The effect of phlorizin on p-aminohippuric acid (PAH) uptake in rat kidney cortex slices was examined in vitro. Slices were incubated in Krebs-Ringer bicarbonate buffer containing 10 mM acetate and PAH-^{3}H, 0.08 mM, at 25°C. Section freeze-dry autoradiographs were prepared as previously described (Kid Int 3:250, 1973). Phlorizin, 0.5 and 5.0 mM, increased the slice-to-medium (S/M) concentration ratio for PAH-^{3}H after 60 and 180 min incubations. Following only 15 min incubation, however, 5.0 mM phlorizin reduced the PAH-^{3}H S/M. The amount of preloaded PAH-^{3}H lost during 90 min washout was reduced from 53% to 35% by phlorizin. Autoradiographs demonstrated that phlorizin blocks the secretion of PAH-^{3}H into proximal tubule lumens. Autoradiographs prepared from slices incubated with phlorizin-^{3}H showed accumulation of phlorizin-^{3}H in cells and lumens of proximal tubules. These studies demonstrate bidirectional PAH transport inhibition by phlorizin at the luminal and peritubular surfaces of proximal tubules. Phlorizin-^{3}H autoradiography showed that phlorizin has access to both luminal and basilar cell membranes. PAH efflux inhibition at the luminal membrane was demonstrated autoradiographically by the absence of PAH-^{3}H from the lumens of proximal tubules in the presence of phlorizin. Influx inhibition at the peritubular membrane was demonstrated by the reduced PAH-^{3}H S/M after short incubations. Efflux inhibition at the peritubular surface was demonstrated by delayed PAH-^{3}H washout. The latter effect causes increased cellular accumulation of PAH-^{3}H after prolonged incubations. These effects of phlorizin on PAH transport suggest that common carriers may be involved in organic acid secretion, amino acid reabsorption, and sugar reabsorption in kidney.

727 CHLORIDE EXCHANGE AND THE EFFECT OF SITS IN EHRLICH ASCITES TUMOR CELLS.
Felice Aull, Martin S. Nachbar and Joel D. Oppenheim, New York University School of Medicine, New York, N.Y. 10016

The action of the amino- reactive agent, 4-acetamido-4'-isothiocyano-stilbene-2,2'-disulfonic acid (SITS) on Cl self-exchange was studied by determining the Cl efflux coefficient (k_e) in steady state cells from ^{36}Cl uptake. For control cells at 20-24 C, k_e was 2.83 hr^{-1} ± 0.16 (SE) (n=13). In 156 mM Cl Ringer, 6 x 10^{-4}M SITS reduced Cl exchange by 37% ± 2 (SE) (n=3) but K self-exchange was unaffected (n=1). SITS was at or near maximal effectiveness at 3-6 x 10^{-4}M. Washing the cells with 0.5% albumin-Ringer completely reversed SITS action. When external Cl was lowered to 32 mM by replacement with acetate or nitrate, the efficacy of SITS was reduced. Since acetate and nitrate themselves depressed the k_e for Cl, these anions probably compete with Cl for a common exchange mechanism, thereby reducing the amount of Cl transfer susceptible to SITS. Supported by the National Cancer Institute, USPHS grant CA 10625.

728 REVERSAL OF ETHINYL ESTRADIOL (EE) INDUCED ABNORMALITIES IN HEPATIC BILE SECRETION AND LIVER SURFACE MEMBRANE LIPID (LSM) COMPOSITION, Francis R. Simon, Manuel Gonzalez and Roger A. Davis, Dept Medicine, Univ Colorado Medical Center, Denver, CO 80262.

The liver transports bile acids (BA) against a concentration gradient by a saturable, energy and sodium dependent process. Administration of EE, a synthetic estrogen, to rats impairs this process as described by maximum capacity to excrete BA (T_m) and decreased bile flow (BF). We examined whether these changes are due to alterations in content of specific LSM proteins or rather due to changes in the concentration of membrane lipids. EE (5 mg/kd/d x 5d) was administered to male rats and LSM fractions isolated. The maximum number of BA binding sites (N) and their affinity (K_d) was determined using ^{14}C-cholic acid (JCI 57:496, 1976). Although EE reduced BF (61%) and BA T_m (55%) compared to control, neither N or K_d was significantly altered. In contrast, EE alters the lipid composition of LSM fractions. The phospholipid concentration was unchanged; however, EE significantly increased triglycerides (TG) (1.6x), cholesterol (C) (1.3x), and cholesterol esters (CE) (4x). It has previously been shown that Triton WR-1339 (22.5 mg/100g) reverses hepatic lipid abnormalities caused by EE. 15 hours after administration of Triton WR-1339 to EE treated rats, the LSM C and CE content was reversed to normal and TG reduced to 38% of control. Coincident with these changes, bile flow was significantly increased to 108% and BA T_m to 110% of control. In contrast, administration of Triton WR-1339 did not change either LSM lipid composition, bile flow or BA T_m in normal animals. These results suggest that abnormalities in LSM lipid composition may reduce the ability of specific receptors to translocate BA, and may explain why EE treatment causes such diverse changes in LSM enzyme function.

729 EFFECT OF cAMP ON GROWTH AND CELLULAR STRUCTURE OF *PHYCOMYCES*. S.K. Malhotra and J.C. Tu, Biol. Sci. Electron Microscopy Lab., Univ. of Alberta, Edmonton.

Cyclic AMP when added to the culture medium affected the growth of sporangiospores of *Phycomyces blakesleeanus* and the effect became more apparent as the concentration of cAMP was increased from 10 μM to 40 μM in the medium. The controls contained no exogenous cAMP. The results of adding cAMP were manifested as follows: (1) The appearance of germ tubes was accelerated. (2) The mycelia increased in their diameter by as much as 10 times as compared with the controls and the branching of mycelia became more profuse. (3) The cell wall of the mycelium became thicker, in some cases about 5 times more than in the controls. (4) Electron micrographs showed a decrease in glycogen aggregates in the cytoplasm which appeared to be consistent with biochemical analysis. (5) Small clusters of intramembranous particles on the convex fractured face of the plasma membrane and corresponding depressions on the concave fractured face became apparent in the replicas of freeze-fractured mycelia. Though the mechanism of action of the externally added cAMP on *Phycomyces* requires investigation, it is apparent that the overall glycogen content and synthesis of cell wall (and presumably chitin synthesis) are affected. Incubation experiments on germinating and non-germinating spores in medium containing ^{3}H-cAMP suggest that the ^{3}H-cAMP is taken up by the germinating spores.

730 Mg-ATPase and HCO_3^--ATPase DISTRIBUTIONS IN RAT LIVER FRACTIONS PREPARED BY ZONAL CENTRIFUGATION, Kenneth Izutsu, Ivens Siegel and Edward Smuckler, Univ. of Wash., Seattle, Wash., 98195.

A plasmalemmal HCO_3^- + Mg^{2+}-ATPase has been proposed as a mechanism for achieving nonequilibrium transmembrane pH gradients through the transport of bicarbonate or hydrogen ions. This hypothesis has been criticized by certain investigators who feel that the plasmalemmal HCO_3^- + Mg^{2+}-ATPase activity is an artifact of sample preparation and represents contamination by a mitochondrial enzyme. The present results may be applicable to this question. We have found a biomodal Mg^{2+}-ATPase distribution following zonal centrifugation of a pellet prepared from rat liver homogenate, and the peaks of activity were correlated with the mitochondrial and plasmalemmal fractions. The two enzyme activities responded differently to experimental manipulation of the incubation conditions.

The mitochondrial enzyme was: 1) more labile during cold storage, 2) more sensitive to product inhibition, 3) more sensitive to changes in pH, 4) more sensitive to changes in the Mg^{2+}/ATP ratio, and 5) more sensitive to changes in ionic strength than the plasmalemmal enzyme. In addition, only the mitochondrial Mg^{2+}-ATPase activity was stimulated by the presence of the HCO_3^-.

The finding that no significant differences could be demonstrated between the Mg^{2+}-ATPase activities in the supposed mitochondrial and plasma membrane fractions prepared from other tissues is curious in light of the many differences obtained in the present study. This suggests that these previous plasmalemmal fractions may have had significant mitochondrial contamination.

731 URIDINE TRANSPORT IN GROWING AND QUIESCENT HAMSTER EMBRYO CELLS. Yael Eilam. Depart. of Membrane Research. The Weizmann Institute of Science, Rehovot, Israel.

The maximum velocity of uridine uptake in hamster embryo cells decreased to half when the cultures became quiescent. This decrease was reversed by the additon of serum to the confluent cultures. The number of ribonucleoside carriers was estimated from the number of inhibitor binding sites with the aid of nitrobenzyl 6-mercaptoinosine (NBMI), a specific, high affinity inhibitor of ribonucleoside transport. This number was similar in quiescent and serum stimulated cells, 3.6 and 3.4×10^4 sites/cell respectively. It is therefore concluded that serum stimulation led to increased carrier transport turnover and not to a change in the number of carriers per cell. Further properties of the uridine carriers in growing and quiescent cells were elucidated in kinetic studies of NBMI inhibition. The rate constant of NBMI binding to the carriers was found to be four times as high in growing as compared to quiescent cells (1.6 and 0.38×10^{-8} $min^{-1}M^{-1}$). Whereas the Km values for uridine uptake in the absence of NBMI were similar in both types of cells (40μM), the apparent Km values differed in the presence of NBMI (138μM for growing and 80μM for quiescent cells). Conceivably these growth-related differences in the properties of uridine carriers may have resulted from differences in carrier conformation.

732 A ROLE FOR MONOGLYCERIDE ACYL TRANSFERASE IN THE TRANSPORT OF LIPID INTO THE LIVER, M. Waite, R. El Maghrabi, L. Rudel, Bowman Gray School of Medicine, Winston-Salem, NC

It has been demonstrated that the liver plays a role in the uptake of components of the remnant lipoprotein. The remnant lipoprotein is formed by the action of the lipoprotein lipase (LPL) on chylomicra. Some evidence suggests that the remnant contains a high concentration of monoglyceride, the product of the lipase action. We have described an enzyme in the plasma membrane of liver that catalyzes the hydrolysis of monoglyceride (MG) and phospholipids (PL). This enzyme is stimulated by heparin and catalyzes a transacylation in which the acyl group of a lipid is transferred to MG to form diglyceride (DG). Since the preferred substrate is MG, and DG is the main product initially, we term the enzyme monoglyceride acyl transferase (MGAT) and suggest that this enzyme plays a role in the transport of MG and PL in the liver. To test this hypothesis we have prepared chylomicra and VLDL which contains [^{3}H] glycerol in triglyceride (TG). Treatment of this with LPL purified from milk produces MG rich fractions. When low concentrations of albumin are present, the MG remains with the lipoprotein whereas high concentrations of albumin bound the MG and removed it from the lipoprotein. We found that MGAT which had been solubilized from the membrane by heparin could attack MG in the remnant as well as that bound to albumin. LPL, on the other hand, did not attack either form of MG. The TG in chylomicra, VLDL or remnant lipoprotein was not degraded by MGAT whereas LPL was active on TG in these sources, so long as the peptide activator was present. These properties demonstrate that MGAT is different from the lipases reported thus far. Further, our data is consistent with the hypothesis that MGAT plays a role in the transport of lipid into the liver cells.

733 INHIBITION OF THYMIDINE UPTAKE IN CULTURED CELLS BY NITROBENZYLTHIOINOSINE, Carol E. Cass and Alan R.P. Paterson, Cancer Research Unit and Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

Nitrobenzylthioinosine (NBMPR), an inhibitor of nucleoside transport in human erythrocytes which binds with high affinity to specific receptor sites in the plasma membrane, was found to be a potent inhibitor of thymidine uptake by logarithmically proliferating HeLa cells. Rates of thymidine uptake, measured in monolayer cultures at 20°C, were constant between 10 and 40s after addition of thymidine. Under these conditions, TTP was the major metabolite of thymidine, and the thymidine phosphates accumulated at constant rates. NBMPR had no effect on thymidine kinase activity, or on the relative proportions of thymidine metabolites in cell extracts, suggesting that NBMPR inhibited thymidine transport into the cell. In the presence of 2 μM NBMPR, mediated entry of thymidine was eliminated and a significant diffusional component was evident. Maximal velocities of 10-21 nmoles/min/10^6 cells and an apparent K_m of 0.5 μM were obtained from reciprocal plots of the mediated component of uptake and extracellular concentration. When HeLa cells exposed to NBMPR were transferred to NBMPR-free media, inhibition of uptake persisted, suggesting that NBMPR was firmly bound to the transport-inhibitory receptor sites.

Supported by the National Cancer Institute and the Medical Research Council of Canada.

734 HORMONE-INDUCED AGGREGATION OF INTRAMEMBRANOUS PARTICLES, Richard M. Hays, Sherman D. Levine, William A. Kachadorian and Vincent DiScala, Albert Einstein College of Medicine and U.S.P.H.S. Hospital, New York.

A dramatic alteration in cell membrane permeability is produced by the hormone vasopressin (antidiuretic hormone) within minutes after attachment to receptors on the basolateral cell surface. Vasopressin (via cyclic AMP) increases the permeability of the opposite (luminal) cell surface to water 50-fold, and to solutes such as sodium and urea 2- to 10-fold in tissues such as toad bladder. Independent membrane sites for water, urea and sodium transport appear to be activated by vasopressin, since inhibitory agents such as phloretin and certain anesthetics selectively block hormone-stimulated urea or water transport.

Until recently, electron microscopy revealed no structural changes in the luminal cell membrane following vasopressin. However, with the freeze-fracture technique, a reorganization of the protoplasmic (P) leaflet is seen, in which vasopressin produces an aggregation of membrane-associated particles. These particles are believed to be membrane proteins. Particle aggregation appears to be related specifically to the hormone-induced increase in water flow, since aggregation is inhibited by selective inhibitors of water flow (anesthetics), but unaffected by a selective inhibitor of solute movement (phloretin). Aggregation takes place whether or not an osmotic gradient is present, and can therefore be regarded as the initiator, rather than the result of water flow. Our current studies are directed towards an understanding of the aggregation phenomenon and its relationship to water flow across the cell membrane.

735 THE MOLECULAR MECHANISM OF ACTION OF UNCOUPLERS OF OXIDATIVE PHOSPHORYLATION, S. McLaughlin, F. Cohen, J. Dilger and M. Eisenberg. Dept. Physiology & Biophysics Health Sciences Center, SUNY, Stony Brook, N.Y. 11794.

The chemiosmotic hypothesis predicts that all molecules which increase the proton permeability of artificial bilayer membranes should act as uncouplers of oxidative and photophosphorylation in biological systems. New experimental evidence will be presented which supports this prediction. To predict how a given uncoupler will affect the membrane potential and internal pH of a topologically closed vesicle, it is necessary to understand its molecular mechanism of action. Specifically, it is necessary to know whether the reactions which occur at the membrane-solution interfaces are in equilibrium, to know the adsorption coefficients of both the undissociated acid and its anion, and to know the membrane permeabilities of both the neutral and the charged permeant species. These parameters have been measured on artificial bilayer membranes for several uncouplers.

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626 CHARACTERIZATION OF TWO MEMBRANE PROTEINS OF RAT LIVER ROUGH MICROSOMES INVOLVED IN RIBOSOME BINDING, Gert Kreibich and David D. Sabatini, NYU School of Medicine, Department of Cell Biology, New York, N.Y. 10016

A careful comparison of SDS acrylamide gel patterns from rough (RM) and smooth microsomes (SM) isolated from rat liver showed that in addition to proteins of bound polysomes, RM contain two polypeptides (approximate molecular weight 65,000 and 63,000 daltons) which are absent in SM. These correspond to two integral membrane proteins which can be iodinated by lactoperoxidase added to the intact microsomal vesicles. The RM polypeptides appear to have a transmembrane disposition and a short carbohydrate moiety exposed on the luminal aspect of the membranes (experiments with Dr. E. Rodriguez-Boulan). Antisera raised against the RM glycoproteins inhibited the in vitro binding of 60S ribosomal subunits to RM stripped of ribosomes (experiments with Dr. W. Mok and Ms. R. Grebenau). The RM proteins were isolated together with the membrane-bound polysomes by solubilization of the RM with the nonionic detergent Kyro EOB (2.5×10^{-2}M). When analyzed in sucrose density gradients, the Kyro EOB residue produced patterns similar to those of large polysomes. These, however, corresponded to pseudopolysomes insensitive to RNase, but sensitive to high salt treatment and to mild proteolysis. Electron microscopy showed that ribosomes within the Kyro EOB pseudopolysomes were attached to a filamentous network representing a residual membrane structure. In intact RM the proteins could be crosslinked to the ribosomes using low concentrations (0.2mg/ml) of methyl-4-thiobutyrimidate. The RM proteins could then be released from detergent-prepared ribosomes by reversing the crosslinks through reduction with mercaptoethanol. Our results suggest that an intramembranous protein network characteristic of RM provides binding sites for the membrane-bound ribosomes. The protein network may also serve to restrict the mobility of ribosomal binding sites in the plane of the membrane. Supported by grants GM21971 and GM20277 from the NIH. GK is an awardee of an RCDA (NIH GM00232).

627 THE ROLE OF MEMBRANE GLYCOPROTEINS IN RECOGNITION PHENOMENA, Gilbert Ashwell, Laboratory of Biochemistry and Metabolism, NIAMDD, NIH, Bethesda, MD 20014

Earlier studies, in vivo, revealed a protective role for the terminal sialic acid residues of serum glycoproteins in regulating their survival time in the circulation. The receptor responsible for the recognition and removal of those proteins from which part, or all, of the sialic acid had been removed was identified originally as an intrinsic constituent of the hepatic plasma membranes of mammalian species. Subsequently, the mammalian receptor was isolated from rabbit liver and characterized as an aqueous soluble glycoprotein composed of two subunits with molecular weights of 48,000 and 40,000, respectively. Pronase digestion of the intact protein resulted in quantitative recovery of the carbohydrate moiety which was shown to consist of two glycopeptides of varying composition. The carbohydrate sequence of both was determined and the relative distribution between the subunits was indicated. The availability of the purified binding protein permitted the development of an affinity column capable of detecting microquantities of circulating asialoglycoproteins. The low levels found in normal human serum were doubled or tripled in sera obtained from patients with a clinical diagnosis of cirrhosis or hepatitis. Subsequent examination of avian and reptilian species revealed an absence of the above galactose specific binding protein in these species--a finding which correlated with excessively high levels of circulating asialoglycoproteins. In this case, an alternate regulatory mechanism was found to be operative in that a new binding protein, specific for N-acetylglucosamine-terminated glycoproteins, was isolated and purified from avian liver.

The recent observations that the mammalian binding protein possesses the lectin-like ability to agglutinate erythrocytes and to stimulate mitogenesis in desialylated T-cells suggests a broader role in recognition phenomena than had been recognized heretofore. The implications of the latter finding will be discussed.

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628 LIPOPROTEIN UPTAKE AND HYPERCHOLESTEROLEMIA, Michael S. Brown and Joseph L. Goldstein, Department of Internal Medicine, University of Texas Health Science Center at Dallas, Dallas, TX 75235.

Cholesterol, a major component of all mammalian plasma membranes is vital to cell growth and survival. Yet, excessive amounts of this sterol can also be lethal as is evidenced by the cholesterol deposition in arterial cells that potentiates the development of atherosclerosis. Thus, mammalian cells are faced with the dual problem of providing sufficient cholesterol for membrane growth and replenishment and at the same time of avoiding excessive accumulation of this insoluble substance.

Recent studies in cultured human fibroblasts have disclosed a new biochemical pathway for control of lipoprotein metabolism, cholesterol synthesis, and cholesterol storage. This pathway has been termed the low density lipoprotein (LDL) pathway because of its most distinguishing feature - namely, a specific receptor on the cell surface membrane that binds plasma LDL, the major cholesterol-carrying protein in human plasma. Binding of LDL to this receptor initiates a multistep process by which the cell picks up the lipoprotein by adsorptive endocytosis, hydrolyzes its protein and cholesteryl ester components within lysosomes, and uses the lipoprotein-derived free cholesterol for membrane synthesis. The lipoprotein-derived cholesterol in turn suppresses the cells own cholesterol synthesis by suppressing the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase and it activates an acyl:CoA:cholesterol acyltransferase that facilitates the reesterification and storage of the incoming cholesterol. The physiologic importance of the LDL pathway is emphasized by the finding that mutations at two sites in this pathway (the LDL receptor in Familial Hypercholesterolemia and the lysosomal acid lipase in Wolman's Syndrome and Cholesteryl Ester Storage Disease) profoundly disrupt body cholesterol metabolism and lead to atherosclerosis.