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## 63. Konferenz der Gesellschaft für Biologische Chemie

**Studiengruppe Membranstrukturen und -transport**

# Deutsches Membranforum

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**Organized by H. Fasold, H. Passow and K.J. Ullrich**

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Abstracts (in the alphabetical order of first authors)

### ATP SYNTHASE OF ESCHERICHIA COLI: RECONSTITUTION OF A FUNCTIONAL $H^+$ CHANNEL.

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The ATP synthase of E. coli is composed of two structurally and functionally distinct entities: the peripheral $F_1$ part which carries the catalytic centers of the enzyme and the membrane-bound $F_o$ complex which functions as a $H^+$ channel. The $F_o$ complex has been isolated and dissociated into the individual subunits a, b, and c in the presence of trichloroacetate and Zwittergent. Reconstitution studies revealed that all three subunits are necessary to form a complex functional in $H^+$ translocation and $F_1$ binding.
In $F_1$-stripped membrane vesicles as well as in $F_o$-containing liposomes protease digestion inhibited $F_1$ binding, while proton-translocating activity remained unaffected. However, liposomes reconstituted with $F_o$ isolated from trypsin-treated membranes were impaired in both binding of $F_1$ and proton translocation. These activities could be restored, when reconstitution was carried out in the presence of native subunit b. From these experiments we conclude that the C-terminal region is necessary for a proper reconstitution of $F_o$ into liposomes.

### PREPARATION AND CHARACTERIZATION OF A NEW ACTIN-BINDING PROTEIN FROM PIG LIVER

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Based on their capacity to co-purify with F-actin repolymerized from a raw preparation of G-actin from pig liver, several actin-binding proteins can be discriminated. The most prominent one of these consists of two polypeptides of almost identical molecular weight ($M_r$=100 000 on SDS-polyacrylamide gels). After dissociation by 0.6 M KI, this protein was separated from actin by gel filtration over Ultrogel AcA 44, freed from myosin by dialysis against low ionic strength buffer and centrifugation, and further purified by chromatography on DEAE-cellulose, hydroxyl apatite, and HPLC. Determination of molecular weight in a non-denatured state by gel filtration (HPLC) showed a $M_r$ of about 430 000 which roughly indicates a tetramer. Isoelectric focussing revealed a single band at about pH 8.7. These data (tetrameric state and basic IP) suggest that this protein is different from alpha-actinin. Also, there was no cross-reactivity with polyclonal antibodies against alpha-actinin from skeletal and from smooth muscel.

### THE CYTOCHROMES OF SULFOLOBUS ACIDOCALDARIUS

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Sulf. ac., a thermoacidophilic archaebacterium, grows at pH 2 - 3 and temperatures of 70 - 80° C. Because of this extreme environment its bioenergetic properties are of great interest, especially regarding the question by what mechanism energy conversion proceeds. Components of the membrane bound energy converting system so far detected are a NADH - dehydrogenase, a succinate - dehydrogenase, ATPases and cytochromes. Redox spectra of Sulf. ac. membranes reveal a superimposed peak in the Soret - region and three distinct peaks in the $\alpha$ - region at 562 nm, 587 nm and 604 nm. Pyridine hemochrome spectra indicate the presence of a and b - type cytochromes, whereas c - type cytochromes are not detected. The peak at 562 nm can be attributed to a b - and/or an o - type cytochrome, the peak at 604 nm to cytochrome $aa_3$. Reduced + $CN^-$ minus reduced spectra as well as pyridine hemochrome spectra of detergent solubilized membranes clearly show that the peak at 587 nm can be attributed to an $a_1$ - type cytochrome. For the purification of the cytochromes Sarkosyl - extracts of Sulf. ac. membranes were separated by HPLC exclusion chromatography. On the analytical scale heme containing proteins with the following molecular weights could be detected : 111 - 116 kD, 102 - 105 kD, 78 kD, 49 - 50 kD and 41 - 43 kD. On the preparative scale a novel species with a peak at 592 nm in the redox spectrum was detected. An attribution of this peak to any of the above described cytochromes could not be achieved, so far.

### $CA^{2+}$- AND TEMPERATURES EFFECTS ON MONOLAYERS OF GANGLIOSIDES

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The influence of $CaCl_2$ and/or different temperatures on the surface requirement of individual gangliosides and phospholipids in monolayers was investigated. In addition surface pressure/area isotherms of defined mixtures of gangliosides and a cyclodepsipeptide (valinomycin) simulated possible glycolipid-peptide-interactions. In contrast to phospholipids gangliosides showed a phase transition between 20 and 30 mN/m at 11 and 20°C. Generally $Ca^{2+}$ had only a condensing effect on gangliosides. In mixtures at a special molar ratio between gangliosides and valinomycin no phase transitions of gangliosides could be detected. Gangliosides exhibited an apparent condensation of the film area in mixed monolayers and this might be taken as a general effect in lipid-peptide-interactions.

## METAL CATIONS AND BINDING OF EXTRINSIC PROTEINS OF BOVINE MYELIN

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The release of extrinsic proteins from myelin of bovine spinal cord by mono- and divalent metal cations ($Cs^+$, $K^+$, $Li^+$, $Na^+$; $Ca^{2+}$, $Mg^{2+}$, $Zn^{2+}$) was studied. Except for $Zn^{2+}$ cations resulted in a concentration-dependent, almost instantaneous release of up to 30% of total myelin protein at 0°C. Soluble protein consisted of myelin basic protein(MBP) exclusively. Still only a portion of total MBP was soluble. Pretreatment of myelin with $Zn^{2+}$ prevented the extraction of MBP by other cations. The results are consistent with electrostatic binding of a portion of MBP that is disrupted by an excess of cations. $Zn^{2+}$ in turn is suggested to enhance hydrophobic interactions of the myelin membrane with MBP thereby overruling the effect of other cations. Thus $Zn^{2+}$ appears to stabilize the myelin membrane as is already known from other biological membranes.

## VOLTAGE GATED POTASSIUM CHANNELS IN ACETABULARIA: A PATCH CLAMP STUDY.

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In the plasmalemma of mechanically prepared protoplasmic droplets from Acetabularia mediterranea single $K^+$ channels have been investigated using patch clamp techniques. The current-voltage relationship (I-V curve) of the open channel is sigmoid over the range between -100mV and +100 mV with saturation currents of about +10 pA. Taking the closed times into account, the mean steady state I-V curve of an individual channel displays outward rectification about the equilibrium voltage for $K^+$ and negative slope conductance at high negative voltages. These two I-V curves are well described by simple reaction kinetic models, the parameters of which are determined to fit the experimental data. The voltage-sensitive activation/inactivation mechanism is discussed to be physiologically important with respect to the $K^+$ balance in the cytoplasm.

## THE INFLUENCE OF THALLIUM IONS ON THE TRANSPORT OF POTASSIUM IN MALPIGHIAN TUBULES

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In most of the insects, the transport of $K^+$ is a prerequisit for urine formation and flow. In Drosophila larvae, this mechanism is influenced by a basolateral $Na^+,K^+$-ATPase and a furosemide sensitive co-transport as well as by an apical $K^+$ pump. Under physiological conditions, isolated tubules of Drosophila hydei produce within 1 h 0.52 nl/min urine at a $[K^+]$ = 211 mmol/l and a transport rate of 109 pmol/min. It is known for many biological structures that $Tl^+$ is competitive to $K^+$ for the same binding sites. Thus, application of $10^{-3}$ mol/l $Tl^+$ to isolated Malpighian tubules decreases the flow of urine to 75 %, the transport rate of $K^+$ to 63 %, and the concentration of $K^+$ to 90 % using the values obtained without $Tl^+$ addition as controls. Application of $Tl^+$ concentrations of $10^{-4}$ mol/l or less were without any influence.

## CHARACTERIZATION AND PARTIAL PURIFICATION OF THE NEURONAL NORADRENALINE CARRIER OF RAT PHAEOCHROMOCYTOMA CELLS (PC-12 CELLS)

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PC12 cells possess the neuronal noradrenaline transport system ($uptake_1$). Noradrenaline transport is selectively inhibited by desipramine (DES). The biochemical properties of the PC12 carrier protein were studied by means of the binding of $^3$H-DES. $^3$H-DES binding (30 min, 25°C) to PC12 membranes is inhibited by a protease (P-5380, Sigma) and by phospholipase $A_2$. Thus, $^3$H-DES binds to a protein which requires lipids for binding. $^3$H-DES binding is also dependent on intact SH-groups and on disulfid bonds within the protein since NEM as well as high concentrations of DTT (or DTE) inhibit $^3$H-DES binding.
The digitonin-solubilized $^3$H-DES binding site was reversibly bound to anion-exchange resins but not to lectin columns, indicating that the binding site is an acid protein (but no glycoprotein). The carrier protein was partially purified by means of reversible binding to hydroxyl apatite and to an affinity column (consisting of an imipramine analog coupled to Affigel 15, Sigma).

## BIOMIMETISCHE LIGANDEN FÜR NUCLEOTID-ABHÄNGIGE BINDUNGSZENTREN

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Vorgestellt wird ein neuartiges Konzept der Molekülkombination, welches die Strategie der Molekülmodifikation (Substratanaloga) und die Strategie der Molekülvariation (Pseudosubstrate) vereint, und welches zu chromogenen Liganden für nucleotidabhängige Bindungszentren führt.
Kombiniert werden hierbei die biomimetischen Strukturelemente von Diarylazo- und/oder Arylmethinfarbstoffen (Pseudosubstrate) mit den wirkortspezifischen Substrukturen (Trägereigenschaften) von Purinnucleotiden. Derartige Pseudonucleotide können einen Beitrag leisten zur molekularen Charakterisierung von Metall-Nucleotid und Protein--Metall-Nucleotid Wechselwirkungen sowie zur Erfassung von chemo- und biofunktionellen Substrukturen von Biomolekülen.

## BINDEPROTEIN-ABHÄNGIGE TRANSPORTSYSTEME IN ESCHERICHIA COLI

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E.coli besitzt zwei Typen von aktiven Transportsystemen für verschiedene Zucker und Aminosäuren. Der erste Typ besteht aus einem einzigen membrangebundenen Protein, das über Protonensymport PMF abhängig das Substrat gegen den Konzentrationsgradient akkumuliert. Der zweite Typ ist ein Mehrkomponentensystem bestehend aus 4 Proteinen von denen das bestuntersuchte das jeweilige periplasmische, wasserlösliche Substrat-Bindeprotein darstellt, die einzige Substraterkennungsstelle des jeweiligen Systems. Zwei weitere Proteine sind stark hydrophobe Membranproteine, die mit substrat-beladenem periplasmischem Bindeprotein reagieren und den eigentlichen Transportvorgang katalysieren. Das letzte Protein ist periphär an der Membraninnenseite lokalisiert und sorgt für die Energiekopplung des Transportvorgangs. Alle bisher untersuchten Proteine dieser Klasse tragen eine ATP Bindestellen-Consensus Sequenz. Energietransfer muß über Konformationsänderung der beteiligten Proteine erfolgen, die zum Einwärtstransport des Substrats führt.

TRANSPORT OF ANIONS THROUGH NEURONAL CELL MEMBRANES BY RECEPTOR-CHANNEL PROTEINS

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Inhibitory postsynaptic potentials in the mammalian central nervous system are mainly caused by the opening and closing of receptor channels that are operated by glycine or GABA. Application of the patch-clamp technique to cultured spinal neurons has elucidated some of the mechanisms that determine ion selectivity and ion transport through these chloride channels. The channels exclude cations but allow the passage of many different anions up to the size of propionate indicating a minimal pore diameter of 5-6 Å. The selectivity of the channels is governed by the interaction of hydrated anions with positively charged groups in the channel wall. Anions are transported across the channels by single-file diffusion where binding occurs at two sites. Glycine and GABA receptor channels display nearly identical ion permeation properties suggesting that the molecular structure of the two channels is similar.

CHARAKTERISIERUNG DES LAMELLAR- INVERTIERTEN PHASENÜBERGANGES MIT HILFE VON OPTISCHER SPEKTROSKOPIE

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Es wird über Untersuchungen zum Phasenverhalten- insbesondere zum Übergang lamellar-nichtlamellar -von natürlichen und synthetischen Phosphatidylethanolaminen und Phosphatidsäuren unter physiologischen Bedingungen (Wassergehalt >95%) bei Variation von Temperatur und Konzentration 2-wertiger Kationen berichtet. Es wird gezeigt, daß weder mit Fluoreszenz- noch mit 90°-Streulicht- oder mit IR-Messungen ein L--H Übergang auf einfache Weise charakterisiert werden kann, sondern daß die typischen Intensitätsänderungen z.B. bei der Lichtstreuung und die Peaklageverschiebungen einiger IR-aktiver Vibrationenpräparations- und konzentrationsabhängig sind. Unter bestimmten, klar definierten Bedingungen ist jedoch eine eindeutige Zuordnung einiger Meßparameter zur lamellaren bzw. invertierten Struktur möglich, so daß auf die Zuhilfenahme aufwendiger Verfahren wie Röntgenbeugung verzichtet werden kann.

CHARACTERIZATION OF MANNOSE 6-PHOSPHATE SPECIFIC RECEPTROS

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The transport of newly synthesized lysosomal enzymes to the lysosomes and the endocytosis of exogenous lysosomal enzymes are mediated by mannose 6-phosphate specific receptors. The two transport pathways share an acidic compartment, CURL, where the ligands are uncoupled from the receptors. The lysosomal enzymes are then delivered to lysosomes, while the receptors are reutilized in further transport. Two receptors have been described which differ in antigenicity, cellular expression, half-lives and cation dependence. The $M_r$ 46000 receptor does not participate in endocytosis. The receptors at the cell surface are in exchange with those in intracellular membranes. Dissipation of pH-gradients in the CURL with weak bases causes a redistribution of the $M_r$ 215000 receptor and its return in ligand occupied state to the cell surface. This receptors recycles continuously in cells depleted of ligands (I-cell disease, cycloheximide treatment) indicating that its movement is constitutive and not triggered by ligand binding or dissociation.

WAVEGUIDE SPECTROSCOPY APPLIED TO THE ION TRANSPORT IN PLANAR LIPID FILMS

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Exploiting the light pipe properties of planar lipid bilayers waveguide spectroscopy (WGS) [H.P.Braun et al. (1984) BBA 773, 61] sensitively detects inhomogeneities of the refractive index caused by ion accumulations. While current measurements only sense moving ions, WGS in contrast, senses and also localizes immobile ions. Comparative studies of membranes containing hydrophobic ions with undoped ones in pure alkali electrolytes exhibit apparent similarities in the charge distribution and transport kinetics, suggesting a similar transport mechanism. WGS applied to membranes doped with carriers show that these molecules facilitate the ejection of the alki ions from the energetically lower interfacial site to the higher states of the bulk electrolyte. This is explained by a reduction of the micropotential due to the high dielectricity constant of the carrier molecules.

SARCOLEMMAL CHLORIDE CONDUCTANCE OF MYOTONOIC MOUSE MUSCLE

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The hereditary neuromuscular syndrome of the mouse, 'arrested development of righting response', ADR, has been shown to be a myotonia in which direct stimulation of muscle fibers elicits runs of action potentials of 1-5s duration (Mehrke et al., J. Muscle Res. & Cell Mot. 7, 85, 1986). The possible involvement of chloride conductance in the ADR syndrome was studied by electrophysiological methods. While membrane time constants in wildtype muscle were 4 times prolonged in chloride-free medium, those of ADR muscle were only 1.4 times longer. This indicated a drastic reduction of chloride conductance of the ADR muscle membrane. At present, $^{36}Cl^-$ flux measurements are performed in order to

a) confirm the electrophysiological results by an independent method and
b) extend the analysis of the anion permeability to non-muscle cells of the mutant.

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**PROTEIN-MEDIATED, ATP-MODULATED FATTY ACID (FA) TRANSLOCATION IN THE HUMAN ERYTHROCYTE (RBC) MEMBRANE.**

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**$^{14}$C-oleic acid was incorporated into the outer membrane layer of RBC and its translocation to the inner layer quantified by the time-dependent decrease of extractability of FA from the outer layer by albumin. The biphasic concentration dependence (between 0.5 - 60 nmol FA/ml cells) of the translocation rates can be described by a saturable ($K_T$ = 6.3 nmol/ml cells, $V_{max}$ = 0.82 nmol/ml cells * $h^{-1}$) and an additional non-saturable (k = 0.016 $h^{-1}$) component. Translocated oleic acid is predominantly incorporated into phospholipids Evidence for a protein-mediated translocation was obtained by SH-reagents. Pretreatment of RBC with diamide and N-ethylmaleimide (= NEM 5 mM, 30 min, pH 8) blocked translocation by about 95%. Incorporation of FA into phospholipids was also markedly suppressed in diamide- and NEM-treated cells. ATP-depletion (by iodoacetate + inosine-treatment), to ≤ 5% of the control, diminished translocation by ≤ 50% indicating an involvement of energy metabolism. Carrier-mediated transfer of FA in the RBC membrane is indicated by these results.**

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DIFFERENCES IN THE WATER DISTRIBUTION BETWEEN THE LIGHT- AND DARK-ADAPTED STATE OF BACTERIORHODOPSIN

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X-ray and electron microscopy studies of purple membranes in the light- and dark-adapted states have revealed very small differences which were hardly detectable in diffraction patterns up to 8 Å. Therefore we started an investigation of these effects by neutron diffraction on stacks of purple membranes fully hydrated with $D_2O$. From the high contrast in density between $D_2O$ and the protein we expected a considerable enhancement of conformational changes between these states reflected by changes in the $D_2O$ or O distribution in bacteriorhodopsin (BR). Indeed the in-plane reflections clearly showed the effect that BR absorbs more molecules of water in the light adapted than in the dark adapted state. Care was taken that the sample in both states was fully hydrated and that no dehydration took place during illumination.

THE $H^+/OH^-$ PATHWAY THROUGH BACTERIORHODOPSIN

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We have performed measurements to examine the existence and the properties of a specific proton/hydroxide ion pathway in the light-driven $H^+$-pump bacteriorhodopsin (BR). Answering the question if and in what way the chromophore retinal of BR participates in the $H^+$-path was the primary aim of this investigation. Transmembrane pH-gradients were quickly established across DMPC vesicles containing BR or the chromophore-free protein moiety bacterioopsin and the induced fluorescence changes of the enclosed pH-sensitive optical probe pyranine were monitored. Our results show that in the presence of the chromophore retinal, passive $H^+/OH^-$ diffusion through BR is blocked. Retinal is either part of the proton/hydroxide ion pathway across the protein moiety of BR connecting both surfaces of the membrane or indirectly controls this path by inducing conformational changes in the protein upon binding.

*KINETIC STUDIES ON THE MULTIDRUG RESISTANCE PHENOTYPE IN CHO CELLS*

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*Cancer chemotherapy often fails due to the emergence of drug-resistant tumour cells. The multidrug-resistant phenotype (MDR) of CHO cells against amphiphilic cytotoxic drugs is caused by the differential amplification of members of the P gene family and the subsequent overexpression of the P glycoprotein in the plasma membrane. The P-mediated exclusion of drug is fast and effective. The uptake of drug to its binding sites in the plasma membrane is a diffusion-controlled process. Bound drug is released within less than ten milliseconds into the medium. - The MDR phenotype of CHO cells can be reversed by the addition of calcium channel blockers which inactivate P glycoprotein.*

CHARAKTERISIERUNG DER MENSCHLICHEN EKTO-5'-NUKLEOTIDASE UND IHRER QUERVERNETZUNGSPRODUKTE IN DER PLAZENTA-PLASMAMEMBRAN

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Das Glycoprotein Ekto-5'-Nukleotidase (5'-N) aus menschlicher Plazenta wurde untersucht sowohl in isolierter gereinigter, als auch in membrangebundener Form mittels ein- und zweidimensionaler Gelelektrophorese, Western-Blot und Markierung mit polyklonalen und monoklonalen Antikörpern. 5'-N liegt im nativen Zustand vor als ein Dimer aus 2 identischen Untereinheiten von je 70 kD. Bedingt durch unterschiedlichen Gehalt an Neuraminsäure existieren mindestens 13 verschiedene Subformen zwischen 66 und 70 kD mit pI-Werten von 5,5 - 7,0. Bei chemischer Quervernetzung mit verschiedenen Reagentien erhält man aus dem isolierten Enzym stets das Dimer (140 kD), in der Membran jedoch die Verknüpfung mit einem Protein von ca. 30 kD. Die Identität dieses Proteins ist unbekannt. Als mögliche Kandidaten aus der Reihe der Zytoskelett-Proteine wurden Aktin und Tropomyosin ausgeschlossen.

VALINOMYCIN AND NYSTATIN BUT NOT CHROMATE MODIFY THE ELECTRICAL BREAKDOWN OF HUMAN RED CELLS

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Chromate was shown to modify the transport properties of human red cell membranes and to crosslink their proteins (Buttner et al., 1986). Using the Coulter Counter technique we measured the electrical breakdown of human erythrocytes according to Zimmermann et al. (1974). Breakdown was indicated by an underestimation of the cell volume above a critical electrical field strength of 1.1 kV/cm. Chromate up to 100 mM did not alter the electrical breakdown nor the cytoplasmic resistivity of the cells (195 Ohm·cm). In contrast, ionophores changed the relation between apparent cell size and field strength: valinomycin rendered the apparent size almost independent of the field strength, whereas nystatin caused a significant decrease in apparent size with increasing field strengths but without a marked breakdown at a distinct threshold. These results are discussed in terms of changes in cell volume, membrane permeability and electrochemical stability.

INHIBITION BY pCMBS OF WATER OSMOTIC ($P_{os}$) AND DIFFUSIVE ($P_d$) PERMEABILITY IN RABBIT KIDNEY PROXIMAL CELLS.

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$P_{os}$ and $P_d$ have been measured in isolated tubules a and cells, respectively, measuring cell volume changes under an osmotic difference ($P_{os}$) (Pflügers Arch 400:343 & 402:337,1984) and proton NMR ($P_d$). Without pCMBS (parachloromercuribenzenesulfonate) $P_{os}/P_d$ =15. The energy of activation ($E_a$) of $P_{os}$ and $P_d$ = 4Kcal/mole. pCMBS inhibits $P_{os}$ and $P_d$ in a dose-dependent manner. pCMBS action is reverted by DTT (dithiothreitol). At pCMBS = 2.5 mM $P_{os}$ is 15% of the control value, $P_{os}/P_d$ = 3 and $E_a$ of $P_{os}$ and $P_d$ = 10Kcal/mole. These results indicate that proximal cell membranes must be pierced by continuous water pathways. From $P_{os}/P_d$ either the pathway's radius is 10A (unreasonable since the reflection coefficient = 1 for molecules with a radius ≥ 5A or their geometry produces single filing of several water molecules. pCMBS alters these pathways. Scatchard plots of $P_{os}$ and $P_d$ vs [pCMBS] give values of N= 4 and 2, respectively. It is possible that 2-4 pCMBS molecules per pathway are required to block water permeability.

**TWO TYPES OF SKELETAL MUSCLE CALCIUM CHANNELS INCORPORATED INTO LIPID BILAYERS.**

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The solubilized and purified dihydropyridine receptor from rabbit skeletal muscle T-tubules was reconstituted into lipid vesicles, and the vesicles were incorporated into lipid bilayers formed at the tip of patch-clamp pipettes. Two types of calcium channels, with slope conductances of ~20 pS and ~10 pS (90 mM Ba), were observed separately or together in single channel recordings from solubilized and purified receptor preparations. Both channel types seem to have at least two open states. The 20-pS channel occupies the open states preferentially at potentials around zero, and closes at strong positive or negative potentials. The open-state occupancy of the 10-pS channel is promoted by nevative and hindered by positive potentials. The open-state probability of the 20-pS channel is decreased by D600 ($ED_{50}$ ~ 14 µM) and enhanced by BAY K 8644 (10 µM) or phosphorylation by cAMP kinase. By contrast, the 10-pS channel does not respond to those interventions. Therefore, both calcium channel types may have similar structures but cannot be separated by dihydropyridine binding alone.

**SOLUBILISATION, PURIFICATION AND RECONSTITUTION OF THE cGMP-GATED CHANNEL FROM ROD PHOTORECEPTORS**

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**The cGMP-gated cation channel from vertebrate rod photoreceptor membranes was solubilised using the zwitterionic detergent CHAPS and reconstituted into the membrane of calcium-containing soybean phosphatidylcholine liposomes using a dialysis procedure. The cGMP-activated efflux of calcium from these liposomes was followed spectrophotometrically using the calcium-indicator dye Arsenazo III. The reconstitution procedure was used to probe for the channel protein during its purification by anion-exchange and dye-ligand affinity chromatography. The purified channel protein has a m.wt. of 63 kDal as ascertained by SDS-PAGE, and probably exists as a homotetramer. The purified channel is cooperatively (n = 3.1) activated by cGMP with a $K_m$ of about 15 µM.**

**ION CHANNELS DETECTED IN INTERNODES OF RABBIT SCIATIC NERVE AFTER PARTIAL DEMYELINATION**

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Voltage-clamp experiments were performed on single internodes isolated from rabbit sciatic nerve. During the demyelination with lysolecithin changes of capacity currents and the occurance of ionic membrane currents were recorded. With progressive demyelination an increase of capacity is accompanied by increasing inward and outward membrane currents. The outward current is mediated by delayed rectifying potassium channels and anion-selective channels, the inward current by sodium channels that have similar voltage-dependent characteristics as nodal sodium channels. At high degree of demyelination the internodal membrane capacity reaches values that are about 10 times larger than the value calulated for the internodal axolemma in the recording pool. An interaction between the surviving membrane of the Schwann cell and the internodal membrane is suggested.

**THE INFLUENCE OF SPHINGOMYELIN ON PSEUDOMONAS AERUGINOSA CYTOTOXIN BINDING TO ERYTHROCYTES**

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Studies on Ehrlich ascites cells have already shown that plasma membrane damage by the pseudomonal cytotoxin (PAC) is initiated by fixation to a high affinity binding site. For red blood cell intoxication the proportion of sphingomyelin located in the outer layer of their membrane plays an important role. Only erythrocytes with low content of sphingomyelin are damaged by PAC.

We have studied the binding of 125I-PAC to sensitive erythrocytes such as those of rabbit and human and to insensitive cattle erythrocytes. Rabbit erythrocytes with a sphingomyelin (SM) : phosphatidylcholine (PC) ratio of 1:2 bind $2x10^4$ molecules/cell and human erythrocytes, SM:PC ratio 2:3, bind $6x10^4$ molecules/cell. No binding was achieved with cattle erythrocytes, SM:PC ratio 12:1. Whereas pretreatment of cattle erythrocytes with sphingomyelinase C (B.cereus) triggered the 125I-PAC binding. We conclude that the PAC binding site in plasma membranes is located close to and masked by high concentrations of sphingomyelin.

**PALMITOYLCARNITINE: A NEW PROBE FOR FAST ANALYSIS OF TRANSBILAYER REORIENTATION OF LIPIDS IN THE ERYTHROCYTE (RBC) MEMBRANE**

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**The flip of lysophospholipids from the outer to the inner membrane layer of RBC can be measured by incorporation of trace amounts of labelled lysophospholipids into the outer layer and quantification of the time-dependent increase of the lysophospholipid fraction not extractable by albumin. A disadvantage of this method consists in the acylation of lysophospholipids to their diacyl analogues at the inner membrane surface. This problem can be overcome by the use of PAF, which has, however, a low flip rate, and by the use of palmitoylcarnitine (16:0 Car) introduced here. The flip of 16:0 Car is fast ($t_{1/2} \cong 2$ h at 37°C, pH 7.4) and the probe is therefore suitable to measure slow flip processes as, e.g. in ox erythrocyte membranes. Like lysophospholipid flip, the flip of 16:0 Car is not energy-dependent and is enhanced by modification of membrane proteins and after insertion of channel-forming antibiotics into the membrane. As a pecularity flip of 16:0 Car is enhanced by lowering the pH and exhibits inter-individual variability.**

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**TRUNCATED OmpC PORINS ARE NOT EXPORTED INTO THE OUTER MEMBRANE**

**OF E. coli. I. Cruse, H. Eberle and P.G. Wood, Univ. Rochester, Rochester, NY, USA, M.P.I. f. Biophysik, Frankfurt/M.**

Mild Bal 31 digestion at the Spe 1 site within the ompC gene in plasmid MY150 (Inouye) and subsequent ligation and transformation of E. coli produces gene products which are either about the same size or 1/2 the size of the intact OmpC porin. When digestion and ligation creats a reading frame shift, a stop codon is encountered within a few codons. The truncated plasmid codes for only about the first 184 amino acids of the total 336 amino acids of the pore protein. The resulting truncated protein is only transiently present and is degraded in either the cytoplasm or periplasm. In contrast in-frame codon deletions within the Spe 1 region yields porins shortened by 1-2 amino acids in a central hydrophobic tract of the protein which are exported into the outer membrane (OM). The observations suggest either the presence of signal information in the C terminal half of the protein which is required for the export of porin into the OM or that the spontaneous global conformation of the nascent full length protein provides the stability and necessary information for its export into the OM.

## DEMONSTRATION OF DIFFERENT TRANSPORT SYSTEMS FOR NICOTINIC ACID IN HUMAN ERYTHROCYTE GHOST MEMBRANE AND BRUSH BORDER MEMBRANE OF RAT SMALL INTESTINE

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In order to characterize the transmembrane transport of nicotinic acid (NA; $pK_a$ 4.91), we used two different membrane preparations; type II erythrocyte ghosts were prepared according to Schwoch and Passow, brush border membrane vesicles (BBMV) of rat jejunum according to Kessler. Concentration dependent kinetics of NA influx in erythrocyte ghosts under equilibrium exchange conditions (t:5 sec/ 34°C) showed dual characteristics (saturable component with $K_t$= 2.3 mM+linear component). Both components were pH sensitive: the linear component due to non-ionic diffusion of NA, the saturable component due to a $OH^-$/NA antiport system which could be completely inhibited by DIDS. The concentration dependent uptake of NA (t : 10 sec/25°C) into BBMV in the presence of a voltage clamp showed linerity. Influx was not effected by different cation gradients but by anion gradients and was highly pH sensitive. A transmembrane pH gradient ($pH_{in}$8.0; $pH_{out}$5.75) lead to a transient overshoot of NA which could not be inhibited by DIDS or SITS. It could be concluded that intestinal NA uptake is due to non-ionic diffusion and therefore favored by low mucosal surface pH.

## FACTORS CONTROLLING $H^+$-ION SECRETION IN THE SMALL INTESTINAL MUCOSA

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The mucosa of the proximal small intestine secretes $H^+$-ions into the intestinal lumen. The mechanisms responsible for this secretory process could be the $Na^+/H^+$ exchange system and/or a lactate recirculation across the brush border membrane. In the secretory state the normal $H^+$-ion secretion is abolished or turned to alkalinization. Stimulus-secretion-coupling is related to changes in the cellular levels of cyclic nucleotides. Therefore we examined in vitro the effect of elevated c-AMP levels on $H^+$-ion secretion and glucose metabolism in rat jejunum. All the experiments were carried out in a gas-tight incubation chamber in presence of luminal glucose (10mM $^{14}$C-U-D-glucose) without effector (control) or with 20 µM forskolin+10 mM theophyllin. By incubating the tissue with the effector, c-AMP levels were increased 3-4 times compared to the control, resulting in an increased $H^+$-ion and lactate appearance in the mucosal medium. The effectors significantly increased glucose uptake but decreased $CO_2$ production from glucose, serosal lactate appearance and lactate accumulation in the cells. Increased mucosal $H^+$-ion appearance may be linked to increased lactate efflux. From this study it cannot definitely be concluded that increased mucosal c-AMP levels are responsible for inhibition of the $H^+$-ion secretory process.

## THE POSSIBLE EXISTENCE OF A Ca ACTIVATED K CHANNEL IN THE CAT CAROTID BODY IN VITRO

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It is well accepted that Ca plays an important role in the stimulus secretory mechanism responsible for hormone release by the carotid body during hypoxia. To further define the action of Ca, cat carotid bodies were superfused in vitro and extracellular calcium ($Ca_e$) and potassium ($K_e$) activities were determined. Decreasing $Po_2$ immediately causes a decrease in $Ca_e$ and a biphasic $K_e$ increase. The changes are accompanied by afferent nerve excitation and are affected in their pattern by the Ca content in the medium ($Ca_m$). They are also present after poisoning the nerve with tetradotoxin. Various Ca channel blockers inhibit the changes in $Ca_e$ and $K_e$ to a different extent and dependent on $Ca_m$. Lowering $Ca_m$ to a critical level (0.1 mM) reverses $Ca_e$ decrease and depresses the biphasic $K_e$ increase during hypoxia. These results suggest that hypoxia stimulates Ca influx which in turn activates a K channel and triggers hormone release.

## SPONTANEOUS INSERTION OF MEMBRANE PROTEINS INTO LIPID VESICLES

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A novel technique has been developed for fast, easy, and gentle insertion of membrane proteins into lipid vesicles (1). Reconstituted protein-lipid vesicles are spontaneously formed upon mixing aqueous suspensions of long-chain phospholipids (DMPC, DPPC, or soybean PC) with the micellar short-chain phospholipid diheptanoylphosphatidylcholine (20 mol% total lipid) in the presence of biological membranes or isolated membrane proteins. We have successfully applied this procedure for functional reconstitution of both isolated membranes containing only one protein species, i.e., bacteriorhodopsin in the purple membrane (1), and purified membrane proteins with a small amount of detergent, i.e., a $H^+$-ATPase from yeast plasma membrane (N.A. Dencher and A. Wach). The observation of spontaneous reconstitution might be of relevance to our understanding of how membrane proteins insert posttranslationally into biological membranes.

N.A. Dencher, Biochemistry 25, 1195-1200, 1986, (1)

## INHIBITION OF THE ANION EXCHANGE SYSTEM OF THE HUMAN ERYTHROCYTE (RBC) MEMBRANE BY SH-REAGENTS

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**The anion exchange system (band 3 protein) of RBC has generally been regarded as "SH-independent" on the basis of its insensitivity to mercurials and alkylating agents. This system, as characterized by oxalate self-exchange, is, however, inhibited by the highly SH-specific class of diazine dicarboxylic acid derivatives (diamide etc.), which oxidize thiols (2 $S^- \rightarrow$ S-S). A maximal inhibition of only 50% can be obtained, which is not an artifact due to the formation of unspecific leaks since noncovalent inhibitors still fully suppress oxalate fluxes after diamide. Inhibition can partly be reverted by dithioerythritol, and is prevented when $CN^-$, which cleaves and cyanylates disulfides (S-S→SCN + $S^-$), is present during the modification. Cyanylation itself is not inhibitory but blocks SH-groups for further inhibitory modifications. This allows to test whether SH-reagents of lower specificity inhibit via SH-modification or not. The thiols in the 35 K C-terminal fragment of band 3 are probably responsible for the inhibitory effects of diamide.**

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## REACTION MECHANISM OF THE ISOLATED AND RECONSTITUTED ASPARTATE/GLUTAMATE CARRIER FROM BEEF HEART MITOCHONDRIA

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**The carrier protein catalyzing aspartate/glutamate counterexchange in the inner membrane of mitochondria was solubilized and purified by hydroxyapatite HPLC. The protein was incorporated into liposomes. With this reconstituted system the transport affinity constants ($K_m$) were determined. Their values turned out to be clearly different with respect to the two sides of the membrane demonstrating an unidirectional incorporation of the reconstituted protein. Bisubstrate analysis of the exchange reaction led to results which are consistent with a sequential mechanism of substrate binding during exchange indicating the formation of a ternary complex. A model for aspartate/glutamate translocation is proposed including a separate binding site for the proton that is cotransported with glutamate.**

## SPECTROSCOPIC STUDIES OF THE PEROXIDASE REACTION OF PROSTAGLANDIN H SYNTHASE

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Prostaglandin H (PGH) synthase catalyzes the conversion of arachidonic acid (AA) to $PGG_2$ (cyclooxygenase) and the reduction of $PGG_2$ to $PGH_2$ (peroxidase). The purified enzyme requires one hemin/polypeptide for the reconstitution of both activities. We study the peroxidase reaction with rapid photometry and EPR spectroscopy. After mixing of $PGG_2$ with purified enzyme at $1^{o}C$ we observed two spectral intermediates, which resembles compound I and compound II of horseradish peroxidase (HRP). Concomitantly to the second species observed also with conventional photometry at $-12^{o}C$, a new transient EPR signal at g=2.005 was detected. The structure of the second intermediate is formally similar to the compound ES of cytochrome C peroxidase. From the similarity with the tyrosyl radical of the ribonucleotide reductase, this signal was tentatively assigned to a tyrosyl radical due to the higher oxidation state of the enzyme.

## NMR AND ESR STUDIES ON THE INTERACTION OF SOLUBILIZED MITOCHONDRIAL MEMBRANE PROTEINS WITH PHOSPHOLIPIDS

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It has recently been demonstrated (1) that the solubilized mitochondrial ADP/ATP carrier contains rather large amounts of tightly bound cardiolipin (CL). It is shown by $^{31}P$-NMR that the protein-bound phospholipid is accessible to phospholipase A2, whereas the resulting lyso-CL remains bound to the protein surface. Weak phospholipid binding was observed after addition of spin labeled CL to the solubilized carrier. Spin label titrations suggest that on the surface of the carrier protein, two molecules of CL bind with low affinity in addition to the tightly bound CL detected by $^{31}P$-NMR. Immobilized phospholipids were also observed in the solubilized $bc_1$ complex of the mitochondrial respiratory chain. Release of the immobilized lipid after addition of Triton X-100 may be tentatively explained by cleavage of aggregated states of the complex.

(1) K.Beyer and M.Klingenberg (1985) Biochemstry 24, 3821.

## A SECONDARY CHLORIDE TRANSPORT SYSTEM IN HALOBACTERIUM HALOBIUM

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Besides halorhodopsin, the known primary light-driven chloride pump [1], *H. halobium* possesses a secondary, oxygen-dependent uptake system for chloride [2]. $^{36}Cl^-$-influx experiments with intact cells allowed to characterize this system as follows: Secondary chloride transport is energy-dependent, but not correlated with internal ATP-level or rate of respiration. It needs no external electron acceptors or specific ions save for $Na^+$. Inhibitor experiments showed a dependence on $\Delta\psi$, but not on $\Delta pH$. The data suggest a $Na^+$-coupled, $\Delta\psi$-driven symport system.

1 Schobert, B. & Lanyi, J.K. (1982) J. Biol. Chem. 257, 10306-10313.
2 Duschl, A. & Wagner, G. (1986) J. Bacteriol. 168, 548-552.

## PHOSPHATE UPTAKE IN OOCYTES OF XENOPUS LAEVIS

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The transport of inorganic phosphate into full-grown, prophase-arrested oocytes of Xenopus laevis takes place by two independent transport systems, P-1 and P-2.
P-1 is sodium-independent, inhibited by the SH-reagents NEM and PCMBS and leads without mixing with the phosphate pool in the cytoplasm to phosphate incorporation into a compartment which can be separated from an oocyte homogenate by centrifugation (48000g, 30 min).
P-2 is sodium-dependent and leads to phosphate uptake into the cytoplasm. P-2 is saturated at high phosphate concentration with Km and Vmax in the range of 0.2 mmol/l and 10 pmol/oocyte/h, respectively. The effect of sodium seems to be saturable. P-2 is inhibited by NEM and PCMBS and down-regulated during progesterone-induced oocyte maturation.
We further describe variations of phosphate uptake amongst oocytes of the same and of different females.

## STIMULATION OF CALCIUM AND ANION ACCUMULATION BY ADP IN SARCOPLASMIC RETICULUM VESICLES

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In the presence of ADP SR vesicles accumulate $Ca^{++}$ ions and anions. This process is dependent on the ADP concentration and exhibits half maximal stimulation at 15 µM ADP. $Mg^{++}$ ions are also absolutely necessary. The amount of accumulated $Ca^{++}$ is very much increased by the use of precipitating anions like oxalate and phosphate compared to non-precipitating anions like $Cl^-$ or succinate. Other purine nucleosidediphosphates e.g. GDP and IDP barely stimulate the $Ca^{++}$ accumulation at $Ca^{++}$ concentrations in the physiological range. The non-hydrolyzable ATP analogue AMP-PNP does not have the same effect as ADP stimulation. The question arises if this coupled transport of $Ca^{++}$- and anions across the SR membrane is mediated by the $Ca^{++}$ pumping ATPase itself or by a different ADP stimulated carrier protein.

## FUNKTIONAL RELEVANTE AMINOSÄUREN DES BAKTERIORHODOPSINS

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Bakteriorhodopsin, das einzige Protein der Purpumembran, benutzt die Energie des Lichtes, um Protonen über die Membran zu pumpen. Zur Aufklärung des Mechanismus des Protonentransfers wurden Isotopen-markierte Aminosäuren ($^{18}O$-Tyr, 4-$^{13}C$-Asp und $^{13}C$-guanido-Arg ) biosynthetisch in das Bakteriorhodopsind eingebaut und mit Festkörper (MAS)-NMR bzw. zeitaufgelöster IR-Spektroskopie untersucht. Die aus diesen Experimemnten gewonnenen Daten kann man mit einem Protonentransfer Mechanismus erklären, der auf einzelnen, diskreten Schritten beruht.Ein Mechanismus dagegen, der auf Ketten von Wasserstoffbrückenbindungen basiert, ist unwahrscheinlich . Die Zahl, der in diesen Arbeiten beschriebenen funktionellen Aminosäuren würde ausreichen, ein Proton von der einen Seite der Membran zur anderen zu transportieren.

## RELATIONS BETWEEN TOTAL INTRACELLULAR CALCIUM AND NA TRANSLOCATION PATHWAYS IN INTACT HUMAN RED CELLS

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The influence of the amount of Ca bound to the inner side of the red cell membrane (reflecting 80-90% of total intracellular Ca ($Ca_i$)) on Na translocation pathways has been investigated on intact human red cells. In erythrocytes of different donors negative relations were seen between $Ca_i$ and the activities of the Na-K pump, the Na-K cotransport, the Na-Li exchange and the Na-leak. In order to investigate whether these relations are causal, red cells were either depleted by incubation with A23187 and EGTA (yielding a 60-80% reduction of $Ca_i$) or loaded with the intracellular Ca-chelator quin-2. Ca depletion or quin-2 loading did not affect the activities of the Na-K pump, the Na-K cotransport and the Na-Li exchange. Both quin-2 loading and Ca-depletion resulted, however, in significant elevations of the Na- as well as the Rb-leak. Thus reduction of Ca binding to the inside of the red cell membrane affects only the unspecific permeability to Na and K and not specific Na-transport systems.

## A NEW METHOD FOR MEASURING HIGH MEMBRANE PERMEABILITIES

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An improved version of the capillary principle (see Osberghaus et al., J.Membr.Biol.,68,29,1982) for obtaining membrane permeabilities from bulk diffusion coefficients ($D_{eff}$) in packed cell columns has been developed. A new generalized theoretical approach extends the validity of the model theory to variable packing density (0.98-0.65) permitting application to cell or vesicle populations that cannot be tightly packed for geometry or stability reasons. $D_{eff}$ is derived from the movement of labelled solutes between two strictly unstirred compartments consisting of cylindrical half cells brought into contact at t=0. The setup avoids errors due to washout of solute from open capillary ends. Cell systems investigated were suspensions of human erythrocytes (RBC) and spheric vesicles obtained from heatfragmented RBC. Permeability coefficients are presented for ethylene glycol, water and hexanol. For low permeabilities ($<10^{-3}$cm/sec) precision is rather low, the resolution of the technique increases for higher permeabilities. Data obtained agree with results of other approaches requiring much more costly equipment.

## pH-REGULATION IN SINAPIS ROOT HAIRS AND RICCIA RHIZOIDS. A COMPARATIVE STUDY CARRIED OUT WITH DOUBLE-BARRELED pH-MICROELECTRODES

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Continuous measurements of cytoplasmic pH in Sinapis root hairs and Riccia rhizoids have been carried out to gain information on translocation of protons across membranes and cytoplasmic pH-control. (i) The cytoplasmic pH changes no more than 0.1 $pH_c$ per external pH-unit changed, regardless whether the $H^+$-pump works or not. (ii) Weak acids rapidly acidify $pH_c$ and hyperpolarize, while weak bases cause the opposite effect. (iii) $CN^-$ acidifies $pH_c$, but alkalizes the vacuole. (iv) In alkaline media CCCP has no significant effect on $pH_c$, but acidifies $pH_c$ in the acidic range. In the presence of CCCP, $CN^-$ still acidifies $pH_c$. The modes of proton reentry and the effects of active and passive proton transport on cellular pH-regulation are discussed. It is concluded that the proton leak and the proton pump are of minor importance, but intracellular $H^+$-production to be of major relevance for cytoplasmic pH-changes.

## ELECTROGENIC PROPERTIES OF THE $Na^+K^+$-ATPase

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Purified $Na^+K^+$-ATPase from pig kidney was attached to black lipid membranes and ATP induced electric currents were measured. The pump was activated by an ATP concentration jump generated by photolysing caged ATP by an UV light flash.

Transient and stationary currents were measured under various conditions. In particular even in the absence of $K^+$ a stationary current was obtained. This is a direct demonstration of electrogenic transport of the $Na^+$-ATPase. It is proposed, that in the absence of $K^+$ $Na^+$ can replace $K^+$ in dephosphorylating the enzmye, but binds about 1000 times weaker than $K^+$.

Using a fast UV laser pulse to photolyse the caged ATP the currents generated by the $Na^+K^+$-ATPase could be measured at a time resolution of 1 ms. This provides at the same time information about the electrogeneicity as well as the rate constants of individual reaction steps of the enzyme.

## WHICH MOLECULES CONSTITUTE THE GLUE IN RED CELL MEMBRANE CROSS BONDING?

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Internal membrane contact induced in red cells by shrinking is normally reversed upon swelling. After heating or chemical treatments known to modify spectrin this contact can become permanent by a mechanism termed membrane cross bonding. In transmission electron micrographs the phospholipid bilayers in the cross bonded region are parallel. Their distance greatly varies from cell to cell (37 to 120 nm). This rules out that cross bonding is mediated by a single layer of cross linking molecules. Oligomerization of a molecular species present in sufficient abundance (Hb or spectrin) seems more likely. Hb can be excluded because cross bonding takes place in white ghosts. Since cross bonding pertains after reversal of chemical modification or after decrease of temperature it is concluded that an irreversible denaturation of spectrin is responsible for membrane cross bonding. Membrane cross bonding could serve as an indicator of spectrin denaturation.

## SIGNAL TRANSDUCTION FROM PHOTOSYSTEMS TO OPENING OF POTASSIUM CHANNELS IN THE GREEN ALGA EREMOSPHAERA

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Transient changes of membrane potential and conductance in the unicellular green alga Eremosphaera viridis indicate a chemically induced action potential (CAP), which is released by light-off and is caused by a temporary opening of potassium channels in the plasmalemma of this alga. In contrast to action potentials of other plants, the CAP is independent of a membrane potential threshold value. It can be released by photosynthetic inhibitors like DCMU ($10^{-3}$ mol*m$^{-3}$) or DBMIB ($10^{-2}$ mol*m$^{-3}$) in the light. The triggering of a CAP by light-off is suppressed in presence of DCMU and DBMIB. These experiments give evidence for a signal perception by photosynthetic systems. Further on the release of a CAP depends on light-intensity and duration of the light-phase used before darkening. Direct measurements of cytoplasmic pH ($pH_c$) with pH-sensitive microelectrodes suppose, that $pH_c$ is involved in signal transduction from chloroplasts to potassium channels. Light-off causes a rapid acidification, always before start of the CAP ($\Delta pH_c \approx 0.2$, in 1.6 s) and a slower regeneration of $pH_c$ back to the former value ($pH_c$ in the light: 7.56). Experiments with $Ca^{2+}$ and $Ba^{2+}$ indicate that there are further internal signals to release a CAP. A sudden increase of external $Ca^{2+}$ causes a CAP. $Ba^{2+}$ ($10^{-2}$ mol*m$^{-3}$) releases repetetive CAP's in the dark as well as in the light. A preceeding change of $pH_c$ cannot be observed. Higher concentrations of $Ba^{2+}$ inhibit the CAP.

Lit.: K. Kohler, W. Steigner, J. Kolbowski, U.P. Hansen, W. Simonis, W. Urbach (1986). Planta 167, 66-75.

Membranständigkeit der Dihydrolipoamid Dehydrogenase (E3) der Pyruvatdehydrogenase (PDH

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E3 (EC 1.6.4.3), offensichtlich gemeinsamer Bestandteil der MEKs PDH, 2-KGDH und BCDH, löst sich in Gegenwart von Triton vom Komplex. Die Membranständigkeit dieser am Aufbau der Komplexe beteiligten Untereinheit wird untersucht. // (1) Gereinigter PDH-MEK (Muskulatur Rattenhinterkörper) nach STANLEY et al. (BJ 191 (1980) 147) und (2) solubilisierte Mitochondrien (2% (v/v) Triton X-114, 50 mmol TAPS /l, pH 7,4) nach Phasentrennung wurden auf lineare Dichtegradienten (0,2 - 2 mol Saccharose / l, TAPS, 0,2% Triton X-100) aufgebracht, (3) submitochondr. Partikel (SMP) präp. nach NALECZ (FEBS Lett. 196 (1986) 331). PDH-Aktivität wurde nach TX-114 Phasenverteilung in der wässrigen Phase gefunden, E3 Aktivität sowohl in der wässrigen als auch in der Triton X-114 Phase, SMP enthielten E3 Aktivität. // Es wird geschlossen, daß der PDH-MEK in situ Bestandteil eines komplexeren membranständigen Systems ist, dessen Eigenschaften durch verwendete Isolationsmethoden drastisch verändert werden (s.a. FÖRSTER: BC Hoppe-Seyler 367 (1986) Suppl., 361).

DECREASE OF MITOCHONDRIAL THIOLS UNDER UNCOUPLING?OLIGOMYCIN INHIBITED AND AGING CONDITIONS:PROTECTIVE PROPERTIES OF ANTI-ANGINAL DRUGS

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Thiol reactivity in rat heart mitochondria was determined under the above conditions. For detection the following chromophores were used:non permeable q-bromobimane MQ, moderately membrane penetrating dithionitrobenzaote($Nbs_2$) and permeable m-bromobimane MB.In all cases investigated the reactivity and/or the amount of -SH groups titrated with MB decrease.Furthermore,in parallel with increase of uncoupling activity of the uncouplers thiols located in a presumably more intermediate and progrediently in a more polar region of the membrane become unreactive.The active metabolite of molsidomine,SIN-1,and to an even more general extent the thiol reagent 2-mercaptopropionylglycine(MPG)exhibit a considerable protection against the decrease of -SH groups under conditions mentioned above.

STRATEGIES FOR PURIFICATION OF THE RAT RENAL $Na^+/H^+$ ANTIPORTER

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The $Na^+/H^+$ exchanger in the brush-border membrane of proximal tubular epithelial cells is responsible for $H^+$ secretion into the tubule lumen. Amiloride-protectable labeling with [$^{14}$C]-dicyclohexylcarbodiimide (DCCD) revealed that a membrane protein with app. MW 65,000 and an IP of 6.2 is involved in $Na^+/H^+$ exchange. This protein can be enriched, but not completely purified, by affinity chromatography of solubilized brush-border membrane proteins on a column with amiloride as ligand. A second strategy for purification involves separation of brush-border membrane proteins by SDS-PAGE and electroelution of polypeptides of the MW 65,000 band. Two-dimensional re-electrophoresis of the eluate revealed the presence of eight polypeptides. Only one of them showed an amiloride-protectable [$^{14}$C]DCCD labeling. Alternatively, the eluted MW 65,000 polypeptides can be separated by reversed phase HPLC. Again, one single peak with an amiloride-sensititve [$^{14}$C]DCCD labeling is recovered suggesting complete purification of the rat renal $Na^+/H^+$ exchanger.

STRUCTURE AND INHIBITORY ACTIVITY OF ORGANIC ANIONS ACTING ON CONTRALUMINAL TRANSPORT SYSTEMS IN THE PROXIMAL RENAL TUBULE

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The contraluminal transport of sulfate, succinate and para-aminohippurate (PAH) has been inhibited by aliphatic and aromatic anions.
The sulfate transport system accepts short dicarboxylates (oxalate) and sulfonates with NH- or OH-groups in ortho-position.
The succinate transporting system is inhibited by aliphatic and aromatic bivalent anions having a distance within 6.5 Å and 10 Å between the two charges. Anions with one ionic negative and one partial negative charge (mono-methylester of succinate and glutarate) are also accepted but with reduced activity.
The PAH transport system accepts aliphatic and aromatic substrates with two ionic negative or even two partial negative charges (dialdehydes) at a distance higher than 7.5 Å. It also accepts monovalent anions if they have a hydrophobic moiety at least as large as the benzene ring or as the hydrophobic part of valerate.

SINGLE SITE ATP-HYDROLYSIS CATALYSED BY RECONSTITUTED $CF_OF_1$

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$CF_OF_1$ is isolated and reconstituted in asolectinliposomes.The enzyme exists in different states.Only the active state is able to catalyse ATP-hydrolysis/synthesis. ATP binds to the enzyme under single site conditions in a first order reaction,implicating that the enzyme makes a complete turnover under single site,single turnover conditions.The concentrations of free and enzyme bound ATP,ADP and $P_i$ were measured as a function of time.Both, ADP and $P_i$ are released from the enzyme indicating a fast turnover under these conditions.Repetitive additions of ATP(maintaining the single site conditions)show that the enzyme is able to make at least 10 turnovers.These results are in contrast to similar measurements with isolated $MF_1$,where the ADP-release is extremly slow. This difference may reflect the change of catalytic properties by the binding of the $F_O$ part to $F_1$.

INVESTIGATION OF THE PROTONPUMP MECHANISM OF BACTERIORHODOPSIN (bR) BY FTIR-SPECTROSCOPY

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Internal carboxyl groups are involved in the transduction of light energy into protontransfer reactions in bR (1,2).

External carboxyl groups are deprotonated during the transition from cation-free-blue to cations-bound-purple bR and seems to be the binding sites for divalent cations (3). The role of external caboxyl acids for the proton pump mechanism is investigated.

(1) Engelhard, M., Gerwert, K., Hess, B., Kreutz, W. and Siebert, F. (1985) Biochem. 24, 100.
(2) Gerwert, K. and Siebert, F. (1986) EMBO 5, 805.
(3) Gerwert, K., Ganter, U.M., Siebert, F. and Hess, B. (1987), FEBS, in press.

## SINGLE CHANNEL RECORDINGS IN BASOLATERAL MEMBRANES OF RABBIT PROXIMAL TUBULES

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Segments of proximal straight tubules were cannulated and perfused on one side. At the non-cannulated end the lateral membrane could be approached by a patch pipette. In cell attached patches with NaCl Ringer both in pipette and bath, outward single K currents could be resolved after depolarization of the patch by about 20 mV. The open probability $P_o$ of single channel current increased with depolarizing clamp potentials. A similar voltage dependence of $P_o$ was observed for $K^+$ channels in cell excised patches, when the pipette was filled with NaCl and the bath with KCl solution. Decreasing bath $Ca^{++}$ concentration from $10^{-6}$mol/l to less than $10^{-9}$mol/l, did not influence single channel open probability. In excised patches a voltage-dependent channel frequently occurred with a single channel conductance of about 30 pS. This channel is equally permeable for $Na^+$ and $K^+$ and about 3 times less permeable for $Cl^-$. Decreasing bath $Ca^{++}$ concentration from 1 mmol/l to 0.01 mmol/l inactivated this channel. The physiological significance of this channel remains still unclear.

## UDP-GLUCOSE INDUCED DISSIPATION OF THE TRANSMEMBRANE PROTON-GRADIENT IN VESICLES FROM A MICROSOMAL FRACTION OF A HIGHER PLANT CELL

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Membrane vesicles isolated from protoplasts of photoautotrophic suspension cells of Chenopodium rubrum by hypotonic lysis and differential centrifugation produce an ATP-dependent intravesicular acidification. This activity is not separable by gradient centrifugation (sucrose, Percoll) from the golgi marker latent IDP(UDP)'ase. Upon UDP-glucose addition this pH-gradient is slowly dissipated, accompanied by a release of phosphate, but not of glucose or sucrose. Phosphate release is inhibited by DIDS, but not atractyloside. Thus there are certain common characteristics with the transport of sugar nucleotides into animal-derived golgi vesicles; however, these latter systems are not considered to be linked to transmembrane ionic-gradients as is suggested by our experiments.

## DETERMINATION OF RATE CONSTANTS IN CYCLIC REACTION SYSTEMS OF ION TRANSPORT FROM ANALYSIS OF SS I(V) CURVES.

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The eight rate constants of a cyclic 4-state model (with one voltage dependent reaction step) for uniport of ions can be determined from (at least three) different steady state current voltage curves as measured under various internal and external substrate concentrations. This can directly be demonstrated by algebraic means based on ordinary reaction kinetics. Extended application of this method can also provide the entire set of 14 rate constants of a 6-state model for a competitive ion transporter. This is shown by application to patch clamp data from an open, semiselective $K^+/Na^+$ channel (Schroeder et al. 1984, Nature 312:361) where reorientation of the uncharged, empty binding site becomes rate limiting at large voltage displacements.

Lit.: Gradmann, D., Klieber, H.G., Hansen, U.P. 1987, Biophys. J. in press

## SPONGE AGGREGATION FACTOR: IN SITU LOCALIZATION BY FLUORESCENT MONOCLONAL ANTIBODY TECHNIQUES

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The aggregation factor (AF) from sponges mediates a heterophilic interaction of homologous cells. Applying electron microscopical means, we succeeded only very rarely in identifying the 90S AF particle in tissue slices from Geodia cydonium. By means of fluorescent monoclonal antibody (mab) techniques, we have localized the cell binding domain of the AF in situ. Mab 5D2-D11, recognizing the 47-kDa cell binding protein of the AF and mab 7D5, directed against a 92-kDa protein in the AF complex, were chosen. By using mab 5D2-D11, the plasma membranes of cells could be brightly stained. However, mab 7D5 reacted only very weakly with the sponge surfaces. Moreover, it was demonstrated that p47 is involved also in cell-matrix interaction.

## HOLISTIC CHARGE SANDWICH MODEL OF THE ERYTHROCYTE MEMBRANE

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**The molecules building up the erythrocyte membrane are highly mobile in the plane and to some extent (cholesterol) between the layers. So the charge sandwiched bilayer forming an inside and an outside equipotential surface is a first approach to calculate electrostatic characteristica of the membrane.**
**We are showing that the mean mean curvature (MMC) of erythrocyte models is directly related to the sequence of shape changes which can be found in erythrocyte deformation. The free electrostatic energy (FEE) of such a membrane can be calculated from the MMC and the electrostatic magnitudes of the system. The FEE depends on the MMC in a parabolic way. So there is a minimum of FEE for one characteristic MMC which means a preferred erythrocyte shape.**
**With increasing deviation from the biconcave shape there is an increase in variation of local mean curvature. This induces in equipotential surfaces an corresponding change in local charge density. Thereby free chemical energy is produced which has to be taken into account when computing the total free energy.**

## STUDIES ON THE ACTIVE SITE OF THE CHLOROPLAST PHOSPHATE TRANSLOCATOR

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The phosphate translocator of the inner envelope membrane enables the export of fixed carbon from the chloroplasts. Binding of one molecule pyridoxal-5'-phosphate (PLP) per active site results in its complete inactivation. Experiments where both the incorporation of $^3$H-PLP into the protein and the inhibition of transport were measured revealed that 100 % inhibition required the binding of about 0.8 mol PLP/mol of the dimeric translocator protein. This indicates that both subunits share only one common substrate binding site.
4,4'-Diisothiocyano-2,2'-stilbendisulfonic acid (DIDS) was shown to be another potent and specific inhibitor of the phosphate translocator reacting with the same lysine residue as PLP. Both, $^3$H-PLP and $^3$H-$H_2$DIDS can therefore be used for specific labeling of the active site. The translocator protein can be totally digested with BrCN to five 3 - 9 kD peptides. Digestion of the labeled translocator should therefore result in one labeled peptide. The identification of this active site peptide will be shown.

## TEMPERATURE DEPENDENCE OF $Ca^{2+}$-ACTIVATED $K^+$ CURRENTS IN HUMAN RED CELL MEMBRANES

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Conductance and gating of $Ca^{2+}$-activated $K^+$ channels were studied in cell-free membrane patches of human erythrocytes in temperature range 0-50°C. The single-channel conductance decreases monotonically with decreasing temperature from about 65 pS to about 10 pS. Arrhenius plot of this dependence gives an activation energy of about 31 kJ/mol. The calcium dependence of the channel opening probability is much steeper at 0°C then at 20 °C. Half maximal activation is reached between 0.3-0.4 µM $Ca^{2+}$ at 0 °C as compared to 2.5 µM at 20 °C. The presented data from the patch-clamp experiments are compared with previously published data from $K^+$ flux experiments at different temperatures.

## POTENTIAL DEPENDENCE OF "ELECTRICALLY SILENT" ANION EXCHANGE MEDIATED BY THE BAND-3 PROTEIN

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The anion exchange protein (band-3) from mouse red cells has been expressed in the full-grown, prophase-arrested oocytes of Xenopus laevis. The electrically silent chloride efflux mediated by the band-3 protein was measured under voltage-clamp by means of microinjected $^{36}Cl$. Over the voltage range from -10 to -100 mV the efflux increased by a factor of about 1.5. The results are compatible with the interpretation that, under the experimental conditions, the anion binding sites of the substrate-loaded transport protein are primarily inwardly oriented. The voltage dependence of the electrically silent anion exchange can be related to a recruitment of the anion-loaded transport protein by the electrical field; this involves reorientation of an effective charge of 0.1 elementary charge in the electrical field during the transition from the inwardly to the outwardly oriented conformation.

## PUMPING EFFICIENCY AND PHOTOCHEMISTRY OF AGGREGATED AND MONOMERIC BACTERIORHODOPSIN

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Light-induced proton release in purple membrane suspensions (1) and vesicular systems reconstituted with bacteriorhodopsin (BR) has been investigated using the pH-indicator dye pyranine and single-turnover flash spectroscopy. The number of protons detected by pyranine is inversely proportional to the buffering power of the medium and 1.1 protons are released per photocycling BR (pH 7.0, 20°C). Increasing amounts of phosphate buffer or KCl accelerate the detection of the released protons, whereas the reuptake by the purple membrane is slowed down by KCl. At high ionic strengths (250 mM KCl) and moderately high buffer concentrations (1 mM phosphate) the proton release lags slightly behind the formation of the intermediate M-412 and proton reuptake by the purple membrane parallels the slow decay component of M-412 or the decay of O-640. In reconstituted vesicular systems proton release and proton uptake by BR as well as passive proton redistribution across the membrane could be followed after a single flash for monomeric and aggregated BR. Within 20% the number of protons released per photocycling monomeric BR equals the proton release of the BR aggregates.

(1) S. Grzesiek and N.A. Dencher (1986) FEBS Lett. 208, 337-342.

## PHOTOAFFINITY LABELING OF BACTERIAL ATP SYNTHASE IN ENERGY-COUPLED PROTEOLIPOSOMES

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We have coreconstituted monomeric bacteriorhodopsin and ATP synthase from R. rubrum into soybean lecithin liposomes. The photoinactivation of ATPase and ATP synthase activity of these proteoliposomes in the presence of 8-azido[$\alpha^{32}$P]ATP and 8-azido[$\alpha^{32}$P]ADP has been investigated. Both nucleotides showed a preferential and specific labeling of the $\beta$ subunits of the ATP synthase. The photoinactivation of ATPase and ATP synthase activity is more efficient when the coreconstituted liposomes are energized by simultaneous illumination with visible light. The photoinactivation of the non-energized system corresponds directly with the incorporation of the photoaffinity label into the $\beta$ subunits only, whereas the photoinactivation of the energized enzyme is directly correlated to the total amount of the covalently bound photoaffinity label both in the $\alpha$ and the $\beta$ subunits.

## ISOLATION AND CHARACTERIZATION OF PORCINE LIVER MYOSIN AND ACTOMYOSIN

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The isolation and purification of liver myosin and actomyosin is based on cycles of high ionic strength extraction and low ionic strength precipitation, and gel filtration. The purified myosin consists of a $M_r$~200.000 heavy chain and light chains of $M_r$~20.500,~19.000 and~14.500. The Ca-ATPase activity of this protein in 0.5 M KCl is higher than the EDTA-ATPase activity.
The superprecipitation of actomyosin prepared from porcine liver shows no step-like character in the presence of 0.1 M KCl but the rate of the development of superprecipitation is influenced by the addition of phalloidin. Actomyosin reconstituted from F-actin ( prepared from porcine liver ) and myosin filaments in 0.05 M KCl is also influenced in turbidity changes upon addition of this cyclic peptide.

## GRAMICIDIN INDUCED ENHANCEMENT OF TRANSBILAYER REORIENTATION OF LIPIDS IN THE ERYTHROCYTE MEMBRANE.

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**Binding of the channel-forming antibiotic gramicidin to the membrane of human erythrocytes highly enhances rates of reorientation (flip) of lysolecithin and palmitoylcarnitine to the inner membrane layer after their primary incorporation into the outer layer. Despite the high increase of flip rates by gramicidin the orientation of the inner membrane layer phospholipids phosphatidylethanolamine and phosphatidylserine is stable. The flip increase becomes detectable when $10^3$ copies of gramicidin per cell (gramicidin to phospholipid ratio 1:2,000) have been bound. This is a 1,000-fold higher concentration than that required for an increase of channel mediated $K^+$-permeability. The flip increase is markedly dependend on structural details of gramicidin. Formylation of its tryptophan residues abolishes the effect. On top of forming cation-selective channels gramicidin produces aqueous leaks in the erythrocyte membrane permeable to ions (choline, oxalate) and non-electrolytes (mannitol, sucrose) at gramicidin concentrations at which flip is enhanced.**

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**KALIUMTRANSPORT BEI *ESCHERICHIA COLI*: SUBKLONIERUNG, PHYSIKALISCHE KARTIERUNG UND ÜBEREXPRESSION DES *trkA*-GENS**

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TrkA ist die Hauptkomponente des konstitutiven Kaliumtransportsystems von *Escherichia coli* und vermutlich an der Kaliumbindung beteiligt. Es wurden neue Plasmide konstruiert, die nur noch das *trkA*-Gen oder Teile von diesem trugen. Die Transkriptions- und Translationsrichtung wurden bestimmt und das N-terminale Ende des Proteins auf Protein- und DNA-Ebene sequenziert. Das Membranprotein TrkA liegt in Wildtypzellen in äußerst geringer Menge vor. Zur Erhöhung der Ausbeute an diesem Protein wurde das *trkA*-Gen unter die Kontrolle des Lambda-$p_L$-Promotors auf dem Expressionsvektor pPLa2311 gebracht. Die zusätzliche Gegenwart einer *tem*-Promotorregion vor dem Strukturgen erhöhte die Effizienz der Überproduktion soweit, daß TrkA einen hohen Anteil am Gesamtmembranprotein erreichte und zum Hauptzellprotein wurde. Diese Überproduktion wirkte letal und führte zur Zellyse. Durch Entfernen des *tem*-Promotors und Verkürzen des Abstandes zwischen $p_L$ und *trkA* ließ sich eine konstitutive Überexpression erreichen.

**SINGLE CHANNEL PROPERTIES OF A PURIFIED AND RECONSTITUTED NEURONAL ACETYLCHOLINE RECEPTOR**

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An α -toxin binding protein from insect brain, which is probably an homooligomeric protein consisting of four or five identical subunits (Mw = 65 kD), has been purified and incorporated into planar lipid bilayers. Addition of cholinergic agonists resulted in an activation of ionic channels, which have been investigated in some detail. Although the neuronal acetylcholine receptor appeared to be quite similar in its channel parameters compared to other (peripheral) acetylcholine receptors, we found some remarcable differences. This channel is obviously activated by one agonist molecule and is very insensitive to hexamethonium. Additionally, in the preparation we investigated, we found channel conductance sublevels, as well as different Ach-activated channel types.

**RECONSTITUTION OF THE cGMP-ACTIVATED CHANNEL FROM PHOTORECEPTOR MEMBRANES INTO PLANAR LIPID BILAYERS**

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The cGMP-activated channel has been purified from membranes of bovine rod outer segments and incorporated into planar lipid bilayers. We examined the cGMP and the voltage dependence by recording single-channel activity of the purified protein. The channel is only weakly selective for monovalent cations and also allows the passage of calcium ions. The unit conductance of the channel ( = 26 pS in 150 mM NaCl) is independent of the cGMP concentration but is *not ohmic*. At medium cGMP concentrations a variety of conductance sublevels (8-10 pS) were observed. The channel is cooperatively activated by cGMP with a Hill coefficient of n=2.3 and a $K_m$=31 uM. The reconstituted channel has properties similar to the channel *in situ*.

IV-CURVE ANALYSIS OF THE $K^+$-CHANNEL IN NITELLA

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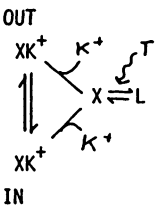
The $K^+$-transporter in the alga Nitella displays a sigmoid IV-curve, if voltage-dependent deactivation processes do not interfere. Curve-fitting of the IV-curves measured at outside $K^+$-concentration from 0.1 to 100 mMol by means of a 3-state model enables the determination of charges, of stoichiometry and of the relative rate constants, which indicate that the loaded complex is very unstable. IV-curve measurements at different temperatures lead to the study of activation and deactivation of transport by control signals from the chloroplasts which influence the exchange of the active cycle with a lazy state L. This model predicts an inductive component which is found in the electrical impedance.




J. Fisahn, U.P. Hansen, and U.P. Hansen, J. Fisahn (1987). J. Membrane Biol. (in press).

THE MAJOR PEROXISOMAL MEMBRANE POLYPEPTIDES (PMPs)

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Peroxisomes of normal rat liver contain 6 major integral PMPs (1). During peroxisome proliferation induced by hypolipidemic drugs or thyroid hormones the concentration of PMP 69 (69 kDa) is increased similiar to the peroxisomal fatty acid ß-oxidation (2). The physiological function of the PMPs is unknown. Experimental evidence indicates to the existence of a peroxisomal porin. The most likely candidate for it is PMP 22 (3). The biosynthesis of PMP 69 and PMP 22 was found to occur on membrane free polysomes. Therefore, these PMPs were released posttranslationally as water-soluble precursors into the cytoplasm from where they are taken up into preexisting peroxisomal membranes. Targeting to them is not mediated by cleavable signal sequences (4). The ER seems not to be involved in the biogenesis of these PMPs.

1 Hartl et al. Arch.Biochem.Biophys. 237, 124 (1985)
2 Hartl et al. (1987) Arch.Biochem.Biophys. in press
3 Van Veldhoven et al. (1987) J.Biol.Chem. in press
4 Just et al. in: Peroxisomes in Biology and Medicine (Fahimi, Sies eds.) Springer Verlag (1987).

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GROWTH FACTOR INDUCED CA-FLUXES IN 3T3, 3T6, AND SV40-3T3 MOUSE CELLS

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We have investigated the kinetics of Ca-release from intracellular stores following treatment by different growth factors of normal and transformed mouse cells, using $^{45}Ca$ flux measurements. Comparison of the kinetics of Ca-efflux after stimulation of 3T3 cells by fetal calf serum, bombesin, or vasopressin with equilibrium effluxes of unstimulated cells shows that the slowly exchanging Ca-compartment, attributed to the mitochondrial compartment (1), is unaffected by stimulation. Ca-effluxes of SV40-3T3 cells are not affected by any of the growth factors tested (bombesin, vasopressin, fibroblast growth factor, epidermal growth factor (EGF), or insulin (I) at concentrations stimulative for 3T3 cells). Similar to SV40-3T3, 3T6 cells do not show increased Ca-effluxes after treatment with bombesin or vasopressin, not mitogenic for the latter. However, both 3T6 and 3T3 cells show slow Ca-effluxes after stimulation with EGF or I, which are mitogenic for both cell lines.

Reference: (1) Hartmann, T., K. Seuwen, G. Adam: Exp. Cell Res. 163, 279-286 (1986).

REINIGUNG UND CHARAKTERISIERUNG MEMBRANGEBUNDENER GLYKOSYLTRANSFERASEN AUS SACCHAROMYCES CEREVISIAE

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Die O-Glykosylierung von Proteinen in der Hefe beginnt am ER [1] und verläuft nach den Gleichungen

(1) GDP-Man + Dol-P → Dol-P-Man + GDP

(2) Dol-P-Man + Prot.-Ser → Prot.-Ser-Man + Dol-P

Das Enzym der Reaktion (1) wurde bis zur Homogenität gereinigt. Mit Hilfe eines polyklonalen Antikörpers wird derzeit versucht das Gen zu klonieren. Das Enzym der Reaktion (2) wurde bisher über 300fach angereichert. Durch Ausschaltung der beiden entsprechenden Gene soll versucht werden, die Rolle der Protein-O-Glykosylierung in Hefen zu klären. Eine mögliche Funktion der Dolicholaktivierung von Zuckern könnte in deren Translokation durch die ER Membran zu sehen sein. Durch Einbau des gereinigten Enzyms (1) in Liposomen konnte in der Tat eine transmembrane Verlagerung von Mannosylresten nachgewiesen werden [2].

[1] A. Haselbeck, W. Tanner (1983) FEBS Letters 158, 335

[2] A. Haselbeck, W. Tanner (1982) Proc. Natl. Acad. Sci. USA 79, 1520

PEROXIDATIVE MEMBRANE DAMAGE IN ERYTHROCYTES TREATED WITH IODOACETATE + VANADATE + FERRICYANIDE

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**Oxidation of membrane proteins and lipid peroxidation are known to cause degradation of membrane barrier properties. Cells possess elaborate systems to protect themselves against oxidative stress. The anti-oxidative capacity of human RBC has now been shown to be overcome by incubating iodoacetate (0.2 mM)-pretreated RBC with permeant vanadate (0.5 mM) and impermeant ferricyanide (5 mM). The cells develop aqueous leaks and undergo colloid-osmotic lysis. Leak formation can be stimulated dramatically by nucleoside metabolism via the hexose monophosphate shunt, even while glycolytic NADH production is inhibited to at least 95%. Membrane damage depends on the availability of molecular oxygen, goes along with peroxidation of membrane phospholipids and can be prevented by anti-oxidants. It is proposed that oxy-radical formation is involved originating from reduction of $O_2$ by NADPH, while vanadate and ferricyanide are required to amplify the peroxidative reactions to an extent exceeding the cellular anti-oxidative capacity.**

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PM II ACTIVITY IN $D_2$-RECEPTOR-RICH MEMBRANE VESICLES

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The eucaryotic protein methylsae II is especially enriched in brain and neuronal tissue. Here investigations of the transmethylation reaction in membrane vesicles derived from bovine striatal tissue are described. We characterized the enzyme (pH-dependence, time courses of basalactivity, dependence of divalent cations, subcellular location) and binding kinetics of the agonist NPA.Stimulation via the dopaminergic pathway yielded a decreased acticity when compared to the time course of basal activity. We will discuss the effect as a potentially modulatory function of this posttranslational protein modification.

FLUORESCENCE STUDIES ON MEMBRANES FROM HUMAN SKIN FIBROBLASTS DEFICIENT IN PLASMALOGENS.

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Human skin fibroblasts from patients affected with the cerebro-hepato-renal (Zellweger) syndrome have a significantly reduced content of plasmalogens (1-O-alkenyl--2-acyl-sn-glycero-3-phospholipids). The effect of plasmalogen deficiency on the fluidity of the plasma membrane was monitored by fluorescence anisotropy,using trimethylammonium diphenylhexatriene as a probe. Measurements were carried out with cells grown on glass cover slips and cell suspensions. Compared with the control, cells deficient in plasmalogens showed increased fluidity.

Transfer of phospholipids from vesicles to cells was measured using fluorescent diphenylhexatrienylpropionyl phospholipids. Plasmalogens were transferred more rapidly into fibroblast membranes than were the diacyl analogs (PE and PC). Cells deficient in plasmalogens were found to take up plasmalogen from sonicated phospholipid dispersion at a much higher rate as compared to control cells.

FUNCTIONAL RECONSTITUTION OF THE ADENYLATE CYCLASE SYSTEM: MECHANISTIC ASPECTS OF SIGNAL TRANSMISSION.

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The ß-adrenoceptor-responsive adenylate cyclase system consists of ß-adrenergic receptor (ßR), stimulatory G-protein ($G_S$) and the catalytic moiety of the enzyme. $G_S$ is a heterotrimer composed of three subunits ($\alpha$,ß, y) where only the $\alpha$-subunit binds GTP. We examined the role of ß,y-subunits, derived from different G-proteins, in hormone-dependent $G_S$-activation. The purified ß,y-subunits from bovine brain and bovine rod outer segments (transducin ß,y) were added in excess to a reconstituted ßR-$G_S$ system. Addition of transducin ß,y had no effect on ßR-$G_S$ coupling efficiency as measured by hormone-dependent GTPase activity. On the other hand, addition of 10-20 fold excess of bovine brain ß, y-subunits over $G_S$ actually stimulated the GTPase activity. To the contrary, addition of large amounts of ß,y-subunits inhibited ßR-$G_S$ coupling. The mechanistic implications of the ß,y-effects for the regulation of hormone-stimulated adenylate cyclase activity are discussed.

STIMULATION OF $Ca^{++}$ CHANNELS IN CARDIAC AND ADRENAL CORTICAL CELLS

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The whole cell Ca current ($I_{Ca}$) of cardiac cells (collagenase-isolated from guinea pig ventricles) and of murine adrenocortical cells (Y1) was measured using single suction pipettes.

Superfusion of the cardiac cells with isoprenaline caused an about 3 fold increase of $I_{Ca}$. This effect was mimicked by intracellular application of cAMP or the catalytic subunit of the cAMP dependent protein kinase, suggesting a cAMP dependent phosphorylation of a Ca channel's related protein.

An increment of $I_{Ca}$ (2-3 fold) was also observed in the adrenocortical Y1 cells when stimulated with angiotensin II. However this effect could not be explained by a cAMP dependent phosphorylation of Ca channels since extracellularly applied forskolin, as well as intracellular applied cAMP did not alter $I_{Ca}$. The angiotensin II effect was abolished when cells were pretreated with pertussis toxin, indicating that $N_i$ or $N_o$ might be involved.

PURIFICATION AND RECONSTITUTION OF DESENSITIZED ß-RECEPTOR FROM TURKEY ERYTHROCYTES.

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Heterologous desensitization of ß-receptor stimulated adenylate cyclase activity is demonstratable in membranes prepared from turkey erythrocytes pretreated with isoprenaline. Hormone-dependent stimulation of adenylate cyclase and GTPase activity were markedly reduced. It has been demonstrated that desensitization in this system can be correlated with the incorporation of phosphate into the receptor molecules. In the present study we have purified receptors from desensitized and untreated erythrocytes using alprenolol sepharose affinity chromatography and reconstituted them into phospholipid vesicles with $G_s$ purified from turkey erythrocyte membranes. Functional R-$G_s$ coupling as assessed by hormone-dependent (a) $G_s$-activation by GTPγS, (b) GTPase activity, demonstrated the desensitized-phosphorylated receptor to be as effective in coupling to $G_s$ as the control receptor. We conclude that receptor phosphorylation alone is insufficient to explain the functional changes in the adenylate cyclase system in the course of desensitization.

FUNKTIONELLE DOMÄNE DES NIKOTINISCHEN ACETYLCHOLINREZEPTORS

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Der nikotinischen Acetylcholinrezeptor (AChR) ist ein heteropentameres Glykoprotein vom Molekulargewicht 290.000 (1). Signalerkennungsdomänen (Acetylcholin-Bindungsstellen) und Effektor-Teil (Ionenkanal) sind integrale Bestandteile dieses Proteins. Wir markierten letzteren kovalent mit einem radioaktiven Photoaffinitätsreagenz ($^3$H-TPMP$^+$) und lokalisierten ihn durch Mikrosequenzierung der radioaktiven Peptide in den Primärstrukturen der Rezeptoreinheiten. Aus den gefundenen Sequenzen konstruierten wir ein Strukturmodell des Ionenkanals (2), das uns plausibler erscheint als die von anderen vorgeschlagenen (3).

1. F. Hucho, Eur. J. Biochem. 158, 211-226, (review article), 1986.
2. F. Hucho, W. Oberthür & F. Lottspeich, FEBS Lett. 205, 137-142, 1986.
3. J. Finer-Moore & R.M. Stroud, Proc. Natl. Acad. Sci. USA 81, 155-159, 1984.

MEMBRANE FLUIDITY OF LYMPHOCYTES IN DUCHENNE MUSCULAR DYSTROPHY (DMD)

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In DMD lymphocyte membranes (n=10) diphenylhexatriene fluorescence anisotropy (DPH-FA) was decreased (0.212±.028) versus controls (n=60, 0.231±.012, p<0.001). Linear regression analysis of creatine kinase activity in sera and DPH-FA in lymphocytes showed a negative correlation (r=-0.93, p<0.001). When incubated in DMD sera, DPH-FA of DMD lymphocytes (n=4) decreased from 0.211±.012 to 0.180 ±.028, and DPH-FA of control lymphocytes (n=5) decreased from 0.228±.017 to 0.179±.025. When incubated in control sera, DPH-FA of DMD lymphocytes increased to 0.224±.012, and DPH-FA of control lymphocytes decreased to 0.208 ±.013. The difference of DPH-FA after incubation in DMD versus control sera was significant (p<0.025 for DMD, p<0.001 for controls). These results suggest the presence of a toxic serum factor in DMD, which attacks lymphocyte membranes and possibly muscle membranes at the same time.

INTERACTION OF ADENINNUCLEOTIDES WITH CYTOCHROME C OXIDASE

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Photolabelling of isolated beef heart cytochrome c oxidase with 8-azido-ATP increases the $K_m$ for cytochrome c, as measured after reconstitution in liposomes. This increase is prevented if ATP but not ADP is present during photolabelling. Similar results are obtained by photolabelling of the enzyme after reconstitution in liposomes. Free ATP, added to proteoliposomes, increases the $K_m$ for cytochrome c correspondingly. Photolabelling of the isolated enzyme with 8-azido-$\gamma$-$^{32}$P ATP at very low (40 nM) and up to millimolar concentrations results in a complex labelling pattern as visualized by autoradiography. At all concentrations significant radioactivity was found in subunits I, II, III, IV, V, VI and VII. This contrasts results of Montecucco et al. (Biochem. J. 234 (1986) 241-243). After labelling of the reconstituted enzyme, radioactivity is found at least in subunits II, IV and in a small subunit.

SHORT CIRCUIT OF THE PROTON PUMP IN PHOTOSYSTEM II BY DCCD

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N,N'-Dicyclohexylcarbodiimide (DCCD) covalently reacts with carboxy groups. In thylakoids it interacts with the ATP-synthase ($CF_0$ and $CF_1$) and inhibits linear electron transport at the level of plastoquinone reduction and oxidation. We found another effect, short-circuiting of the proton pump associated with photosystem II and water oxidation, which was evident from the following observations: (1) diminished proton uptake from the medium during quinone reduction, (2) diminished proton release into the lumen by water oxidation, (3) unaffected rate of oxygen evolution and initial extent of the electrochromic absorption changes, (4) rapid decay of the transmembrane electric potential difference (half decay time about 1 ms instead of about 100 ms in controls) and (5) shorter lag time of P700 - reduction; this lag time is ascribed to the protonation of reduced bound plastoquinone. In conclusions, DCCD seemed to induce a direct transfer of protons produced during water oxidation across the thylakoid membrane to the bound quinones at the acceptor side of photosystem II.

THE MECHANISM OF PROTON TRANSPORTED COUPLED ATP-SYNTHESIS IN CHLOROPLASTS

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The chloroplast ATP-synthetase can exist in - at least - four different states: an inactive oxidized state, an inactive reduced state and both forms can be brought into an active state by membrane energization. Using an artificially generated $\Delta pH$ and $\Delta\psi$ with a rapid mixing quenched flow system the rate of ATP-synthesis and ATP-hydrolysis was measured as a function of $\Delta pH$ and $\Delta\psi$ and the substrate concentrations. It is shown that for the different states of the enzyme different functional dependencies on $\Delta pH$ are found. The catalytic reaction can be measured only when the enzyme is in the active reduced state. The rate as a function of substrate concentrations differs under these conditions markedly from those measured earlier. The maximal enzyme turnover is 700 $s^{-1}$.

## PROTOLYTIC REACTIONS IN PHOTOSYNTHESIS

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Proton pumping and proton translocation coupled with ATP-synthesis are important elements of biological energy transduction. Thylakoids of green plants are particularly well suited for physicochemical studies of protolytic reactions since 1.) proton pumps can be pulse-stimulated by light flashes and 2.) backflux of protons, e.g. via the ATPsynthase, $CF_0CF_1$, or its channel portion, $CF_0$, can be "completely tracked" by spectrophotometric techniques for measuring pH-transients in both aqueous phases and transients of the transmembrane voltage. We treated the following quantitatively: Is the lateral diffusion of protons along a membrane/water interface enhanced or delayed? Are there special pathways for protons between pumps and ATPsynthases (localized coupling?)? Is the very high turnover number of $CF_0$, $6\times10^5$ $H^+/s$, compatible with a hydrogen bonded chain? Which factors are rate limiting coupled proton translocation by the ATPsynthase?

## REGULATORY PROPERTIES OF THE CYTOCHROME C OXIDASE COMPLEX

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The activity of cytochrome c oxidase is strongly influenced by anions. ATP was found to increase the $K_m$ for cytochrome c specifically by interaction with the cytosolic domain. An unspecific and biphasic effect of anions on the activity is obtained by interaction with the two matrix-oriented domains of the y-shaped enzyme complex. The effect is proportional to the charge, but independent on the type of anions. The change of activity is paralleled by a change of the visible spectrum, indicating an alteration of the ligand sphere of heme a and/or $a_3$. From hydropathic plots of the amino acid sequences of the beef heart subunits an anisotropic charge distribution on both sides of the inner mitochondrial membrane is concluded (outside negative, inside positive). A model of cytochrome c oxidase is presented suggesting movement of the two matrix-oriented domains against each other in the mechanism of proton translocation across the membrane, and in regulation of activity.

## DOES THE cGMP-DEPENDENT PHOTORECEPTOR CHANNEL EXIST IN TWO DIFFERENT CONFORMATIONS ?

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The photoreceptor channel exists in the plasma and disk membrane of rod outer segments. We studied the properties of this channel by spectrophotometrically following the cGMP-stimulated $Ca^{2+}$ efflux from disks. The $Ca^{2+}$ efflux exhibits two kinetic phases of different cGMP-sensitivity and pharmacological behaviour. A fast kinetic phase is activated with a Michaelis constant $K_m$=23 uM and a slower component with $K_m$=170 uM. The activation of both kinetic phases is cooperative (Hill coefficient $n$=2.0-3.5). The slower phase is blocked by the drug *l*-cis-diltiazem, whereas the fast component is not affected. We interpret the two kinetic components to indicate that the cGMP-dependent channel exists in two different conformations.

## PATCH CLAMP STUDIES OF ION CHANNELS RECONSTITUTED INTO LARGE LIPOSOMES FORMED BY DEHYDRATION-REHYDRATION OF LIPID FILMS

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The preparation of giant liposomes suitable for patch clamp experiments by the "hydration technique" is reported. Essentially, the method consists of carefully controlled dehydration of a suspension of small vesicles followed by rehydration of the residue resulting in formation of large liposomes. Here, we use the hydration technique to characterise the solubilised and reconstituted $K^+$-channel of sarcoplasmic reticulum (SR) from rabbit skeletal muscle. Single channel properties determined after the dehydration-rehydration cycle are in good agreement with the ones observed for SR $K^+$-channels reconstituted in planar lipid bilayers or freeze-thawed liposomes. In reconstituted preparations of purified synaptosomal membranes from rat brain, a voltage dependent cation channel is characterized. This observation and the measurement of single ion channels reconstituted from plant membranes indicate, that the hydration technique is a generally applicable tool to study ion channels from a variety of membrane preparations.

## ELECTROGENIC $Ca^{2+}$-UPTAKE MECHANISM IN RAT PANCREATIC ENDOPLASMIC RETICULUM

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$(Ca^{2+}+K^+)$-$Mg^{2+}$ATPase promoted $Ca^{2+}$-uptake into exocrine pancreatic endoplasmic reticulum (ER) was investigated by studying ATPase activity and phosphorylation of its intermediate using $AT^{32}P$. The data showed an anion-dependent stimulation sequence of $(Ca^{2+}+K^+)$-$Mg^{2+}$ATPase activity and $^{32}P$-incorporation into a 100 kDa-phosphoprotein in intact vesicles: $Br^- > Cl^- > NO_3^- > SCN^- >$ cyclamate$^- > SO_4^{2-} > SO_3^{2-}$. In Triton X 100 (0.015%) treated vesicles both increased to the level obtained with $Cl^-$. An electrical membrane potential difference generated by preformed $K^+$-gradients in the presence of $K^+$-ionophore valinomycin ($10^{-6}$M) caused either inhibition ($K_o^+ > K_i^+$) or stimulation ($K_o^+ < K_i^+$) of $(Ca^{2+}+K^+)$-$Mg^{2+}$ATPase activity. We conclude that $Ca^{2+}$-uptake into ER is electrogenic and that permeable anions are necessary for charge compensation of $(Ca^{2+}+K^+)$-$Mg^{2+}$ATPase promoted $Ca^{2+}$-transport into exocrine pancreatic ER.

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## PHLORIZIN AFFINITY COLUMNS AND PURIFICATION OF THE SODIUM-GLUCOSE COTRANSPORTER

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Phlorizin is a powerful reversible inhibitor of the sodium-glucose cotransport system present in the brush border membranes of mammalian kidney and small intestine. In an attempt to purify the sodium-glucose cotransporter, phlorizin was derivatized to 3-aminophlorizin and subsequently coupled to Affi-Gel 15 (column 1) or Sepharose 4B (column 2). Pig kidney brush border membranes were solubilized with Triton X-100 and the sample applied to column 1. Most of the solubilized membrane protein was washed from the column with buffer whereas several proteins were specifically eluted with D-glucose but not with D-mannose. The latter proteins were applied to a Sephadex G-25 gel filtration column, to remove D-glucose, and then to column 2. SDS-PAGE demonstrated an almost pure 53,000 Da protein obtained by D-glucose elution of the second column. This protein does not bind to the unsubstituted resins. Reconstitution experiments are in progress to determine, whether this protein is the sodium-glucose cotransporter or a component thereof.

## MITOCHONDRIAL PORIN FROM NEUROSPORA CRASSA: STRUCTURE AND IMPORT INTO MITOCHONDRIA

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Porin, an integral outer membrane protein of mitochondria forms channels, allowing the diffusion of small molecules.
Full-length cDNA clones were obtained from a Neurospora library by expression cloning in pEX vectors (1). Nucleotide sequencing revealed that porin is a polar protein, consisting mainly of sided β-sheets, except an α-helical region at the extreme amino-terminus which is suggested to be the non-cleavable import signal. Import into mitochondria depends on ATP and a proteinaceous receptor on the mitochondrial surface.

(1) Stanley, K.K. and Luzio, J.P. (1984) EMBO J., 3, 1429-1434

## GATING KINETICS OF A VOLTAGE DEPENDENT $K^+$ CHANNEL IN ACETABULARIA MEDITERRANEA

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Data from a patch clamp study of a $K^+$ channel in the plasmalemma of Acetabularia mediterranea were used for an analysis of electric channel-gating.

The steady-state lifetime distributions of open and closed states at various holding potentials indicate a minimal number of two conducting and two nonconducting states and the voltage dependence of their mean lifetimes.

Averaging of the single channel currents after voltage steps under voltage clamp conditions reveals an exponential decay of mean channel current with at least two time constants and amplitudes of the gating reaction clearly visible.

The results of both approaches are merged into a reaction kinetic model for channel gating.

## TRANSPORTSYSTEME IN MEMBRANEN CHOLINERGER SYNAPSEN

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An Synaptosomen und synaptosomalen Membran-Vesikeln aus dem cholinergen Nervengewebe von Insekten sind der hochaffine Cholintransport und die $Ca^{2+}$-abhängige Freisetzung von Acetylcholin untersucht worden. Dabei hat sich gezeigt, daß die hochaffinen Cholin-Carrier durch Ionengradienten und das Membranpotential energetisiert werden; der Transport-Prozeß kann durch extrazelluläres ATP sowie über die Proteinkinasen A und C reguliert werden. Die durch depolarisierende Bedingungen ausgelöste Freisetzung von Acetylcholin wird via präsynaptischer muscarinischer Autoreceptoren inhibiert. Diese synaptosomalen muscarinischen Rezeptoren wurden in Bindungsstudien als M2-Subtypen identifiziert. Über Heteroreceptoren und via Proteinkinase C konnte eine verstärkte Acetylcholin-Freisetzung aus isolierten Nervenendigungen induziert werden.

## GENERAL OCCURRENCE OF A POOL OF THE Mr 32000 QB BINDING POLYPEPTIDE - D-1 PROTEIN - IN PHOTOSYNTHETIC MEMBRANES?

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In thylakoids of green algae and higher plants the existence of a relatively large pool of the Mr 32000 QB binding polypeptide has been demonstrated. Following pulse labelling, the loss of radioactivity from this protein shows a lag phase of about four hours in Chlamydomonas (Wettern, Plant Science 43, 173-177 (1986)).
Such a lag phase could not be observed, however, in the light-dependent loss of radioactivity of the QB binding protein in thylakoids of the cyanobacterium Synechococcus spec. PCC 6301 (Anacystis nidulans). In the case of this organism the loss of radioactivity begins right after the end of the labelling period. From this observation it is tentatively concluded, that in contrast to the situation with green algae and higher plants, in the prokaryotic organism there is no "transient pool" of non-integrated Mr 32000 D-1 protein.

## IDENTIFICATION OF COMPONENTS OF THE RENAL $Na^+$-D-GLUCOSE COTRANSPORTER

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The covalently binding D-glucose analog 10-N-(bromoacetyl)amino-1-decyl-ß-D-glucopyranoside (BADG) was synthesized. BADG is a reversible and competitive inhibitor of the $Na^+$-D-glucose cotransporter. After long incubation also a D-glucose protectable, irreversible inhibition was demonstrated. In pig kidney brush-border membranes with BADG polypeptides with molecular weights and isoelectric points of 82 000, pH 5.6; 75 000, pH 5.4, pH 6.9; 64 000, pH 5.2 and 47 000, pH 5.4 were labeled. Since labeling of the 82 000 and 75 000 $M_r$-polypeptides was protected by D-glucose these polypeptides are thought to be components of the $Na^+$-D-glucosecotransporter which contain D-glucose binding sites. Two monoclonal antibodies against the 82 000 $M_r$-polypeptide crossreact with the 64 000 and 47 000 $M_r$-polypeptides in pig. In fresh rat membranes crossreaction was only observed with a 47 000 $M_r$-polypeptide. Thus the 64 000 $M_r$-polypeptide is supposed to be a proteolytic splitting product whereas the 47 000 $M_r$-polypeptide may be subunit of the $Na^+$-D-glucose cotransporter.

## CATION CHANNELS OF NON-ENZYMATICALLY ISOLATED HELIANTHUS TUBEROSUS VACUOLES.

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Helianthus was sliced in 0.8 M glycine-betaine solution pH 7.8. After filtering the vacuoles were liberated by this procedure, then incubated in a solution with 400 mM KCl, 1 mM $CaCl_2$ and 25 mM Hepes/NaOH pH 7.5 on polylysine coated coverglass. Experiments were performed in petri dishes containing 3 ml solution after transference of the adhering vacuoles on the coverglass. The pipette was filled with 100 mM KCl and 1 mM $CaCl_2$. In 11 experiments with excised patches we measured a conductance between 100 ps and 160 pS and a reversal potential ($E_r$) between 9 and 20 mV. 3 mM TEA reduced the conductance in 2 experiments from 143/159 pS to 104/66 pS. In the same experiments the reversal potential decreased from 18/13 mV to 9/8 mV. In two other experiments a smaller channel of 87/66 pS conductance could be found in addition to the other. In most of the cases the I/V-curves show a rectification. As an additional finding, there seems to be a voltage-dependence of open probability and of channel lifetime. All facts found so far indicate, that we found potassium channels in the tonoplast of Helianthus with a comparable conductivity and a similar inward-rectification (current flows in the vacuole) as found by Kolb et al. (87) in vacuoles of barley.

Lit.: H.-A. Kolb, K.Köhler, E. Martinoia, J.M.B. (87) in press.

DETERMINATION OF INNER VOLUME OF LIPID VESICLES FROM DYNAMIC LIGHT SCATTERING DATA

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Size distributions of phospholipid vesicles (prepared by dialysis of mixed octyl-glucoside/egg lecithin micelles) are determined from dynamic light scattering measurements. Mean vesicle diameters of about 200 nm and widths around 50 nm were characteristical for these distributions. The knowledge of size distribution allows to calculate the volume enclosed by the spherical, unilamellar vesicles. The results are in very good agreement with those obtained from absorbance and fluorescence measurements of trapped carboxyfluorescein. Thus, for the determination of size distribution and inner volume of vesicles the dynamic light scattering technique offers the advantages to be faster than most of the other methods, to be non-invasive, and to be able to work also with dispersions of low lipid content (≤ 0.1 mM of monomeric lipid).

**TRANSPORT OF ß-LACTAM ANTIBIOTICS BY INTESTINAL BRUSH BORDER MEMBRANES**

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**In order to investigate the molecular mechanisms of intestinal absorption of ß-lactam-antibiotics, kinetic and photoaffinity labeling studies with small intestinal brush border membrane vesicles were performed. The uptake of [$^3$H]benzylpenicillin was $Na^+$-independent and cis-inhibited by ß-lactam antibiotics and dipeptides, whereas amino acids and glucose had no effect. Photoaffinity labeling with [$^3$H]labeled benzylpenicillin and N-(4-azidobenzoyl)-derivatives of glycyl-L-proline and cephalexin resulted in the labeling of several membrane polypeptides with a predominant labeling of a 127 kDa polypeptide. The labeling of this polypeptide was decreased by ß-lactam antibiotics and dipeptides, whereas amino acids, glucose or bile salts had no effect. This suggests that a 127 kDa polypeptide is a component of the small intestinal transport system shared by ß-lactam antibiotics and dipeptides.**

POTASSIUM TRANSPORT IN *ESCHERICHIA COLI*: IDENTIFICATION OF THE KDP-ATPase ACTIVITY.

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The Kdp system of *Escherichia coli* is a high-affinity $K^+$- transport system ($K_m$ = 2 µM). The enzyme complex, consisting of the three subunits KdpA (59,189 Da), KdpB (72.112 Da) and KdpC (20.267 Da) is located in the cytoplasmic membrane (1). For reproducible measurements of the Kdp associated $K^+$ stimulated ATPase activity in inside out vesicles, the mutant strain TKR 1000 (*kdpA42*, Δ*unc*) was constructed. The measurements revealed a striking discrepancy between the $K^+$ transport capacity *in vivo* and the low $K^+$-stimulated ATPase activity in inside-out vesicles. This low enzymatic activity could be stimulated 6-to 7-fold by addition of a protein factor obtained by fractionated $(NH_4)_2SO_4$ precipitation of cell cytoplasm. Mutant analysis and immunoblotting with Kdp specific antisera revealed that the activator protein is not identical with one of the three Kdp subunits. Up to now it is unclear whether the phenomenon reflects an unspecific stabilization of the enzyme complex or whether a specific regulatory mechanism is involved.

(1) Laimins, L., Rhoads, D. B., Altendorf, K. and Epstein, W. (1978) Proc. Natl. Acad. Sci. USA 75, 3216-3219

THE CALCIUM ATPASE OF PLASMA MEMBRANES STRUCTURAL AND FUNCTIONAL PROPERTIES

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Three approaches will be described to characterize some structural principles and related functional properties of the plasma membrane $Ca^{2+}$-ATPase
1) Circular dichroism and fluorescence spectroscopy provided evidence for conformational differences between the $Ca^{2+}$ high affinity state (=$E_1$) and the $Ca^{2+}$ low affinity state (=$E_2$) of the enzyme.
2) Controlled proteolysis of the purified $Ca^{2+}$-ATPase ($M_r$=138kDa) using trypsin, chymotrypsin or the $Ca^{2+}$-dependent protease calpain permitted the identification of a number of peptide fragments (between 12 and 124 kDa) of different functional properties.
3) Calmodulin-derived fragments and chemically modified calmodulin were used to identify essential parts (e.g. C-terminal half of calmodulin) and amino acid residues (e.g. ARG, MET) essential for competent interaction of calmodulin with the $Ca^{2+}$-ATPase as its target.

RECONSTITUTION AND FUNCTIONAL CHARACTERIZATION OF MITOCHONDRIAL CARRIER PROTEINS

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**The inner membrane of mitochondria contains a variety of carrier systems for nucleotides, phosphate and substrate anions. In the course of the years many of these translocators have been solubilized, purified, and reconstituted into artificial membranes. Reconstituted systems proved to be well suited tools for the functional characterization of these carriers without any interference which may be caused by other enzymes and translocators. Methodical aspects of the reconstitution procedures and results of the functional analysis of reconstituted carriers shall be discussed mainly with respect to the ADP/ATP carrier, the aspartate/glutamate carrier, the phosphate carrier and the ketoglutarate carrier.**

*INTERACTION OF POLYMYXIN B NONAPEPTIDE WITH BACTERIAL AND MODEL MEMBRANES*

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*The adjuvant antimicrobial compound polymyxin B nonapeptide (PMBN) sensitizes gram-negative bacteria to a wide range of hydrophobic antibiotics by increasing outer membrane permeability. PMBN binds to the anionic sites of the cell envelope. If Pseudomonas aeruginosa strains are grown under magnesium depletion, the protein pattern, LPS, and lipid composition of the outer membrane changes, and the sensitization by PMBN is lost. - The interaction of PMBN with anionic phospholipids was studied by calorimetry, CD, ESR, NMR, and fluorescence spectrometry, electron microscopy, fusion and leakage assays. PMBN caused the interdigitation of phosphatidylglycerol bilayers suggesting that the penetration of hydrophobic side chains from a peptide bound electrostatically on the surface is sufficient to induce this phenomenon. Stopped-flow experiments revealed that in the presence of PMBN small vesicles fused with a frequency of 100 $s^{-1}$, large vesicles with that of 10 $s^{-1}$.*

## ACTIVATION OF THE PLASMA MEMBRANE $H^+$-ATPase IN YEAST CELLS BY $K^+$ AND $NA^+$

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Several effectors modulate the plasma membrane $H^+$-ATPase activity in yeast cells such as ATP, ADP, $Mg^{++}$, $Ca^{++}$, pH, glucose and phosphorylation of the enzyme (see A. Goffeau 1986). The role of monovalent cations as effectors in whole cells was not clear. We have found that the $H^+$-ATPase is activated by $K^+$, for which an intrinsic efficient transport system (channel) exists, and by $Na^+$, if an ion channel is generated by external electric field pulses. The oscillatory coupling between the membrane potential and glycolysis during activation indicates a complex non-linear dynamics of the components involved. The role of the membrane potential will be discussed.

A. Goffeau, in "Molecular Basis of Membrane Transport", Prague 1986, A. Kotyk, ed., pp. 32-37.

## BINDING OF OUABAINE TO THE Na-K-ATPase IN OOCYTES OF XENOPUS LAEVIS IS VOLTAGE-INDEPENDENT

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Voltage-clamp experiments were performed on full-grown, prophase-arrested oocytes of Xenopus laevis. The activity of the electrogenic Na-K pump in the oocytes depends on membrane potential, and can selectively be inhibited by ouabaine or dihydroouabaine (DHO). The degree of inhibition of the current generated by the Na-K pump was determined for different concentrations of DHO (.5 to 10 µM) and for different membrane potentials (-150 to +80 mV). In addition, the amount of radioactively labled ouabaine bound to the membrane of single oocytes was determined under voltage clamp. The results show that neither the inhibition produced by a given amount of bound DHO nor the amount of bound ouabaine depends on membrane potential.

## TWO FUMARATE REDUCTASE GENES OF WOLINELLA SUCCINOGENES

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The anaerobic bacterium W. succinogenes synthesizes ATP by electron-transport-coupled phosphorylation with formate plus fumarate (reaction a). Fumarate reductase catalyses

$$\text{Formate} + \text{fumarate} \rightarrow CO_2 + \text{succinate} \quad \text{(a)}$$

the terminal step of the fumarate respiration. The enzyme has been isolated, characterized and reconstituted in liposomes. To obtain further information on the structure of fumarate reductase and its function in electron-transport-coupled phosphorylation, we have cloned the structural genes in E. coli. Two genes (frdA, frdB) of the fumarate reductase which encode the FAD-protein (Frd A) and the iron-sulphur protein (Frd B) were cloned together with a W. succinogenes promoter. The gene order was promoter - frdA - frdB. The identity of the frdA gen was verified by comparing the amino-terminal sequence of the Frd A protein with the nucleotide sequence of frdA.

## TRAPPING IN PHOTOSYSTEM I

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The efficiency and kinetics of exciton trapping in photosystem I (PS I) was studied by electrical detection of the primary charge separation in pea chloroplasts. A comparison of the amplitude of the photovoltage evoked by laser flashes of either 12 ns or 30 ps duration showed, that trapping competes efficiently with annihilation and other loss processes, even at high excitation energy. This is also found if most of the traps are in the closed state. Kinetic analysis of the photovoltage recorded with our highest time resolution yielded a decrease in the trapping time from 90 ps at low energy to < 50 ps at high energy. This suggests that trapping in PS I is diffusion limited. If PS II is closed, there is no spillover of excitation energy to PS I.

## IRREVERSIBLE INHIBITION OF $Ca_o^{2+}$ ACTIVATED $K^+$ CHANNELS IN THE RED BLOOD CELL MEMBRANE BY $Ca_o^{2+}$

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In previous work from this laboratory (1) it was shown that the $K^+$ selective channels in the red cell membrane that are responsible for the so-called Gárdos effect require for their activation external $K^+$ ($K_o^+$) and internal $Ca^{2+}$ ($Ca_i^{2+}$) (1). It was further shown that incubation of the cells in media free of $K_o^+$ leads to an irreversible loss of susceptibility to activation by $K_o^+$ and $Ca_i^{2+}$ (2). Using suitable chelators and solutions made from ultrapure $K^+$ and $Na^+$ salts, we now demonstrate that the inactivation in $K_o^+$-free media takes place only when traces of $Ca_o^{2+}$ ( 1-10µM) are present. Thus low concentrations of $K_o^+$ protect the $K^+$ channels against irreversible inactivation by $Ca_o^{2+}$.

References: (1) Knauf, Riordan, Schuhmann, Wood, Passow (1975) J. Membr. Biol. 25: 1-22 (2) Heinz and Passow (1980) J. Membr. Biol. 57: 119-131

## THE UNIT CONDUCTANCE OF $CF_0$

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A method has been developed to determine the unit conductance of ionic channels in thylakoid membranes by spectrophotometric measurements under single flash excitation of proton pumps. The unit conductance of Gramicidin evaluated by the method was 2.7 pS in good agreement with the literature. For $CF_0$, the proton channel of the ATPsynthase, we found a unit conductance of appr. 1 pS. This was by orders of magnitude greater than reported in the literature and also than theoretically expected for a single hydrogen-bounded chain. Proton flow across $CF_0$ was completely tracked by parallel measurements of pH-transients in the lumen and in the medium and, in addition, the voltage decay. The protonic unit conductance of $CF_0$ showed no pH-dependency, but an isotope effect of 1.7 after substitution of $D_2O$ for $H_2O$. The protonic unit conductance of $CF_0$ was that big, that proton diffusion through the aqueous phases became rate limiting if the water structure was disturbed.

## THE SMALL, NON-CATALYTIC SUBUNITS OF THE MITOCHONDRIAL $bc_1$-COMPLEX

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Besides subunits carrying redox centres, the energy-transducing complexes of the inner mitochondrial membrane in all eucaryotic systems investigated so far contain several small proteins which are not present in the bacterial bc complexes. The fact that these subunits are constituent parts of the complexes has been verified and amino acid sequences of these subunits have been determined during the past few years. However, the function of the small subunits has not yet been elucidated.

A refined hydropathy analysis of the $bc_1$ complex of beef heart mitochondria, with special regard to the occurence of amphiphilic α-helices and ß-strands, revealed possible membrane-spanning helical stretches in all small subunits. The hydropathy patterns of homologous subunits are conserved in different species. Some of the subunits have striking features which are observed also in yeast mitochondria; e.g., the 9.2 kD subunit (homologous to the 17 kD subunit of yeast) has a strongly acidic domain which may be involved in the binding of cytochrome c.

## $MG^{2+}$ EFFECT ON CHARA MEMBRANE ELECTRICAL CHARACTERISTICS

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Excitable giant internodal cells of the green alga Chara have proved to be convenient objects for electrophysiological investigations. Resting membrane voltage exceeds diffusion potentials by far, due to Mg-ATP-dependent H-exporting pump activity. Mg and Ca were isoosmotically exchanged in the bathing medium. Increasing Mg:Ca relation up to 4:1 had no effect on $V_m$, further increase depolarized the plasma membrane. A concomitant shift of the action potential peak was observed and explained by Ca equilibrium potential change. At inward current producing clamp voltage, I-V curves display Mg-induced conductance increase, whereas depolarizing clamp voltage will not change I-V relationship under Mg. DCCD experiments yielded Mg to act mainly on passive transport sites, with the Mg-dependent I-V relation being similar to that obtained in the dark.

## $CA^{2+}$ ACTION UPON SINGLE $K^+$ - CHANNELS IN THE CHARA TONOPLAST

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Cytoplasmic droplets released from amputated Chara internodal cells into isotonic solution are surrounded by the original tonoplast membrane. Highly conductive K-channels (150 pS) are resident in the tonoplast, as visualized by patch clamp technique, and membrane voltage equals K equilibrium potential. The activity of this type K-channel is insensitive to Ca over a wide concentration range (~0.1 µM up to 10 mM), facing the vacuolar side of the membrane, whereas elevating the Ca concentration above 0.1 µM at the cytoplasmic face dramatically retards channel conductance, and, judged from preliminary observations, affects the closed-open transition rate. The inhibitory Ca effect seems to be asymmetric, i.e., K outward current is suppressed, whereas inward K current proceeds via a largely unchanged channel conductance.

## A (2)H-NMR STUDY OF TRYPTOPHAN DYNAMICS IN GRAMICIDIN A

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Gramicidin A (GA), a pentadecapeptide which forms ion channels across lipid membranes, was deuterated in its four tryptophan ring sidechains and incorporated into bilayers containing either lyso 16:0 PC, DMPC or DOPC. The (2)H-NMR lineshape, intensity and $T_{2e}$ were used to judge the type and rate of motion observed as a function of temperature. By comparing these three phospholipid/GA mixtures we could demonstrate that the dominant mechanism of motional averaging for the tryptophan ring deuterons was rotation of the whole GA molecule about its long helical axis perpendicular to the membrane plane. Furthermore, different lipids altered this rotational rate by influencing lateral GA-GA interactions parallel to the membrane plane.

## STRUCTURE AND DYNAMICS OF PLASMALOGEN MODEL MEMBRANES

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Deuterium was used to investigate the structure and dynamics of the sn-2 hydrocarbon chain of semi-synthetical choline and ethanolamine plasmalogens in bilayers containing 0,30,and 50 mol% cholesterol. The sn-2 acyl chain was found to adopt a similar conformation as observed in the corresponding diacyl phospholipid, however, the flexibility at the level of the C-2 methylene segment of the plasmalogen was increased. Deuterium NMR spectra of bilayers composed of ethanolamine plasmalogen yielded quadrupolar splittings of the C-2 segment much larger than those of the corresponding diacyl lipids, suggesting that the sn-2 chain is oriented perpendicular to the membrane surface at all segments. Cholesterol increased the ordering of the choline plasmalogen acyl chain to the same extent as in diacyl lipid bilayers. $T_1$ relaxation time measurements demonstrated only minor dynamical differences between choline plasmalogen and diacyl lipids in model membranes.

## 8 Å RESOLUTION ELECTRON MICROSCOPY PICTURES OF PORIN FROM THE OUTER MEMBRANE OF E. COLI

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In the past, electron microscopy studies on negatively stained lattices of porin have provided limited resolution of 20 Å (A. Engel et al., Nature 317 (1985) 643). Considerable improvements in lattice preparation combined with modern cryoelectron microscopy technique has resulted in a break through with respect to resolution in the case of porin. Phospholipase treatment gave porin lattices of high quality with unit cell dimensions as small as 74 Å. The liquid helium cooled objective lense allowed 11 electrons/Å$^2$ in low dose experiments. Three types of embedding materials were used: gold-labelled glucose, pure glucose and negative staining with uranyl acetate. The glucose embedded pictures showed for the first time very clearly the boundaries between the lipid, the protein and the channel areas in projection parallel to the membrane plane.

## STRUCTURE, ANTIGENIC SITES AND FUNCTION OF THE HIV-1 ENVELOPE PROTEIN

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Using a computer program that predicts the secondary protein structure and superimposes values for hydrophilicity, surface probability and flexibility we analyzed the envelope protein sequences of 11 independent human immunodeficiency virus (HIV) isolates linked to AIDS syndrome, three of which represent sequential isolates from a single patient. Several potential antigenic sites were identified; in the exterior protein part (gp120) the majority of those predicted epitopes were found in regions of high sequence variability among independent and also in the sequential virus isolates which are interspersed with highly conserved regions. This property of predicted structure variation in antigenic sites may play an important role in the virus' pathology. gp41, the membrane-associated peptide, contains no highly variable regions; about 80% of the amino acids were found to be conserved and only two epitopes could be predicted. This analysis may give a first hint to the secondary and possibly tertiary structure of the variant HIV envelope proteins. To prove this model and to characterize the functions of single protein domains we synthesized oligopeptides from several regions and tested them for their ability to induce neutralizing antibodies and receptor binding.

## STUDIES ON THE EXPRESSION AND FUNCTION OF THE EB VIRAL-MEMBRANE PROTEIN BNLF1

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The Epstein-Barr viral protein encoded by the open reading frame BNLF1 at the right end of the viral genome was suggested to be the target for cytotoxic T-cell reaction directed against EBV-infected lymphocytes and is the only viral gene product which is transcribed in latently infected cells and has characteristics of membrane proteins. Using sera against synthetic oligopeptides derived from the amino acid sequence we could show that this protein is synthesized in Burkitt's lymphoma cell lines in a truncated form lacking 138 amino acids at the $NH_2$-terminal end. Proliferation of autologous T-cells could be achieved *in vitro* by a further peptide from this region whose location is predicted in a short amino acid loop between two transmembrane regions at the outer side of the cell membrane. Using antipeptide sera in immunofluorescence tests in latently infected cells, a possible reaction in about 20%-30% of the cells could be obtained; a similar amount of BNLF1-MA producing cells were identified by *in situ* hybridization using $^3$H-cytidine labeled DNA probes. We suggest that BNLF1-MA may not be a typical latent EBV product but a protein expressed in 20%-30% of the cells. *in vivo* those cells are eliminated by cytotoxic T-cells; in rare cases and in combination with additional factors only the truncated form of the protein my be produced and those cells may develop into lymphomas.

## QUANTITATION OF ENDOGENOUS DIGITALIS IN SERUM AND ITS CORRELATION TO BLOOD PRESSURE IN PATIENTS WITH ESSENTIAL HYPERTENSION

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Endogenous digitalis was determined by incubating isolated ($Na^+$+$K^+$)-ATPase with [$^3$H]ouabain and serum. Using this receptor assay sera of normotensives showed 76.3 ± 9.3 nM ouabain equivalents while that of hypertensives showed 234.8 ± 48.7 nM ($p < 0.001$). The "endogenous digitalis" (x) correlates to the diastolic blood pressure (y) as y(mm Hg) = 40,6 + 23.3 log x (nM). Boiling of the serum at pH 5.5 for 15 min. led to a 65% reduction of the concentration of "endogenous digitalis" and to the loss of concentration differences between normotensives and hypertensives. Despite of the demonstration of increased levels of "endogenous digitalis" with the receptor assay in the serum of patients with essential hypertension, no such difference could be detected by the use of digoxin antibodies.

## IMPORT OF HONEYBEE PREPROMELITTIN INTO THE ENDOPLASMIC RETICULUM

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Honeybee prepromelittin (PPM) can be inserted into and correctly processed by dog pancreas microsomes independently of signal recognition particle (SRP) and docking protein (DP). Which features in the primary sequence of PPM are responsible for this behaviour? We addressed this question by constructing a series of plasmids encoding hybrid proteins of PPM and the cytoplasmic dihydrofolate reductase as well as plasmids encoding truncated forms of PPM. We conclude that the signal sequence in PPM can interact with SRP per se and that it usually does not do so because of the size of PPM. A working model for the import of PPM into the endoplasmatic reticulum will be presented.

## FUNCTIONAL DISSECTION OF NUCLEAR ENVELOPE mRNA TRANSLOCATION SYSTEM

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Unidirectional transport of poly(A)+mRNA through the nuclear envelope pore complex is thought to be an energy-dependent process which involves a nuclear envelope nucleoside triphosphatase (NTPase). In the intact envelope, this enzyme is regulatable by poly(A) binding and by poly(A)-dependent phosphorylation/dephosphorylation of other components of the mRNA translocation system. One monoclonal antibody (mab) was elicited which markedly decreased the efflux of rapidly labeled RNA and of one specific mRNA (ovalbumin) from isolated nuclei. It markedly increased the binding of poly(A) and the maximal catalytic rate of the NTPase, but did not alter the apparent $K_m$ of NTPase or the extent of its stimulation by poly(A). The mab reacted with both a p83 and a p65 in the nuclear envelope.

## CRYSTAL STRUCTURE OF THE NONIONIC DETERGENT NONANOYL-N-METHYLGLUCAMID AND COMPARISON WITH STRUCTURES OF OTHER AMPHIPHILIC COMPOUNDS

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We have crystallized and solved the crystal structures of Nonanoyl-N-methylglucamid (MEGA-9) and of Heptyl-, Octyl- and Decylgluconamide. The alkylgluconamides differ from MEGA-9 mainly by a reversed amide group and by lacking the N-methyl group.

In solid state both types of molecules are packed parallel in sheets with adjacent sheets being arranged in a head-to-tail fashion.

Packing, torsion angles and hydrogen bonding schemes as well as hydrophobic contacts are discussed in order to explain why MEGA-9 is a detergent whereas the alkylgluconamides form micelles only at temperatures above 80°C.

## NEUTRON- AND X-RAY SMALL ANGLE SCATTERING OF FREE AND MEMBRANE BOUND BACTERIAL $H^+$ATPASES.

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Aqueous solutions of $F_1$ATPases from Micrococcus luteus, Micrococcus species and Escherichia coli, detergent solubilized ATP-synthase from Rhodo - spirillum rubrum and reconstituted proteoliposomes from ATP-synthase from Rhodospirillum rubrum and deuterated lecithin have been investigated by X-ray and neutron small angle scattering. For the investigation of the membrane bound enzyme a recently developed technique for the neutron scattering of matched proteoliposomes has been applied. By combination with biochemical data, from the results some distances of subunits were derived. The reconstituted ATP-synthase was present in monomeric form and resembled the detergent solubilized enzyme. The $F_1$ATPases showed similar distances of the large subunits. The enzyme from Micrococcus luteus showed structural changes by variation of the temperature and by reaction with an irreversible inhibitor.

## SORTING OF PROTEINS INTO MITOCHONDRIA

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Transport of proteins coded for by nuclear DNA and synthesized cytosplasmic ribosomes has been dissected into a number of distinct steps. The following of these steps will be discussed: (i) Recognition of precursors of mitochondrial membrane proteins by receptors on the surface of the mitochondria. (ii) Translocation of precursors through "translocation contact sites" in which outer and inner membranes are closely and firmly linked. (iii) Energy dependence of import of precursors into and across the inner membrane. A requirement was found for nucleoside triphosphates (ATP or GTP) with all precursors studied, including fusion proteins containing non-mitochondrial sequences. In the case of inner membrane proteins this NTP requirement is in addition to the requirement for the electrical membrane potential. (iv) Proteolytic processing of precursors by the soluble processing peptidase in the mitochondrial matrix.

## THE EFFECT OF LIGHT STRESS ON THE TURNOVER OF THYLAKOID MEMBRANE POLYPEPTIDES

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The turnover of thylakoid membrane polypeptides and total fatty acids of the green alga Chlamydomonas reinhardii was studied by pulse-/chase-experiments. During an incubation time of 90 min at 3000 $\mu E/m^2/sec$ white light intensity the cells are irreversibly damaged, whereas a light intensity of 2000 $\mu E/m^2/sec$ results in reversible loss of photosynthetic capacity and in an accelerated turnover of various membrane polypeptides and total cellular fatty acids. Under these conditions preferential degradation of the 32 kDa herbicide-binding protein occurs (and minor damage of some proteins being part of PS II and the LHC). This polypeptide is degraded and continuously synthesized at the same time. During the light stress condition the specific radioactivty of different fatty acids declines. Among the fatty acids measured (16:0, 16:1tr, 18:0, 18:1, 18:2, and 18:3) 16:1tr is the one which is mostly effected. Cells recover from this light stress at normal light intensities (200 $\mu E/m^2/sec$).

## EFFECT OF GTP AND OF cGMP ON THE PLASMA MEMBRANE OF RETINAL RODS

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The plasma membrane of retinal photoreceptor cells is known to contain a light-sensitive, cGMP-dependent channel. We report on experiments with single rods isolated from the retina of the frog Rana esculenta. The whole-cell patch clamp technique was employed for both electrical recording and internal dialysis. When a simple intracellular medium with potassium as the principal ion was used as pipette filling solution, the plasma membrane gradually hyperpolarized from - 25 mV to - 55 mV and the light-sensitivity deteriorated. We attribute these observations to loss of either GTP or cGMP or both by diffusion from the cell interior into the recording pipette. Adding of either of the two nucleotides to the pipette filling solution prevents the hyperpolarization and the decay of the photoresponses but the concentrations required differ considerably, i. e. less than 1 µM cGMP vs. more than 100 µM GTP. The effects are discussed within the framework of current knowledge about the mechanism of phototransduction.

## TRANSIENTLY METHYLATED PROTEINS IN ACETYLCHOLINRECEPTOR-RICH VESICLES

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Protein methylase II is an ubiquitous enzyme throughout all eucaryotic kingdoms. It is identified by the intrinsic instability of the transferred methyl groups. We have shown a transient increase in the amount of the methyl groups incorporated into proteins of acetylcholine receptor (AChR)-rich vesicles upon stimulation.

Several endogeneous methyl group accepting proteins (MAPs) were demonstrated by transmethylations done either after separation of the proteins by SDS-polyacrylamide electrophoresis (SDS-PAGE) followed by transfer on to nitrocellulose (NC) membranes or by transmethylations prior to the separation of the methylated proteins by 16-BAC-PAGE. In both instances more than 20 labelled protein species could be resolved, but their MWs differed markedly, suggesting binding of labelled proteins to those fixed on the NC membrane.

Sample preparation for 16-BAC-PAGE rendered part of the proteins of the AChR-rich vesicles detergent insoluble. These insoluble proteins included all the transferred methyl groups, yielding essentially the same kinetic of methyl group transfer as found in the filter assay.

The methylated protein aggregates were too large to enter the gel when subjected to 16-BAC-PAGE or SDS-PAGE. This is taken as evidence for the formation of extensive crosslinks between proteins of the soluble and the membrane fraction upon transmethylation.

Some of the proteins constituting the aggregate were identified by immunoblots.

## AFFINITY CHROMATOGRAPHY OF NEURONAL TRANSMITTER PROTEINS USING A BIOTINYLATED G- PROTEIN AS AFFINITY LIGAND

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DCHAPS, a zwitterionic derivative of deoxycholic acid, can be used for high yield solubilisation of dopamine D2 receptors (1). Regulation of the receptor high affinity agonist binding state by guanine nucleotide regulatory protein in solubilisate from bovine striatum is demonstrated by Scatchard analysis of $^3$H- NPA binding in the presence or absence of the non- hydrolysable GTP- analog GppNHp.

Another component of this transduction system (guanylnucleotide binding proteins $G_i/G_o$) has been purified from bovine cortex via sequential DEAE-, AcA- and octylamine sepharose chromatography to a $GTP_\gamma S$ binding activity of 1-3 nmol/mg.

For use as a molecular probe and affinity ligand the G- protein has been biotinylated. The $GTP_\gamma S$ binding activity retained after the modification serves as a parameter for the ability of the protein to be coupled in solution. After reconstitution with solubilisate the preparation is purified on an avidin column.

V. Soskic, J. Petrovic Biochem.Pharmacol 35,4229(1986)

## INVESTIGATIONS ON THE TRANSEPITHELIAL TRANSPORT OF STEROIDS IN THE MUCOSA OF THE GUT

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The cardiotonic steroid $^{3}$H-cymarol and its aglycon $^{3}$H-strophanthidol were administered either to the luminal or to the blood side of isolated mucosae of guinea-pig jejunum, ileum or colon mounted in a flux chamber. Transepithelial fluxes in both directions as well as steroid uptake were measured. Both steroids revealed net secretion, which value increased in distal direction with reference to the gut segments. In contrast to strophanthidol the permeation of corticosteroids (cortisol, dexamethasone) in the jejunum was compatible with simple diffusion, whereas in the colon also net secretion of these compounds was found. Tissue content of the cardiotonic steroids was always higher after blood side administration than after luminal side administration.

For separating luminal and blood side transport mechanisms, studies with brush border vesicles of guinea-pig small intestine were started. The influx of $^{3}$H-ouabain (Na-independent) and $^{3}$H-cymarol was compared to the transport of $^{14}$C-D-glucose in double label experiments. For both steroids influx into an osmotically reactive space was found. At 30 min the ratios of the apparent spaces not corrected for unspecific binding were ouabain/D-glucose approx. 0,5 and cymarol/D-glucose approx. 1.

## LABELLING OF MEMBRANE PROTEINS ON ISOLATED RAT HEPATOCYTES AND LIVER CELL PLASMA MEMBRANES BY PHOTOACTIVATED $^{3}$H-BUMETANIDE

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The loop diuretic bumetanide is a competitive inhibitor of taurocholate uptake into hepatocytes (Am J Physiol,243,G48,1982) and a potential photoaffinity reagent for the renal Na-K-Cl cotransport system (Am J Physiol,250,C799,1986). We intended to identify binding of $^{3}$H-bumetanide to hepatocytes after photoactivation and to correlate the binding to its assumed uptake via the bile acid transport system. On intact hepatocytes proteins with 25, 30, 42, 49, 54, and 146 kDa became labeled. Labelling was strongly NaCl dependent. 1 mM (cold) bumetanide or furosemide blocked protein labelling while 10 mM probenecid and 1 mM cholate were ineffective. Protein labelling was not observed on AS-30D hepatoma cells which lack the bile acid transport system and which do not take up bumetanide. On purified liver plasma membranes $^{3}$H-bumetanide was bound to proteins of 25, 33, 51, and 68 kDa. This labelling was not NaCl dependent. The results indicate multiple binding proteins for bumetanide on hepatocytes. These proteins may represent a hepatic bumetanide uptake system.

## IMPORT OF CYTOCHROME c1 INTO MITOCHONDRIA INCLUDES A DETOUR THROUGH THE MATRIX

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Cytochrome c1 is located at the outer surface of the inner mitochondrial membrane and is largely exposed to the intermembrane space. It is synthesized in the cytosol, post-translationally imported into mitochondria and proteolytically processed in two steps. Import of c1 can be studied by the use of a coupled in vitro transcription/translation assay with a full-length cDNA clone. Under appropriate conditions precursor could be accumulated in the matrix which could subsequently be chased to intermediate and mature sized c1. Thus, sorting of cytochrome c1 involves translocation across both mitochondrial membranes and retranslocation across the inner membrane.

## IS PLUTONIUM BOUND TO THE SAME MEMBRANE PROTEINS AS IRON IN HEPATOCYTES?

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The uptake of plutonium into liver cells appears to be governed by a similar mechanism to that of iron, but in other cell lines there are substantial differences. Investigations with membrane fractions and solubilized membrane proteins suggest that in rat liver cells both metals are bound to integral membrane protein(s). The affinity of plutonium for that protein appears to be smaller than that of iron: it is easier split off in the presence of chelating agents like EDTA or desferrioxamine. The binding between iron and its membrane proteins, on the other hand, is more labile at low pH values.

## CALCIUM CHANNEL RECONSTITUTION INTO SOLVENT-FREE LIPID BILAYER MEMBRANES ON THE TIP OF GLASS PATCH PIPETTES

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Reconstitution allows the study of functional properties of biochemically defined proteins in an in vitro recording system. However, the usefulness of conventional bilayer techniques for the analysis of single-channel events of low amplitude and fast kinetics, such as Ca channel activity, is limited by problems such as electrical noise, limited resolution, fragility and contamination of membranes with non-volatile hydrocarbons. Thus, PE (70%)/PS (15%)/cholesterol (15%) membranes (1 mg lipid per ml n-hexane) were formed at the tips of glass patch pipettes. Pipette-bilayer seals, electrical noise of bilayers and amplitude and time resolution of channel openings in bilayers were of a similar order of magnitude as the equivalent parameters in biological membranes. Bilayer artifacts were distinguished from protein-related channel openings by their lack of pharmacology and their broad amplitude distribution. Ca channels were reconstituted by fusion of protein-containing vesicles with the bilayer. Two types of Ca channels with different functional properties were observed.

## LATERAL PROTON FLOW BETWEEN PUMPS AND ATP-SYNTHASES IN STACKED THYLAKOIDS

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It is under debate whether proton flow is enhanced or delayed at membrane surfaces. We measured the relaxation of a pH-pulse, which was generated between thightly appressed thylakoid membranes by stimulation of the intrinsic proton pumps (photosystem II) by flashes of light. The alkalization arrived only slowly in the medium ($t_{\frac{1}{2}}$ =100 ms). It was however accelerated if the membranes were unstacked ($t_{\frac{1}{2}}$ =2.7 ms). Description of this diffusion problem revealed, that the delayed propagation of the pH-pulse was quantitativly attributable to the buffering capacity between stacked membranes, presupposing a "true" diffusion coefficient for $OH^-$ or $H^+$ as in bulk water. This refuted speculation on enhanced diffusion at membrane surfaces and led us to an estimate of the lateral loss of protonmotive force of some 0.3 pH-units, which was small in comparison with the transmembrane force.

## THE MEMBRANE STRUCTURE STABILIZES ONE CONFORMATION OF THE ACH RECEPTOR

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The binding of a fluorescent agonist to the membrane-bound acetylcholine receptor from T. marmorata was monitored in the time range of milliseconds, seconds and minutes. In the time range of milliseconds, the second order association reaction is independent of the membrane structure. In the time range of minutes, there is a slow reaction of first order ($k \approx 0.005$ s-1) which contributes to 60-80 % of the amplitude. It is also observed when the association of [3H]-acetylcholine is monitored by means of a filtation assay. This component is related to the saturation of the second binding site. It is lost when the membrane structure is disturbed by the addition of detergents, lipids or alcohols. It can be attributed to a conformational change of the active to the desensitized form of the acetylcholine receptor, indicating that the active conformation is stabilized by the membrane structure.

## Two proteins at the inner surface of nuclear envelopes recognize poly(A) sequences.

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To identify binding proteins in the nuclear envelope that serve to recognize poly(A), a mixture of ADP and (8-azido)ADP was subjected to polymerisation by polynucleotide phosphorylase. In the photoreactive polynucleotide(100.000 MW) the (8-azido)ADP content ranged between 5% and 10%. The purified poly(A) was labeled in 5'-position with a radioactive phosphate group. In nuclear membranes, prepared by DNase I treatement under strongly hypotonic conditions, our photolabel generated a protein-nucleic acid complex of approx.270 kd molecular weight. This could only be detected in very small amounts, when resealed nuclear envelopes were used. A further control was furnished by a RNA, polymerisized from(8-azido A,A,G,C,U)DP. Upon digestion with RNase $U_2$ the complex yield two proteins with molecular weights of 50 kd and 30 kd.

## IDENTIFICATION OF BINDING SITES INVOLVED IN THE SELECTIVE MODIFICATION OF BAND 3-MEDIATED $SO_4$-TRANSPORT BY DANSYL-CHLORIDE

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Dansylation of the red cell membrane causes a partial inhibition of band 3-mediated Cl exchange and an enhancement of $SO_4$ exchange. The pH-dependence of $SO_4$ exchange with a maximum at pH 6.3 is replaced by a plateau above pH 7.0, while the pH-dependence of the partially inhibited Cl-transport and the $HSO_4$/Cl net-exchange remain unaltered. The effects are due to the dansylation of three different amino acid residues. Two of these are only accessible for dansylation when the anion transport inhibitor APMB is present. One of these two sites is located on the chymotryptic 17 kDa-fragment, the other on the 35 kDa-fragment of band 3. SH, $\epsilon$-$NH_2$- and tyrosine residues are not involved. We suggest that the effects are due to the dansylation of two histidine residues.

## TRANSLOCATION OF CARBOXYPEPTIDASE Y INTO ENDOPLASMATIC RETICULUM OF YEAST IN VITRO

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Microsomes from Saccharomyces cerevisiae have been isolated and studied for the protein import in a homologous cell-free system. Northern blot hybridization analysis of poly(A)$^+$RNA isolated from membrane-bound and free polysomes showed that PRC1 transcript (coding for vacuolar glycoprotein carboxypeptidase Y (CPY) contains information for segregation which is expressed during translation. A yeast cell-free system has been derived, consisting of S48-yeast extract, mRNA template and rough microsome fraction which promoted translocation and processing of nascent protein. The in vitro synthesized precursor of the CPY (59 KDa) was translocated and core glycosylated (67 KDa) when yeast microsomal membranes were added. Protease protection experiments showed translocation of the large band of 67 KDa. Furthermore, proCPY synthesized by tunicamycin-treated yeast strain ABYS1, which is disturbed in the maturation of proCPY showed the same electrophoretic migration pattern as the in vitro translated proCPY.

## PROTON GRADIENT COUPLED ACTIVE GLUCOSE TRANSPORT AT THE TONOPLAST OF HIGHER PLANTS

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ATP-dependent proton pumping and $^{14}$C-O-methyl D-glucose (OMG) transport are both inhibited by antibodies against native H+ -ATPase or its catalytic subunit (72 kDa; see Mandala & Taiz 1986 J.Biol.Chem. 261:12850), whereas antibodies against the 62 kDa subunit have little effect. The antibodies raised against the tonoplast H+ -ATPase from Zea mays L. cross-react with polypeptides of identical size from tonoplast of tobacco as shown by Western blotting. As expected proton pumping and OMG transport are inhibited in a similar way in both plant species. The results strongly support the model of a proton gradient driven glucose transport at the tonoplast of higher plants (H+/glucose antiport).

## CARBODIIMIDES INACTIVATE ($Na^+ + K^+$)-ATPase BY MODIFYING THE CATALYTIC SUBUNIT ON THE 56000 TRYPTIC FRAGMENT BEARING THE ADP SUBSITE

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Carbodiimides are candidates for labelling the cation binding sites of ($Na^+ + K^+$)-ATPase [1]. [$^{14}$C]-Dicyclohexylcarbodiimide (DCCD) and the fluorescing N-cyclohexyl-N'-(4-dimethylamino-α-naphthyl)carbodiimide (NCD-4) inactivate this enzyme by modifying the α-subunit. Limited trypsinolysis at 150 mM $K^+$ shows, that this inactivation is not due to the modification of the aspartyl residue accepting the phosphointermediate on the 40 kDa fragment but due to the 56 kDa fragment bearing the adenosine subsite of the ATP binding site. Specific labelling is obtained at 80 µM DCCD and at 100 µM NCD-4. $Na^+$ and $K^+$ prevent the incorporation of these carbodiimides.

1. W.Schoner, H.Schmidt (1969) FEBS Lett. 5, 285-287

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ENERGY METABOLISM AND PROLIFERATION:EFFECTS OF UNCOUPLERS OF OXIDATIVE PHOSPHORYLATION ON 3T3, 3T6, AND SV40-3T3 MOUSE CELLS

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Application of valinomycin (VAL) to rodent cells gives rise to growth arrest, non-specific as to cell cyclusphase, and to cell death in the case of transformed cells, whereas normal cells are arrested in G1/GO-phase without appreciable cell death (1). In the present work, it is shown that this transformation-specific effect of growth arrest independent of cell cycle phase in contrast to normal is found also for true uncouplers of oxidative phosphorylation, such as CCCP. However, such uncouplers (at $\geq$ 10 µM) are cytotoxic both for normal and transformed cells. Mitochondrial sites of action are demonstrated by inhibition of mitochondrial uptake of the cationic fluorescence-dye rhodamine 123. Aerobic glyco-lysis (measured as cellular lactate production) is strongly increased upon uncoupler application. Furthermore, this gives rise to persistent cytoplasmatic acidification by about $\Delta pH \simeq 0.4$ for CCCP ($\geq$ 10 µM) but only $\Delta pH \leq 0.1$ for VAL ($\simeq$20nM). Reference: (1) Kleuser, B., H. Rieter, G.Adam: Cancer Res. 45, 3022-3028 (1985).

FUSION PROTEINS CONTAINING NUCLEAR LOCATION SEQUENCES OF SIMIAN VIRUS 40

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Nuclear location of certain proteins encoded for by Simian virus 40 (SV40) is determined by short amino acid sequences. These are permanent parts of the mature protein not removed during nucleocytoplasmic transport. Examples are residues 125-132 of large T, 1-8 and 12-19 of VP1. We have constructed hybrid genes coding for residues 127-147 of large T or 2-67 of VP1 and the almost complete E.coli protein beta-galactosidase. The presence of nuclear location sequences in the hybrid genes was verified by dideoxy-sequencing in M13 vectors. Fusion proteins were expressed in E.coli and purified by affinity chromatography. Fusion proteins were found to be perfectly soluble and to retain galactosidase activity. Fusion proteins were labeled with fluorescein iodacetamide at a ratio of 1-2 moles chromophore per mole protein. The labeled proteins are currently employed to study mechanisms of nucleocytoplasmic protein traffic in single mammalian cells (see Peters, R. 1986. Biochim. Biophys. Acta 864:305-359).

INCORPORATION OF A SYNTHETIC MITOCHONDRIAL SIGNAL PEPTIDE INTO LIPID MONOLAYERS AND BILAYERS

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The interaction of the chemically synthesized 25-residue signal peptide of subunit IV of yeast cytochrome c oxidase (p25) with synthetic and natural phospholipids has been studied with monolayer and bilayer techniques. This peptide and some mitochondrial signal peptide analogues spontaneously insert into phospholipid monolayers and form amphiphilic structures at lipid-water interfaces. The incorporation of p25, as measured by the monolayer area increase at constant surface pressure, strongly increases in the presence of negatively charged lipids and the measured incorporation isotherms are well described with a simple two-step model involving membrane-water partitioning and an in-plane binding reaction of the negatively charged phospholipids to the partitioned peptide. The peptide inserts with its α-helix long axis parallel to the plane of the monolayer and releases entrapped carboxyfluorescein from phospholipid vesicles in a potential-sensitive manner.

HYDROPHOBIC AND POLAR INTERACTIONS IN MEMBRANE PROTEINS.

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Porin is a protein that forms voltage-gated channels across outer membranes of E. coli, with most of its mass within the membrane boundary. It is significantly different from hydrophobic proteins that span the membrane in alfa-helical segments, with the hydrogen bonding potential presumably saturated within segments. Porin is hydrophilic, and its transmembrane segments span the membrane in beta-pleated sheet structure. Moreover, the folding requires that both ionizable and polar groups occur within the membrane domain. Chemical modifications of the protein demonstrate that significant numbers of such residues appear burried within the membrane core. Full saturation of its hydrogen bonding potential appears a prerequisite of its stability.

DIACYLGLYCEROL KINASE OF E. COLI. PURIFICATION TO HOMOGENEITY AND LIPID DEPENDENCE.

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Diacylglycerol kinase (dgk) was highly purified in organic solvent in a 5-step procedure that included HPLC. The proposed assignment to the dgkA-gene (1) was supported by molecular weight determination (m.w., appr.14,000), N-terminal sequence (Met-Ala-Asn), CNBr-fragmentation and amino acid analysis. As predicted, proline was absent.

The enzyme protein had an absolute lipid requirement for function. A number of structurally different lipids were active, displaying positive kinetic cooperativity. The enzyme survived heating to 100 °C as a lipid/protein complex in water or as delipidated protein in butanol-1.

The low molecular weight, stability and known primary structure make dgk an ideal material for the study of lipid/protein interactions. - - Supported by DFG.

(1) Lightner, V.A., Bell, R.M., Modrich, P. (1983) J. Biol. Chem. 258 10856-10861.

PARTIAL PURIFICATION OF MICROSOMAL G6P-PHOSPHOHYDROLASE ON HYDROXYLAPATITE

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Glucose-6-phosphate phosphohydrolase was effectively solubilized with the nonionic detergent Renex 690 in the presence of sodium chloride from the microsomal membrane. All of the phosphohydrolase activity was found in the solubilized supernatant after ultracentrifugation. Subsequent separation on hydroxylapatite has been proved to be a successful step in the way to purify this enzyme. With the first separation on hydroxylapatite the glucose-6-phosphate phosphohydrolase appeared in the through flow volume. Rechromatography of the through flow volume on hydroxylapatite was accompanied with a significant loss of activity but the remaining phosphohydrolase was very stable under various conditions. The enrichment of phosphohydrolase activity was about 12 fold with a yield of 6 %. SDS electrophoresis of the glucose-6-phosphate phosphohydrolase fractions after the second separation step revealed only 3 - 4 dominant protein-staining bands in the molecular weight region between 17,000 - 45,000.

FLUIDITY CHANGES IN ISOLATED LIVER PLASMAMEMBRANE METIATED BY AN INTEGRAL ENZYME ACTING ON LIPID COMPOSITION
Studies by Fluorescence Polarization, Anisotropy, and Electron-microscopic Autoradiography
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Li··id exchange and transfer proteins are well known to switch bilayer asymetry. In many tissue cells strong evidence exsits for enzymatic conversion of phosphatidylethanolamine (PtdEn) to phosphatidylcholine (PtdChol). The reaction requires three successive N-methylations of the ethanolamine moiety by S-adenosylmethionine (SAM). Although highest activity for this enzyme(s) has been found in rat liver, small importance of methylation pathway in PtdChol is the fact. Yet unclear remains wether the methyltransferase (PMT) is soluble or membrane bound and are there more than one enzyme. In view of this fact, we carefully investigate our plasma membrane fractions using 3-H-methyl-SAM. By TLC analysis of the lipid extract radioactivity could be only detectable for PtdChol. In addition, for a minor part radioactivity has been also found associated with extractable membrane proteins.
Measurement of fluorescence polarization by 1.6-diphenylhexatrien (DPH) as a probe and anisotropy after preincubation with SAM on suspended liver plasma membranes a marked change in membrane microviscosity has been observed. Because caution should be exercised in interpreting results obtained using fluorescence probes to determine membrane fluidity, we evaluate extensively the order parameter excimer quenching with various probes. Therefore it must be concluded a change of the membrane asymetry in the polar headgroup regions on acting of this enzyme.
All enzyme parameters and the velocity constant for S-adenosylhomocystein (SAH), a potent inhibitor for this enzyme has been determined and found to be identical for PMT I (E.C. 2.1.1.17).
Finally, we have been able to demonstrate location of the PMT I into the membrane plane by autoradiography with the electron microscope.
The consequence of these findings in combination with earlier data on hormone action will be discussed.

RECONSTITUTION OF THE ERYTHROCYTE ANION TRANSPORT SYSTEM IN PHOSPHATIDYLCHOLINE AND SPHINGOMYELIN VESICLES
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Band 3 protein, the anion transport protein of the human erythrocyte membrane, was incorporated into phosphatidylcholine bilayers by a novel procedure: (1) From a mixture of band 3, phosphatidylcholine (0.2 %), Triton X-100 (0.2 %) and octylglucopyranoside (0.7 %) the latter detergent was removed by dialysis. This led to the formation of band 3-containing vesicles. (2) Triton X-100 left in the sample was removed by sucrose density gradient centrifugation. When studied by measurements of sulfate efflux, the system assembled shows all the major properties of the erythrocyte anion transport system (Scheuring, U., Kollewe, K., Haase, W. and Schubert, D. (1986) J. Membrane Biol. 90, 123-135). Reconstitution of the anion transport system in sphingomyelin bilayers needed modification of the procedure described. With the modified method, anion transport in the reconstituted system was similar to that in phosphatidylcholine bilayers.

DEMONSTRATION OF COOPERATING α-SUBUNITS IN WORKING $(Na^+ + K^+)$-ATPase by $Co(NH_3)_4ATP$
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The MgATP complex analogue cobalt tetrammine ATP ($Co(NH_3)_4ATP$) inactivates $(Na^+ + K^+)$-ATPase by phosphorylation. The dissociation constant of the enzyme-MgATP analogue complex at 37°C is $K_D = 500$ µM, the inactivation rate constant $k_2 = 0.05$/min. ATP protects the enzyme at the low affinity ATP binding site ($K_D = 360$ µM). It is evident therefrom that $Co(NH_3)_4ATP$ is recognized by the low affinity ATP binding site of $(Na^+ + K^+)$-ATPase. The ability of the inactivated enzyme to bind [$\alpha$-$^{32}$P]ATP at the high affinity ATP binding site and $Na^+$-dependent phosphorylation with [$\gamma$-$^{32}$P]ATP is not affected by the extent of the inactivation. It is concluded that high and low affinity ATP binding sites coexist simultaneously in the membrane bound $(Na^+ + K^+)$-ATPase and that in the catalytic cycle the low affinity ATP binding site must convert to the high affinity binding site. The findings support a model of interacting subunits in the working enzyme.

DIFFERENZIERUNG DER PLASTIDENHÜLLMEMBRAN
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Die Plastidenhüllmembran ist konservativ erhalten während aller Schritte der Plastidendifferenzierung, z.B. im Übergang Proplastid-Chloroplast, Proplastid-Etioplast-Chloroplast. Dem gegenüber unterliegt die Thylakoidmembran außerordentlichen starken Veränderungen. Die wichtigsten Bausteine der Thylakoidmembran werden entweder in der Plastidenhüllmembran synthetisiert oder müssen sie passieren z.B. Proteine. Wir haben Methoden entwickelt, die an der Hüllmembran lokalisierten Schritte der Membranbiogenese zu untersuchen, dazu gehören u.a. die Entwicklung des Proteintransportapparates in der lichtabhängigen Entwicklung des Chloroplasten. Außerdem unterliegt die Hüllmembran selbst starken Veränderungen ihrer molekularen Zusammensetzung.

STRUCTURE AND DYNAMICS OF PHOSPHOLIPID HEAD GROUPS IN THE PLASMA MEMBRANE OF AN EUCARYOTIC CELL LINE
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Mouse fibroblasts from connective tissue, LM cells, were grown on medium containing deuterated choline or ethanolamine. An almost complete labeling of the phosphatidylcholine (~ 48% of total lipid in membrane) or phosphatidylethanolamine lipids (up to 40% of total lipid) was achieved. The addition of deuterated serine led to the formation of deuterated phosphatidylserine. Deuterium and phosphorus nmr spectra were recorded for lipid extracts, whole cells and plasma membranes. The deuterium label was attached at α- or β- head group segment of the corresponding phospholipid. Thus it was possible for the first time to visualize the choline and ethanolamine head group in intact biological membranes. The structure and dynamics of these head groups are similar to those of synthetic lipid bilayers. No specific lipid protein interactions are detectable.

IMPORT OF FROG PREPROPEPTIDE GLa INTO THE ENDOPLASMIC RETICULUM
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Frog prepropeptide GLa is processed and imported by dog pancreas microsomes. These events do neither depend on docking protein nor on the presence of ribosomes. A hybrid protein between prepropeptide GLa and an unrelated peptide fused to the carboxy terminus, however, behaves like a typical secretory protein precursor with regard to docking protein- dependence. This suggests that independence on docking protein, in the case of prepropeptide GLa, can be attributed to the size of the precursor protein. Processing and import of prepropeptide GLa by microsomes are ATP-dependent. Therefore, import of proteins into the endoplasmic reticulum includes an ATP- requiring step not involving a ribosome/ ribosome receptor- or SRP/ docking protein- interaction.

## FATTY ACYLATION OF VIRAL GLYCOPROTEINS

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Fatty acylation of polypeptides is a common hydrophobic modification, which occurs at all levels of organisation during evolution.
Here we report on the fatty acid composition, the structure of the linkage site and the biological function of different viral membrane glycoproteins. Our data show that the fatty acid linkage sites are located near the membrane spanning domain of the polypeptides. Hyroxylamine treatment of non-acylated Sendai virus fusion (F) protein does not affect its hemolytic activity, while the fusing potential of several ortho- and paramyxoviruses is impaired drastically under the same experimental conditions.

## VOLTAGE-DEPENDENT GATING OF $K^+$CHANNELS IN GUARD CELLS

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Stomata in leaves allow plants to regulate the exchange of gases with their environment. Variations of stomatal apertures are mediated by contolled changes of $K^+$ concentrations in the surrounding guard cells. In order to investigate the regulation of $K^+$ fluxes during stomatal aperture changes, the voltage-dependent gating of $K^+$-selective channels in the plasma membrane of guard cells was studied using the patch clamp technique. In whole-cells depolarisations produced outward $K^+$ currents. Hyperpolarisations elicited inward $K^+$ currents. Inward and outward $K^+$ currents were selective for $K^+$ over $Na^+$ and could be blocked by exposure to $Ba^{++}$. In cell-atached and outside-out patches previously identified $K^+$-selective channels were studied (Schroeder, Hedrich and Fernandez (1984) Nature 312). Averaging of single channel currents during voltage pulses resulted in kinetics that were similar to kinetics of whole-cell $K^+$ currents. Estimates show that the voltage-dependence of $K^+$ channels could be involved in the regulation of $K^+$ exchange by guard cells. Blue-light stimulated electrogenic pumps can activate inward $K^+$ channels by hyperpolarization and drive $K^+$ accumulation. Prolonged depolarisation would result in release of $K^+$.

## AN INTEGRATED FLUORESCENCE MICROPHOTOLYSIS APPARATUS FOR SINGLE-CELL FLUX MEASUREMENT

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Fluorescence microphotolysis (FM) can be employed to measure membrane transport and molecular mobility in single cells (Scholz,M. et al. 1985. Eur. Biophys. J. 13:37-55; for review, see Peters, R. 1986. Biochim. Biophys. Acta 864:305-359). These novel possibilities may be of interest to a range of biologists, physiologists and pharmacologists. We have therefore thought to provide a simplified integrated FM apparatus which can be more easily handled and is less expensive than existing experimental set-ups. The apparatus was constructed from an air-cooled argon ion laser, an acousto-optical modulator, an inverted microscope, a single-photon counting system, and a laboratory computer with graphics and plotting capabilities. The computer was directly interfaced with the other components to control the experimental process and to handle the data. The software accounts for flux and diffusion measurements in both the discontinuous (flash) and continuous mode of FM.

## FORMATION OF GIANT LIPOSOMES IN A LIPID-DETERGENT-GRADIENT

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A gradient of octylglycoside (OG) and lecithin (PC) in water at a constant molar total ratio ($R_t$) of 2.5 was formed between two slides. The effective ratio ($R_{eff}$) in the OG-PC-aggregates changes with the total concentration of amphiphiles along the gradient. Therefore the different aggregational states, which arise during detergent dialysis, can be observed simultaneously in a darkfield microscope. There is a clear border between vesicles and the micellar area, but micelles seem to emerge inside the large vesicles when they move towards the micellar border. Close to the micellar zone the smaller vesicles can be seen to fuse to very large vesicles >5µm. Therefore this method is also promising for reconstitution of membrane proteins to be examined in patch clamp or other flux measurements.

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## ROLE OF THE AGGREGATION FACTOR IN THE REGULATION OF PHOSPHOINOSITIDE METABOLISM IN SPONGES

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The aggregation factor (AF) of the sponge Geodia cydonium recognizes the aggregation receptor (AR) which is inserted in the plasma membrane. The 47-kDa cell binding fragment of the AF was used to investigate the phosphoinositide metabolism in this avertebrate system. We found that after binding of p47 to AR a strong stimulation of the phosphate incorporation into phosphatidylinositol occurs, followed by an increased turnover of phosphoinositides in Geodia cells. The consequences of these events are: (i) Increase of the cytosolic $Ca^{2+}$ concentration, resulting in an increased $Ca^{2+}$ efflux rate. (ii) Stimulation of protein kinase C, which ultimately leads to an unusually strong induction of DNA polymerase alpha.

## UPTAKE OF METAL TRANSFERRIN COMPLEXES INTO MULTICELLULAR SPHEROIDS OF RAT HEPATOCYTES

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In vivo Fe-59 and Pu-239 occur as their metal transferrin complexes in the blood and are taken up into the liver and other organs. Experiments with rat hepatocytes, cultured as monolayers failed to show a receptor mediated transport of these metals across the membranes. After desialylation of transferrin, hepatocytes accumulated Fe-59 but not Pu-239. Using a new culture system for rat hepatocytes, i.e. multicellular spheroids we could demonstrate considerable uptake of Pu-239 from its transferrin complex. However, the uptake rate of Fe-59 from the metal transferrin complex is only one tenth compared to that of Pu-239. These results indicate that transferrin bound Pu-239 and Fe-59 possibly enter liver parenchymal cells in different ways.

### A FLUORESCENE METHOD TO STUDY NUCLEOCYTOPLASMIC TRANSPORT IN AMPHIBIAN OOCYTES

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Radiotracer methods are frequently used to study nucleocytoplasmic transport in amphibian oocytes (for review, see R. Peters. 1986. Biochim. Biophys. Acta 864:305-359). We have developed an equivalent fluorescence method. A substance is fluorescently labeled and injected into X. oocytes. After an appropriate incubation period the injected cells are quenched in liquid propan, sectioned at -20°C, fixed with paraformaldehyd at -20°C, and warmed to room temperature in a dessicator. In the sections the nucleocytoplasmic fluorescence ratio Fn/p is measured by microfluorometry. Since the cytoplasm has a strong and variable autofluorescence, non-injected cells are processed simultaneously with injected cells. The method was tested with bovine serum albumin (BSA) and nucleoplasmin (NP), a nuclear non-chromatin protein abundant in Xenopus oocyte nuclei. Fn/p was 0.12±1.97 for BSA and 16±0.1 for NP (cytoplasmic injection, 8-10h incubation at 19°C) indicating that BSA was excluded from and NP accumulated in the nucleus. The tryptic core fragment of NP was also excluded.

### PRESENCE AND PROPERTIES OF A MEMBRANE-BOUND CORRINOID PROTEIN IN METHANOGENIC BACTERIA

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Methanogenic bacteria contain substantial amounts of membrane-bound corrinoids(60nmol/1g cell dry weight), which are non-covalently bound to an integral membrane protein (1). The corrinoid cofactor is a derivative of vitamin $B_{12}$. The corrinoid-containing membrane protein has been isolated after treatment with nonionic detergents. It forms a complex of molecular weight 500 kDa, which contains a minimum of 8 corrinoids and is composed of 3-4 subunits. The corrinoid containing subunit has a mw of 37 kDa and is redox active (Co I/III, midpoint potential -0.15 V). It is proposed that this protein is involved in the terminal step of $CH_4$ formation,

$$CH_3\text{-Coenzyme M} + 2[H] \longrightarrow CH_4 + \text{Coenzyme M}.$$

(1) Schulz H, Fuchs G (1986) FEBS Lett 198: 279-282.

### FAST AND SLOW UPTAKE OF LECTIN MEMBRANEGLYCOCONJUGATE-COMPLEXES INTO HUMAN FIBROBLASTS

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After binding with cross-linking lectins receptors in the plasma membrane of human gingival fibroblasts are concentrated at the membrane surface and consequently endocytosed. Gingival fibroblasts of the 3rd to 6th passage were exposed to different fluoresceinated lectins (Concanavalin A, wheat germ agglutinin, phythemagglutinin L = PHA-L, Ricinus communis agglutinin I & II, soybean agglutinin) for a time span from five minutes up to 24 hrs. All of these lectins bound to the cell surface within five minutes and most of them were endocytosed within the first hour, PHA-L, however, was marked by an unusually slow mode of uptake. Therefore different mechanisms of glycoconjugate uptake have to be discussed.

### A $^{35}Cl$ STUDY OF THE BINDING OF $Cl^-$ IONS TO THE ANION CARRIER OF ERYTHROCYTES

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Chan and coworkers (J. Biol. Chem. 259:6481-6491 (1984)) showed that the linewidth of the $^{35}Cl$ resonance can be used to study the binding of chloride ions to the anion transport system of erythrocytes of the band 3 protein. By using the competitive anion transport inhibitor 4,4'-dinitro-2,2'-stilbendisulfonate (DNDS), the fraction of chloride ions bound to band 3 protein could be determined.

The method is sufficiently precise for the investigation of various inhibitors, allowing distinction of competitive and non-competitive inhibitors. Thus, p-nitrobenzenesulfonate (pNBS) is a competitive inhibitor, whereas it was shown that the anion transport inhibitor 2-(4'-aminophenyl)-6-methylphenylthiazol-3,7-disulfonate (APMB) inhibits the band 3 protein at the inside of the erythrocyte membrane in a different manner compared to the band 3 protein at the outside of the membrane. This result indicates an asymmetry of the anion transport.

### THERMODYNAMIC AND KINETIC ANALYSIS OF INCORPORATION IN A MEMBRANE

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We have carried out experimental and theoretical studies with the voltage-dependent pore forming peptide alamethicin in order to explore the extent and rate of its incorporation in phospholipid bilayers. The diverse approaches used to analyze the data are quite generally applicable whenever one can measure a signal reflecting association of a substrate molecule and a membrane (which may be binding or incorporation). These procedures are demonstrated for the case of alamethicin in aqueous solution to which unilamellar lipid vesicles have been added. Circular dichroism or fluorescence titration curves could be evaluated, resulting in partitioning isotherms which clearly indicate the existence of aggregate formation and thermodynamically non-ideal repulsive interaction of the incorporated peptide. The kinetics can be quantitatively interpreted in terms of a mechanism comprising a diffusion controlled penetration step followed by a possible slower conformational transition and comparatively fast aggregation.

### KINETIC STUDIES OF THE INCORPORATION OF ALAMETHICIN IN PHOSPHOLIPID BILAYERS

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In order to examine how fast the voltage-dependent pore forming peptide alamethicin can incorporate in a membrane, we have carried out pertinent kinetic measurements. A fluorescence label was first attached to the C-terminal end group. The incorporation process could then be monitored by the inherent increase of the fluorescence emission. After mixing of aqueous peptide and lipid vesicle preparations in a stopped-flow apparatus we observed single exponential time functions (at temperatures above the lipid phase transition). The relaxation times fall between about 5 to 500 ms. They increase with decreasing lipid concentration but are independent of the amount of peptide. This can be very well described in terms of a pseudo first order one step incorporation reaction. For DOPC the rate constants turn out to be nearly diffusion controlled (including penetration in the bilayer). A DMPC system, on the other hand, exhibits a somewhat smaller rate which is apparently determined by a slower conversion of the secondary structure.

THE HUMAN B CELL ASSOCIATED CD37 DIFFERENTIATION ANTIGEN. A GLYCOPROTEIN WITH EXTENSIVE N-GLYCOSYLATION

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The B cell associated CD37 antigen has been characterized by the use of monoclonal antibody HD28. The CD37 antigen is strongly expressed on human normal and leukemic B lymphocytes. This glycoprotein consists of a single chain protein core of approx. 25 kd to which two N-linked carbohydrate antennae of various length are linked. The total molecular mass ranges from 40 to 52 kd in most cells and in a lymphoma cell line which is growth-dependent on human serum, the glycosylation is enhanced resulting in a molecular mass of approx. 40 to 64 kd. The glycosylation degree of the CD37 antigen seems to be dependent on the specific growth requirements of the cells. Also, the quantitative cell surface expression of this glycoprotein seems to be correlated with the activation state of the cells studied because, after in vitro stimulation of human tonsillar B cells with BCGF, the expression of the antigen is largely enhanced.

LIPID POLAR GROUPS AS INDICATORS OF ELECTRIC CHARGE AND REGULATORS OF MEMBRANE SURFACE POTENTIAL

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The average orientation of the polar head groups of three commonly occurring phospholipids, i.e. phosphatidylcholine, phosphatidylethanolamine and phosphatidylglycerol has been determined to be parallel to the plane of the membrane. Addition of negatively or positively charged lipids or binding of metal ions, hydrophobic ions, and charged local anesthetics alters the headgroup conformation and orientation in a systematic fashion which can be measured by deuterium magnetic resonance. The observed changes in the nmr parameters are large and linearly related to the amount of electric charge bound to the membrane surface so that the lipid head group becomes a sensitiv electrometer. Lipids may regulate the metal ion concentrations at the membrane surface. Conversely, metal ions at physiological concentrations may change the membrane surface potential by up to 40 mV.

EFFECTS OF PROPRANOLOL ON THE Ca-ACTIVATED K PERMEABILITY IN HUMAN RED CELLS

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The effect of propranolol on the Ca- and/or Pb-activated K permeability was analysed with the patch-clamp technique. The results were compared with flux measurements in cell suspensions. The dependence of the single-channel activity on the cytoplasmic Ca concentration is shifted by an order of magnitude to lower Ca concentrations, and simultaneously, channel activity at saturating Ca concentration is reduced by propranolol. This is compatible with the results from the flux measurements which show stimulation of the K permeability by 1 mM propranolol at Ca concentrations in the nanomolar range and inhibition at micromolar concentrations.

EFFECT OF TRYPTIC DIGESTION OF THE MEMBRANE SKELETON ON ELASTICITY, VISCOSITY AND STABILITY OF THE HUMAN RED BLOOD CELL MEMBRANE

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Trypsin sealed into red cell ghosts causes a degradation of spectrin and an increase of the rate of lateral diffusion of the band 3 protein. After incubation at 15 ng/ml trypsin at 37°C for 1 hr, about 50% of the spectrin is digested and the lateral diffusion of band 3 has reached 50% of the plateau value obtained at very high trypsin concentrations, indicating a considerable disruption of the membrane skeleton. Nevertheless, no significant changes of membrane elasticity or viscosity can be detected. However, the membrane becomes quite fragile such that its viscoelastic properties can no longer be measured at trypsin concentrations > 15 ng/ml. Thus, viscoelastic properties and stability of the membrane are independently controlled by the membrane skeleton. This conclusion agrees with that of Chasis and Mohandes (1986) based on work performed with essentially different techniques.

LOCAL ANAESTHETICS AND PRESSURE.- A COMPARISON OF DIBUCAINE BINDING TO LIPID MONOLAYERS AND BILAYERS.

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The intercalation of the local anaesthetic dibucaine, in its cationic form, into monolayers of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine was studied at pH 5.5. The intercalation caused a monolayer expansion which was strongly dependent on the lateral pressure applied. By making judicial assumptions about the area requirement of dibucaine, the monolayer expansion curves could be transformed into true binding isothermes. Dibucaine binding isothermes were constructed for different monolayer pressures and were compared to a bilayer binding isotherm measured under similar conditions with UV spectroscopy. A monolayer-bilayer equivalence pressure of 30.7 to 32.5 mN/m was found. The analogy of the monolayer and bilayer binding isotherms further suggests that the binding of cationic dibucaine does not change the internal pressure in the bilayer phase. The pressure dependence of the intercalation of cationic dibucaine into lipid membranes may also be of relevance for the phenomenon of pressure reversal in anaesthesia.

THE 165 kDa PEPTIDE - A MAJOR PART OF THE CALCIUM CHANNEL

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The purified voltage-sensitive L-type calcium channel from rabbit skeletal muscle contains peptides of $M_r$ 165±5, 55±3, 32±2, 130±5 and 28±1 kDa in SDS-PAGE. The 130 kDa and 28 kDa peptides migrate as a single 165 kDa peptide under nonreducing conditions. These peptides were separated by HPLC. Peptide maps of the 165 kDa and 130/28 kDa peptide show, that these peptides are not related to each other. Photoaffinity labelling with the dihydropyridine analog ±azidopine and the diphenylalkylamine analog Lu47781 showed, that only the 165 kDa peptide contains high affinity binding sites. The 165 kDa peptide was phosphorylated to 1.5 mole phosphate/mole protein by cAMP-kinase. The isolated 165 kDa peptide was reconstituted to a voltage dependent calcium channel. Suggesting that the 165 kDa peptide is a major part of the L-type calcium channel.

**cGMP-ACTIVATED CHANNELS IN EXCISED PATCHES OF BOVINE ROD OUTER SEGMENT MEMBRANES**

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We recorded cGMP-induced currents across excised patches of the plasma membrane from bovine rod outer segments. Without cGMP the current/voltage profile was flat. After addition of cGMP (10-100 uM) to the bathing solution, the membrane conductance increased and the I/V-curve became non-linear. At 60 mV and 100 uM cGMP maximum currents of about 100 pA were observed. The range of cGMP concentrations that activated this conductance and the rectifying behaviour of the I/V-curves were similar to those, observed in excised membrane patches of amphibian rods.

CELL-TO-CELL CHANNELS IN ISOLATED PANCREATIC ACINAR CELL PAIRS

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Pairs of pancreatic acinar cells were isolated by collagenase treatment of the mouse pancreas. After establishing the double whole-cell patch configuration a junctional conductance of about 40 nS could be measured. A spontaneous uncoupling occured after about 30 min. Occasionally the stepwise opening and closing of several single cell-to-cell channels was identified. The cell-to-cell channel showed an ohmic behavior with an unit single-channel conductance of about 130 pS. Changes of the junctional conductance by about 50 pS were also observed. Uncoupling of the cells could be induced by addition of benzhydrol (0.3 mM) or octanol (0.4 mM) to the bath medium. Partial recovery of coupling could be attained in both cases by wash-out. By application of noise analysis single channel amplitudes and the corresponding kinetic parameters could be derived from multi-channel experiments. Comparison of the single-channel and multi-channel analysis gives evidence for conducting substates of the cell-to-cell channel.

MODULATION OF THE MEMBRANE INCORPORATION OF ALAMETHICIN BY SALT AND CHOLESTEROL.

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The membrane-water partitioning of the pore-forming peptide alamethicin has been investigated as a function of ionic strength and cholesterol content of the membrane. The results give insight into the modulation of peptide incorporation by cholesterol. We are particularly interested in correlations between partitioning and pore-forming properties of alamethicin in order to test the recently developed idea that the voltage gating of the alamethicin pore might be triggered by a dipole-mediated electric field effect on the partition coefficient. This idea is based on a "critical" effect seen in equilibrium titrations and traced to the aggregation of peptide in the bilayer (BBA 861(1986),141; Biochemistry, in press). The "critical concentration" where aggregation in the membrane starts to dominate is decreased by salt and increased by cholesterol in a way which corroborates our new view of the gating events.

FATTY ACID ACYLATION OF ANKYRIN IS REVERSIBLE AT THE ERYTHROCYTE PLASMA MEMBRANE

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The peripheral membrane protein ankyrin mediates the attachment of the membrane skeleton to the plasma membrane in erythrocytes. We recently found that ankyrin contains covalently bound long chain fatty acid (Staufenbiel and Lazarides, Proc. Natl. Acad. Sci. USA, 83, 318, 1986). Now evidence is presented that ankyrin is continuously acylated and deacylated in mature red blood cells. The acyl moiety attached to ankyrin turns over rapidly as compared to the polypeptide backbone which is stable throughout erythrocyte life. This indicates a regulatory significance of the fatty acid modification for the function of ankyrin.

RAPID TRANSIENT CHANGES OF CYTOPLASMIC pH AND THEIR INTERRELATION WITH POTASSIUM CHANNELS IN EREMOSPHAERA

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Electrophysiological experiments with the spherical green alga EREMOSPHAERA VIRIDIS showed transient and voltage-independant changes of membrane potential and conductance, a so-called chemical induced actionpotential (CAP). A CAP can either be released by light-off or by addition of different other factors: (1) DCMU, (2) acid media, (3) sodium acetate, (4) $NH_4Cl$ or (5) $BaCl_2$. Additional measurements with tetraethylammonium and various concentrations of different ions ($K^+$, $Na^+$, $H^+$, $Ca^{2+}$, $Cl^-$) indicate, that the CAP is caused by a temporary opening of $K^+$-channels in the plasmalemma (Köhler et al. 1985). To investigate the role of cytoplasmic pH ($pH_c$) in the signal-transduction from chloroplast to the $K^+$-channels, $pH_c$ was directly measured by fast $H^+$-sensitive microelectrodes. After light-off, as well as after addition of acid media or $NH_4Cl$, a transient acidification of $pH_c$, corresponding to a CAP, could be detected. In contrast, light-on causes a transient alkalisation, but no CAP. In each case of acidification, $pH_c$-change preceeds polarisation of CAP, which clearly indicates, that $pH_c$ plays an important role in internal signal transduction. Addition of $BaCl_2$ causes no preceeding acidification, which may be due to a more direct effect of $Ba^{2+}$- ions on $K^+$-channels.

Lit: K. Köhler, W. Steigner, W. Simonis, W. Urbach (1985), Planta 166,480-499

DENUDATION OF LIMULUS VENTRAL PHOTORECEPTOR CELLS FROM GLIAL CELLS BY ENZYMATIC TREATMENT

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To establish an easy and fast method to prepare photoreceptor membranes for patch clamp measurments, we have denuded the ventral photoreceptor of Limulus polyphemus from extracellular matrix and glial cells. Since not much is known about the specific constituents of the extracellular matrix of arthropods, we first identified collagen and elastin as components by their specific histochemical stains. These were treatet by different enzymes: specific and unspecific proteases and glycosidases. The effects of these treatments were observed with the electron microscope. Best results were obtained with the combination of 2mg/ml collagenase and 0,3mg/ml elastase. The normally observed mechanical resistance of the cell was completely missing so that penetration by microelectrodes was very easy. The response of photoreceptors treated according to this description was not reduced as was shown in an electrophysiological test lasting more than 3 hours.

APPLICATION OF PROTEINS TO PHOTORECEPTOR CELLS BY THE BACKFILLING METHOD
STUDIES WITH THE VENTRAL NERVE OF LIMULUS POLYPHEMUS

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The introduction of monospecific antibodies against membrane and soluble proteins would be a powerful tool for the investigation of the molecular basis of photoreception. Prerequisite for this type of experiments is the application of antibodies with a minimum of cell injury. We exploited the retrograde axonal transport system in Limulus polyphemus. To evaluate the pertinent parameters we established the experiment with horseradish peroxidase, a high molecular weight protein(47 kD). We used the isolated ventral nerve of the crab. The transversely cut proximal end of the nerve was immersed into the test solution containing a minimal HRP-concentration of 2mg x $ml^{-1}$. The transported HRP was stained by an enzymatic dye reaction to calculate the transport-rate. The transport rate was estimated to 150mm per day within a temperature range of 15 -22°C. Peroxidase conjugated antibodies( 200kD)were transported with a slightly lower velocity than HRP alone.

IMPORTANCE OF CARBOXYTERMINUS FOR IMPORT OF APOCYTOCHROME c INTO MITOCHONDRIA

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Apocytochrome c, the precursor protein of cytochrome c is synthesized in the cytosol without a cleavable presequence. It is then post-translationally transferred into mitochondria via specific receptors and converted to holocytochrome c by covalent attachment of heme by the enzyme cytochrome c heme lyase. We have cloned full-length cDNAs from the wild type and a mutant (cyc1-2) of Neurospora crassa. cyc1-2 produces an import incompetent apocytochrome c precursor. Nucleotide sequencing revealed that due to an intron splicing defect, the mutant precursor has an extended carboxyterminus. We suggest that an intact carboxyterminus is required for efficient import of apocytochrome c into mitochondria.

PROPERTIES OF PHALLOIDIN UPTAKE BY BASOLATERAL LIVER PLASMA MEMBRANE VESICLES

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The mechanism and driving forces for hepatocellular phalloidin uptake were studied by a rapid-filtration technique using basolateral liver plasma membrane vesicles.Overshooting phalloidin uptake was observed in the presence of a $K^+$ gradient as well as a $Na^+$gradient.$Na^+$could be replaced by $K^+$or $Li^+$.In the presence of choline chloride a distinct uptake reduction of 57% was seen.Countertransport phenomena suggest phalloidin transport rather than binding.Phalloidin uptake was inhibited significantly by taurocholate,iodipamide and antamanide,but only slightly by α-amanitin.Creation of a negative intravesicular potential by altered accompanied anions or by valinomycin-induced $K^+$-diffusion potentials enhanced the initial uptake rate for phalloidin,demonstrating rheogenic solute uptake. These findings are consistent with the concept of a multispecific sinusoidal transporter:The hepatocellular uptake of phalloidin is due to carrier-mediated transport,very similar to that of bile acids.Hepatic uptake of phalloidin is assumed to be driven by both a monovalent cation gradient and a membrane potential.

**Stereodynamische Kopplung von Lichtenergie und Ionentransport in Retinalproteinen**

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Infrarot, Resonanz-Raman, optische Spektren und andere Beobachtungen weisen auf einen Photozyklus von Bakteriorhodopsin und Halorhodopsin hin, der durch die Folge intermediärer Retinalisomere all-*trans*, → 13,14-*cis* → 13-cis → all-*trans* characterisiert ist. Eine derartige Dynamik sterisch verschiedener Isomere macht aus Retinal eine molekulare Pumpe mit einer extrem schnellen, lichtgetriebenen Vorwärtsreaktion und langsameren, thermischen Rückwärtsreaktionen, die an den $H^+$ und $Cl^-$ Transport gekoppelt sind. Unsere Ergebnisse basieren auf quantenchemischen Berechnungen des Einflusses äußerer Ladungen auf die stereodynamischen Eigenschaften, die Schwingungs- und optischen Spektren der protonierten Schiffschen Base des Retinal.

**"CHEMOMECHANICAL" LEAK FORMATION IN ERYTHROCYTES (RBC) TREATED WITH ETHYLDIMETHYLAMINOPROPYLCARBODIIMIDE (EDC)**

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**To study membrane barrier properties, RBC were treated with the $COO^-$-modifying agent EDC. EDC-treatment (1-20 mM, hct. 10%) abolishes cell deformability by membrane rigidification and induces minimal $K^+$-leakage. When EDC-treated cells are sheared, however, e.g. by resuspension after centrifugation or in a cone-plate viscometer (4/sec, 50% hct.), a rapid release of $K^+$ and colloid-osmotic lysis occur. Untreated cells did not lyse under these shear conditions. $K^+$ is released in a graded fashion, the fraction of leaky RBC depending on EDC-concentration, cell packing density and duration of shear. The EDC-activated $COO^-$ yields an amide when amines are added or hydrolyses when excess EDC is removed. Under both conditions leak formation is abolished without restitution of deformability. Thus, EDC activation of $COO^-$ and frictional forces between rigidified cells are thought to be required for leak formation. The EDC-modified RBC membrane may be an interesting model for studying the features underlying the shear stability of biological membranes.**

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$Ca^{2+}$- AND $H^+$ PUMPS IN $IP_3$ SENSITIVE PANCREATIC ENDOPLASMIC RETICULUM (ER)

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In isolated pancreatic ER MgATP-driven $^{45}Ca^{2+}$ uptake was inhibited by vanadate by 90%. Protonophores (carbonylcyanide-m-chlorphenylhydrazone and nigericin) and the $H^+$-ATPase blocker 7-chloro-4-nitro-2,3--benzoxadiazole (NBD-Cl) reduced $^{45}Ca^{2+}$ uptake by about 20%. The stimulatory effect of the intracellular messenger inositol 1,4,5-trisphosphate ($IP_3$) on $Ca^{2+}$ release was diminished in the presence of protonophores indicating involvement of a $H^+$ gradient in filling of the $IP_3$ sensitive $Ca^{2+}$ pool. MgATP-driven $H^+$ transport as measured by the acridine orange method was abolished by protonophores and NBD-Cl, but not by vanadate. If a $H^+$ gradient was generated by preincubation with MgATP in the presence of vanadate, $^{45}Ca^{2+}$ uptake from a medium without MgATP was stimulated, whereas preincubation with

NBD-Cl prevented $^{45}Ca^{2+}$ uptake. The data show that both a $H^+$- and $Ca^{2+}$ pump are located in an $IP_3$ sensitive $Ca^{2+}$ pool of ER. The $H^+$ pump generates a $H^+$ gradient that can promote MgATP-independent $Ca^{2+}$ uptake. (*Supported by DFG Th345/1-1)

PURIFICATION AND CHARACTERIZATION OF A 22 kDa PHOSPHOPROTEIN FROM RAT PAROTID MICROSOMES

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Stimulation of secretion in the parotid gland by agonists involving cAMP as second messenger leads to the phosphorylation of a particulate protein with apparent molecular mass of 22 kDa (protein III). This protein was found exclusively in the endoplasmatic reticulum and it behaves like an intrinsic membrane protein. It could be extracted from the ER-membranes only with Triton X-100, SDS, or concentrated formic or acetic acid. The purification of this protein involved extraction of the microsomes with Triton X-100, removal of the detergent by acetone precipitation, extraction of the water soluble proteins, the lipids and lipoproteins, preparative SDS-PAGE and finally RP-HPLC on C8-columns with formic acid as mobile phase. Protein III could be phosphorylated "in vitro" with the catalytic subunit of the cAMP-dependent protein kinase to a degree of approximately 0.1 mol $P_i$/mol protein. Tryptic and chymotryptic peptides containing the phosphorylation site of protein III were analyzed by RP-HPLC. The only phosphopeptide obtained after in vitro phosphorylation was identical with the phosphopeptide obtained after stimulation of intact rat parotid gland lobules with isoproterenol. Until now the function of this protein is unknown, but recent results showed that protein III is not only present in exocrine secetory glands (parotis, lacrimalis, submandibularis, pancreas) but probably also in liver cells. This indicates that the phosphorylation of protein III is not exclusively linked to the stimulus-secetion-coupling in secretory cells.

DEVELOPMENT AND RELEASE OF CELL PROTRUSIONS (PLASMA POLYPS, PP)

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We report about a mechanical induction of PP-development in the strips of human placenta post partum and about a chemical induction in the other series (for example monoiodoacetate, acetylsalicylic acid, benzoic acid, diclofenac).

We summarize our results concerning the contents of soluble and membrane-bound proteins in the PP's and the intact placenta, as well as concerning the concentrations of metabolic intermediate products and the protein contents in the endoplasmatic reticulum of both sources.

The paper describes also the determination of enzyme activities of the following enzymes: glycolytic enzymes, transaminases, glucose-6-phosphate-dehydrogenase, glucose-6-phosphatase, carboanhydratase, $Ca^{++}$- and $Na^+$-, $K^+$-ATPase, alkine phosphatase, γ-glutamyltransferase.

MEMBRANE FUSION, SERIES OF EXPERIMENTS ON PLASMA POLYPS OF HUMAN PLACENTA AND LIPOSOMES

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We report about experiments of membrane fusion on plasma polyps of the human syncytiotrophoblast and on fresh prepared, small liposomes.

The results are demonstrated by figures and videorecorder.

IMPORT INTO MITOCHONDRIA OF FUSION PROTEINS BETWEEN PROTEOLIPID AND DHFR

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Fusion proteins were constructed between different parts of the presequence or the total subunit 9 of Fo-ATPase (proteolipid) and mouse cytosolic dihydrofolate reductase (DHFR). The hybrid proteins were efficiently imported in vitro into Neurospora crassa mitochondria. Import depends on the presence of nucleoside triphosphates and a membrane potential. Binding of methotrexate to the DHFR part does not abolish binding to the mitochondrial surface but prevents import. We suggest that the fusion proteins are caught in translocation contact sites between outer and inner membrane (1).

(1) Schleyer, M. and Neupert, W. (1985) Cell 43, 339-350

CORECONSTITUTION OF YEAST PLASMA MEMBRANE ATPASE AND BACTERIORHODOPSIN

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Functional coupling of Bacteriorhodopsin (BR) and the Plasma Membrane ATPase (PMATPase) is a powerful tool to investigate the reactionmechanism of ATP-binding, ATP-hydrolysis and protontranslocation by the PMATPase. Well defined and stable protongradients, generated by BR, enable someone to estimate the kinetics of the PMATPase for a period of minutes.
Four different reconstitution methods were testet in order to gain a system where both proteins are orientated correctly (inside-out) and which is technically easy and reproducible.
To determine whether BR and PMATPase are incorporated into the same liposomal membranes we applicated illumination, ATP and/or protonophores in different orders and followed protontranslocation by using the fluorescence probe ACMA (9-amino-6-chloro-2-methoxyacridine).
ATP-hydrolosis and protontranslocation by PMATPase were inhibited by BR-generated protongradients.

EFFECTS OF CATECHOLAMINES ON MEMBRANES OF DIPTERIAN SALIVARY GLANDS

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The salivary glands of insects have been the subject of a number of genetical, neurochemical and electrophysiological investigations. In the present report membrane potential responses of salivary gland cells of Chironomus thummi were monitored during bath-application of biogenic amines. Adrenaline, noradrenaline and dopamine produced a depolarization of the membrane in a dose-dependent manner. This depolarization could not be abolished by amiloride or ouabain. Addition of some adrenergic receptor agonists and antagonists produced no response or only a marginal response. The response to catecholamines is reduced by prior external application of 4-aminopyridine (a K-channel blocking agent). It is suggested that a direct effect of catecholamines on K-channels of dipterian salivary gland cell membranes must exist.

## EFFECTS OF MEMBRANE ANISOTROPY ON SEROTONIN BINDING IN RAT BRAIN

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Fluorescence depolarization was used to measure the effects of membrane anisotropy on specific [$^3$H]serotonin (5-HT) binding in rat brain homogenates. Membrane anisotropy was differently affected by n-hexanol and ascorbate (5 mM): N-hexanol reduced, while ascorbate increased the anisotropy. Both substances, however, decreased 5-HT binding. Because of the different correlations between specific 5-HT binding and membrane anisotropy the results suggest that anisotropy is a critical factor, however, just one of several determinants in 5-HT binding studies.

## THE INFLUENCE OF SODIUMASCORBATE ON THE TRANSPORT OF POTASSIUM IN MALPIGHIAN TUBULES

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In most of the insects, the transport of $K^+$ is a prerequisit for urine formation and flow. In Drosophila larvae, this mechanism is influenced by a basolateral $Na^+,K^+$-ATPase and a furosemide sensitive co-transport as well as by an apical $K^+$ pump. Under physiological conditions, isolated tubules of Drosophila hydei produce within 1 h 0.52 nl/min urine at a $[K^+]$ = 211 mmol/l and a transport rate of 109 pmol/min. In the present studies, the effect of ascorbic acid (ASC) and its Na- and K-salts on this transport has been investigated. It could be shown that within 1 h Na-ASC ($5x10^{-4}$ mol/l, pH 6.9) decreases $[K^+]$ to 57 % and its transport rate to 44 % using the values obtained without Na-ASC addition as controls. The results obtained with ASC or K-ASC are not consistent. It is believed that the effect might be due to an inhibition of $Na^+,K^+$-ATPase a mechanism proposed by others (s. e.g. Ng et al., Biochem. Pharmacol. 34 (1985) 2425).

## IMPORT OF M13 PROCOAT PROTEIN INTO THE ENDOPLASMIC RETICULUM

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M13 procoat protein is processed to transmembrane coat protein by dog pancreas microsomes after completion of synthesis and in the absence of the SRP/ docking protein- system. ATP is required for fast and efficient processing of procoat by microsomes. Requirement for ATP is also observed in the absence of ribosomes or docking protein. This indicates the existence of a unique assembly pathway for procoat into microsomes which depends on ATP but does not depend on the SRP/ docking protein- and ribosome/ ribosome receptor-systems. We suggest that the ATP-requirement is linked to a so far unknown component in the cytoplasm, acting directly on the conformation of precursor proteins.

## ß-PYRIDOXAL-ADENOSINE 5'-DIPHOSPHATE - AN INSTRUMENT FOR DETECTING AN ESSENTIAL ARGININE RESIDUE IN THE ATP-BINDING SITE OF KIDNEY ($Na^+ + K^+$)-ATPase

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The dissociation constants ($K_D$) for three polyphosphate modified ATP-analogues were determined by measuring the displacement of [$^{32}$P]-ATP from the enzyme's binding sites. $K_D$ Adenosine 5'-tetraphosphate = 2,4 µM, $K_D$ ß-Phenyl-Adenosine 5'-diphosphate = 42 µM, $K_D$ γ-Phenyl-Adenosine 5'-triphosphate = 187 µM. According to these data the γ-Phosphate is not absolutely necessary. We synthesized ß-Pyridoxal-Adenosine 5'-diphosphate for active site labelling of kidney ($Na^+ + K^+$)-ATPase. The oxo-group of the pyridoxal-residue interacts with free amino-residues - as e.g. arginyl-residues - forming a "Schiff-base-complex" which can be stabilized irreversibly in presence of $NaBH_4$. [$^{32}$P]-labelled ß-Pyridoxal-Adenosine 5'-diphosphate inactivates the enzyme and marks probably an arginyl-residue in the polyphosphate binding site located on the α-subunit of the ATPase. Inactivation by ß-Pyridoxal-Adenosine 5'-diphosphate is hindered by addition of ATP or potassium.

## THE LDL-RECEPTOR - A TRANSPORTPROTEIN FOR APOPROTEIN B- AND E-CONTAINING LIPOPROTEINS

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The hepatic LDL-receptor has been shown to bind LDL and VLDL via their component apolipoprotein (apo) B or B and E. Binding of chylomicrons in different metabolic stages and of various HDL-subfractions has been tested by ligand blots and measured by membrane assays. Affinity was lowest for apo B-100 as in LDL, undetectable for B-48 as in chylomicrons, but was vastly increased by apo E as in remnants, the product of lipidhydrolysis of chylomicrons. However, the affinity was modulated by C-apos, which thus regulate the intravascular metabolism of chylomicrons. HDL-subfractions differed in their ability to bind to the LDL-receptor despite their abundance in apo E. $HDL_2$ bound with high affinity to membranes, but not to the LDL-receptor, nor did $HDL_1$, though being rich in apo E. VHDL, a recently isolated subfraction, bound to the LDL-receptor, probably due to structural differences or to its additional content of apo B. Light and electronmicroscopic autoradiographs demonstrated that binding of all lipoproteins led to endocytosis by hepatocytes and transport in endosomes and multivesicular bodies to lysosomal degradation.

## Deformation von kugelförmigen Vesikeln im elektrischen Feld

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Werden Lipid-Vesikeln einem homogenem Feld ausgesetzt, so ändert sich ihre Form. Bei nicht zu hohen Spannungen wird aus einer Kugel ein Ellipsoid. Die Deformation wird durch das Minimum der Summe aus krümmungselastischer Energie und der elektrischen Deformationsenergie gegeben. Es zeigt sich, daß das starke elektrische Feld in der Lipidschicht keinen Beitrag liefert. Für kleine Frequenzen des angelegten Feldes geht die Dicke der Membran nur als Korrektur für kleine Vesikeln ein, für höhere Frequenzen vermag die Lipidschicht die Feldlinien nicht mehr abzuschirmen, entsprechend kleiner wird die Deformation.

EXPRESSION OF ACETYLCHOLINE RECEPTORS IN RAT MUSCLE - MUSCULAR AND FUNCTIONAL CHANGES UPON DE- AND REINNERVATION

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The nicotinic acetylcholine receptor, a pentameric transmembrane protein, contains four different subunit types: in bovine muscle, a developmentally regulated change from the embryonic ($\alpha_2$, $\beta$, $\gamma$, $\delta$)- to the adult ($\alpha_2$, $\beta$, $\varepsilon$, $\delta$) type could be correlated with functional changes. We measured the abundance of $\gamma$- and $\varepsilon$-subunit specific mRNA in rat muscle upon denervation and during reinnervation. Together with the analysis of the receptor channel properties the results show, that motor nerves regulate the expression of different acetylcholine receptor types.

PROCESSING OF THE PRECURSOR OF BACTERIORHODOPSIN

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It was first discovered by Sumper and Herrmann (Eur. J. Biochem. 89 (1978) 229) that bacteriorhodopsin (BR) is formed as a precursor and is not processed to mature BR in spheroplasts. We observed on SDS-PAGE of purple membranes from the early growth phase two slower moving bands of molecular weight increased approx. by 1200 and 1800 in addition to mature BR. Immunodetection with specific antibodies against the pre-sequence and the carboxy terminus of BR identified these bands as two forms of the precursor of BR. In further experiments the dependence of the intensities of these bands on the age of the cell culture was investigated. Young cells showed a strong intensity in the upper band, which changed gradually to the middle one until after 7 days the band of mature BR became the strongest. A papain digestion study in combination with immunodetection provided clear evidence that the observed modification took place within the pre-sequence region of the precursor. All these facts together led to the idea that the precursor of BR is processed in two steps.

NEW EVIDENCE FOR THE ESSENTIAL ROLE OF ARGININE RESIDUES IN ANION TRANSPORT ACROSS THE RED CELL MEMBRANE

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2,3-butanedione, in the dark and in presence of borate, rapidly inactivates sulfate equilibrium exchange across the human red cell membrane. Reactivation occurs spontaneously after the removal of borate, indicating a reaction of butanedione with essential arginine residues. The inactivation exhibits pseudo-first order kinetics. Chloride ions are able to protect the transport system against inactivation with the reagent. This suggests that the modifyable residue (s) are constituents of the substrate binding site. The 60 % inhibited transporter can still bind $^3H_2DIDS$ covalently up to 85 % $\pm$ 12 of its total binding capacity. Studies on the reversible $^3H_2DIDS$ binding have shown that the 75 % inhibited transporter can still bind $^3H_2DIDS$ reversibly up to 71 $\pm$ 7 % of its total binding capacity. These results provide no evidence for a direct interaction between the essential arginine and $H^2DIDS$.

CONTRIBUTION OF MITOCHONDRIAL AND RED CELL MEMBRANE THIOLS TO FLUIDITY

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Variation of pH between 7 and 8 resulted in steep fluidity decreases for mitochondrial membranes between pH 7.3 and 7.0, and between 8.1 and 7.5 for those of red cells. Fluidities at the physiological range are thus considerably different for both membrane types. In both cases the loss in fluidity is accompanied by a decrease in functional membrane thiols. Enormous consequences appear for mitochondrial function in the ischemic or hypoxic state. Membrane lipid blebs, in parallel with generation of disulfide bonds from free radical scavenging action were found previously to occur as an early sign of hypoxic damage. This is a clear indication for loss of membrane asymmetry, which can be kept up only by a constant amount of energy. Most important is the finding that in the absence of functional thiols fluidity of the mitochondrial membrane dramatically decreases even at pH 7.4.

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ATP- EFFECT IN IMPORT OF PROTEINS INTO THE ENDOPLASMIC RETICULUM

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While the ER membrane is the system best understood with respect to specificity of membrane insertion of proteins, the general opinion on the mechanisms involved have recently been revised in the light of new data. There appear to be different requirements for competence for membrane insertion: One requirement is the presence of a signal sequence. Other requirements are related to intrinsic features of the whole polypeptides. The molecules may not be allowed to fold into structures which are thermodynamically favorable. For most proteins, SRP and docking protein and the ribosome and a ribosome receptor may serve to prevent the proteins from folding. With smaller proteins, such a complicated system may not be required. There, an ATP-requiring step seems to be related to preservation of a competent conformation of precursor proteins.