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## A PLASMA MEMBRANE WITHOUT MUCH OF A CELL

### Report from the 3<sup>rd</sup> Meeting of the European Red Cell Club

The European Red Cell Club brings together scientists interested in membrane phenomena that can be studied in the red blood cell.

From June 1 - 5, 1982 its third meeting was held at Schloss Münchenwiler in memory of the late Prof. W. WILBRANDT.

A wealth of new information about the structure and function of the red cell membrane was presented by workers from all parts of Europe. The meeting once again demonstrated how well the red cell membrane lends itself to the study of basic membrane processes. With all due respect for those who study the whole red cell's task in the body, which is oxygen and carbon dioxide transport, membraneologists cherish the erythrocyte for its being an exceedingly simple cell with a fairly complex membrane.



## ANION TRANSPORT

An important step of the  $\text{CO}_2$  transport in the blood consists of the  $\text{Cl}^-/\text{HCO}_3^-$  exchange across the red cell membrane. The exchange of these hydrophilic anions across the hydrophobic membrane is mediated by a glycoprotein with a membrane weight of 96,000 daltons, the band 3 protein. The abundance of this protein (about 25–30% of the total membrane protein) and the ease with which anion transport can be measured have made the study of anion transport very attractive, and the combined efforts of many laboratories around the world led, and still lead, to a rapid advancement of our knowledge about this transport system. A relatively large number of contributions to this subject reflected the current interest.

Experiments of EIDELMAN, GINSBURG and CABANTCHIK (Jerusalem) dealt with the kinetics of anion transport. The hetero-exchange of the fluorescent probe NBD taurine against  $\text{SO}_4^{2-}$  or Cl ions was followed by continuously monitoring the fluorescence change that is associated with the movement of the probe from the intracellular to the extracellular compartment. Most conspicuous was the demonstration of a break in the Arrhenius plot for the temperature dependence of NBD-taurine/Cl exchange at about  $18^\circ\text{C}$ , with an  $E_A$  of 23 kcal/mole below and 40 kcal/mole above that temperature. In contrast, for NBD-taurine/ $\text{SO}_4^{2-}$  exchange the activation enthalpy was 30 kcal/mole and independent of temperature. The authors suggested that the transport protein may exist in two different forms, one as a carrier for monovalent anions, and another for divalent anions, and that the different temperature dependences reflect this difference. From further experiments they derived the conclusion that the binding of the anions to the outer membrane surface is the rate-determining step of the anion exchange mechanism.

P.J. BJERRUM, WIETH, ANDERSEN and BORDERS JR. (Copenhagen) reported on acid based titrations of the transport system where they varied the pH in the medium at a constant intracellular pH of about 7.0. They found that anion translocation is activated by deprotonation of an acidic group with pK about 5 and inhibited by deprotonation of an alkaline group with pK about 12. They infer from the effects of substrate anions on the two pK values that they pertain to parts of the extracellular chloride binding site. Experiments with carbodiimides and phenylglyoxal suggest that the groups titrated at low pH are carboxylic acid groups of aspartic or glutamic acid and the groups titrated at high pH arginine residues. Under the experimental conditions chosen, phenylglyoxal binding was confined to one out of the 44 arginine residues that exist in band 3. The modified residue is located in the chymotryptic 35K fragment of the transport protein. A closer analysis of the data suggests that there may exist allosteric interactions between the two protomeric forms of the band 3 dimer in the red blood cell membrane.

The results were discussed in terms of a "zipper model" of anion exchange whose prominent feature consists of the breaking of salt bridges between carboxylate and arginyl residues by the substrate anions and a subsequent rearrangement of these groups, which transforms the protein from one conformational state in which it is capable to accept a substrate at one surface to another conformation in which it assumes the capability to accept a substrate at the opposite surface.

ZAKI (Frankfurt/Main) used a range of arginine-specific agents to modify the band 3 protein. These agents include, besides phenylglyoxal, 1,2 cyclohexandione and 2,3 butandione. All of these agents produced inhibition. They reduced the capacity of band 3 to bind  $^3\text{H}_2\text{DIDS}$ , a specific inhibitor of anion transport; but there was no simple stoichiometrical relationship between the effect on transport and the  $^3\text{H}_2\text{DIDS}$  binding capacity. The emphasis of her work was on the demonstration of allosteric interactions between binding sites for the substrate anion, the  $\text{H}_2\text{DIDS}$  binding site and the amino acid residues involved in the inhibition by the arginine reagents. When  $\text{Cl}^-$  was present, the inhibitory binding site of the band 3 molecule was protected against modification by the arginine specific reagents at pH 8.0, while  $\text{SO}_4^{2-}$  was without effect, indicating that  $\text{SO}_4^{2-}$  and  $\text{Cl}^-$  binding produced different conformational changes of the transport protein. There was some overlap between her observations and those of BJERRUM et al., lending mutual support to the respective findings.

BERGHOUT, LEGRUM and PASSOW (Frankfurt/Main) showed that dansylation of the red blood cell membrane leads to an inhibition of  $\text{Cl}^-$  transport, while  $\text{SO}_4^{2-}$  transport is accelerated. This effect depends on the state of the  $\text{H}_2\text{DIDS}$  binding site. When this site is occupied by the disulfonic acid APMB, the inhibition of  $\text{Cl}^-$  transport and the acceleration of  $\text{SO}_4^{2-}$  transport are potentiated. When the site is occupied with DNDS, both effects are reduced or prevented, suggesting that the two agents stabilise the  $\text{H}_2\text{DIDS}$  binding site in different conformations. The pH dependence of  $\text{SO}_4^{2-}$  transport across the dansylated membrane does not longer pass through the maximum around pH 6.3 that is typical for the untreated red blood cell, but reaches a plateau above pH 7.1, and thus assumes the same characteristics as the pH dependence for monovalent anion species in untreated red cells. Obviously, dansylation interferes with the  $\text{H}^+/\text{SO}_4^{2-}$  cotransport and converts the band 3 protein into a conformation in which it has no longer the capacity to discriminate between monovalent and divalent anion species.

SIGRIST and ZAHLER (Bern) explored the mechanism of anion transport by the modification of specific side chains in the hydrophobic region of the band 3 protein with arylisothiocyanates and their photoreactive azido derivatives. Phenylisothiocyanate reacts preferentially with a transmembrane C-terminal segment of band 3 (the 35K chymotryptic fragment),

thereby producing inhibition of anion transport. The exposure to phenylisothiocyanate in the presence of DNDS, and subsequent removal of the latter, shows that the capacity to transport anions is preserved. This indicates allosteric interactions between the DNDS binding site and the site that is susceptible for covalent phenylisothiocyanate binding. When the cross-linking azido derivative of phenylisothiocyanate was used, two band 3 molecules were linked together to form a covalent dimer.

PASSOW (Frankfurt/Main) reviewed current ideas about the mechanism by which band 3 mediates anion transport. He focused on the information that can be derived from studies with chemical modifiers like dansyl chloride, 1-fluoro-2,4-dinitrobenzene and H<sub>2</sub>DIDS. He concluded that anion transport as mediated by the stationary transport protein can only take place by conformational changes of a gate that is formed by amino acid residues on adjacent peptide chains that form an aqueous channel across the membrane. The conformational changes at the gate are associated with the reorganisation of other, more distant, portions of the peptide chain and can be monitored by measuring the reactivity of so-called modifier sites towards non-covalently and covalently binding modifiers of transport. He demonstrated matrices that represent the interactions between the substrate binding site and such modifier sites, as well as amongst different modifier sites, and he presented examples that demonstrated such interactions experimentally.

KOEHNE, HAEST and DEUTICKE (Aachen) incorporated purified band 3 protein into vesicles of different lipid composition and studied the DNDS-sensitive (=band 3 mediated) anion transport. The turnover numbers of anion transport were about 80% of those measured in the intact red cell. Transport across PC bilayers remained unaffected when up to 28 mole % of the PC was replaced by cholesterol. Replacement by up to 35 mole % of the PC by sphingomyelin left the transport process also unaltered. However, when this level was exceeded, transport ceased. Turnover numbers in PE vesicles were as high as in PC vesicles. Replacement of the PE by PS up to 33mol% did not affect the transport activity. Above this range, transport activity disappeared. The data suggest that transport activity of band 3 is affected by the fluidity and surface charge of the lipids. The effects of sphingomyelin could account for the differences of anion transport rates in red cells of different animal species which, from earlier work of DEUTICKE and coworkers, are known to be controlled by variations of sphingomyelin content.

PAPPERT and SCHUBERT (Frankfurt/Main) studied the association of purified band 3 protein in solutions of non-ionic detergents by means of analytical ultracentrifugation. They showed that reversible association equilibria exist between monomers, dimers and tetramers, thus confirming their earlier results with a detergent-free band 3 preparation. They discussed the

results in the light of independent observations on the intact red blood cell membrane from other laboratories, and concluded that the association equilibria that they observed with or without detergents may also exist in the red blood cell membrane.

A prerequisite for many biochemical studies is the solubilisation of the band 3 protein. In many cases, it is desirable to solubilise the protein without the application of detergents or to produce fragments of the large peptide chain that remain in solution even in the absence of detergents. HERBST and RUDLOFF (Frankfurt/Main) were able to solubilise the band 3 protein by acylation with dicarboxylic acid anhydrides at low temperatures. The acylated protein could be purified by conventional or high pressure gel filtration chromatography. Enzymatic degradation yielded water soluble peptides that could be separated by reversed phase chromatography and that can be used for further chemical characterisation.

#### Abbreviations used in the foregoing text

DNDS	=	4,4'-dinitro stilbene-2,2'-disulfonate
H <sub>2</sub> DIDS	=	4,4'-diisothiocyanato stilbene-2,2'-disulfonate
NBD	=	(4-nitro-2,1,3-benzoxadiazolyl)-2-amino ethane sulfate
PC	=	phosphatidyl choline
PE	=	phosphatidyl ethanolamine
PS	=	phosphatidylserine
APMB	=	2-(4-amino-3-sulfophenyl)-6-methyl-7-benzothiazol sulfonate

### SODIUM-POTASSIUM TRANSPORT

#### Passive Na-K-transport

The presentations in the session on passive cation transport principally concentrated on separating and defining the various routes by which Na<sup>+</sup> and K<sup>+</sup> cross the red cell membrane, particularly focussing on the true electrodiffusive passive "leak" pathway, and the NaK-co-transport system. Most speakers used Cl<sup>-</sup> dependence and/or furosemide inhibition to define the latter system, and ouabain to eliminate pump mediated fluxes. In the initial contribution M. ZADE-OPPEN used changes in medium pH and chloride concentration to alter membrane potential whilst monitoring Na and K efflux by flame photometry. The furosemide-sensitive Na and K fluxes were Cl<sup>-</sup> dependent, but varied in a complex way with changing membrane potential and pH. The furosemide resistant fluxes showed a high sensitivity to membrane potential (especially when membrane potential became positive) and ionic strength, con-



sistent with an electrodiffusive flux. However, some interaction with  $\text{Cl}^-$  was observed.

I. BERNHARDT (Berlin) also concentrated on the effects of membrane and surface potential on passive K (Rb) efflux in his paper. Using media of low and varying ionic strength, and cells treated with neuraminidase he showed that the passive K efflux was affected by the surface potential as well as the transmembrane potential, analysing the results in terms of DONATH and PASTUSHENKO's equation.

A.R. CHIPPERFIELD concentrated on passive Na fluxes, correlating the affinities for various loop diuretics and pharmacological agents on Na efflux and K influx through the NaK co-transport system. Further data on anion-substitution established that the anion-specificity for Na efflux was the same as for K influx.

In the final paper of the session, J. DUHM presented kinetic data for Na efflux and K influx via the cotransport system in human and rat red cells. He also reported data for human and Wistar Kyoto rats, investigating the possible relationship between hypertension and changes in NaK cotransport in red cells, a problem which is currently receiving a lot of attention.

In Dr. DUHM's rat experiments, an increased cotransport K flux was seen in DOCA-salt hypertension which was shown to be due to a fall in plasma K, since feeding a low K diet gave an even more dramatic increase in cotransport fluxes. Interestingly, in the low K diet experiment, the increased fluxes returned to control values within 24 h of feeding a normal K diet. This indicated the raised flux was due to a change in red cell volume via Na,K concentration changes. Rat red cells, like those of several other species, show a volume sensitivity in their furosemide-sensitive K fluxes.

### The Na-K-pump

In the absence of any Na the pump exchanges 1 K for 1 K ion. This process requires the presence of ATP and of inorganic phosphate ( $\text{P}_i$ ). The explanation for this requirement is that the form of the protein which binds K tightly ("occluded" K-form) called  $\text{E}_2(\text{K})$  is transformed by  $\text{P}_i$  (under phosphorylation) to a form which releases K to the inside. S.J.D. KARLISH and W.D. STEIN showed that without the two ligands K- (or Rb-) flux through the system is of the order of 1 % of the pump flux. The accelerating effects of ATP and  $\text{P}_i$  add together to increase the rate largely. The physiological meaning of the "occluded" K is obviously to minimize leak fluxes. D.A. EISNER and D.E. RICHARDS demonstrated that the kinetic behaviour tallies with the proposed scheme: for ATP there is an optimal concentration which rises with internal K concentration.  $\text{P}_i$  accelerates at high but inhibits at low concentration of ATP. A concentration of  $\text{P}_i$  which stimulates at high external K concentration can inhibit at low external K concentration.



## CALCIUM TRANSPORT AND THE CALCIUM-SENSITIVE POTASSIUM CHANNEL

### Ca-permeability

It has been known for some time that the intracellular free  $\text{Ca}^{2+}$  concentration is below  $10^{-6}$  M owing to a low passive permeability and a rapid extrusion by an ATP fuelled Ca-pump. V.L. LEW, R.Y. TSIEN, C. MINER and R.M. BOOKCHIN reported experiments done with a new chelator for  $\text{Ca}^{2+}$  which can be trapped inside intact cells (R.Y. TSIEN, *Nature* 290, 527-528, 1981) leading to the conclusion that the physiological intracellular  $\text{Ca}^{2+}$  concentration is 10-30 nM ( $\sim 10$  times less than thought before), that the steady state leak influx of  $\text{Ca}^{2+}$  (and hence the steady state pump flux) is  $\sim 50 \mu\text{mole/l cells} \cdot \text{h}$  (5 times more than thought before) and that part of the leak flux is increased by metabolic processes and reduced by a rise in internal  $\text{Ca}^{2+}$  concentration.

L.O. SIMONSEN, J. GOMME and V.L. LEW devoted a large effort to the study of the famous ionophore for bivalent cations, A 23187. By assessing  $^{45}\text{Ca}$  flux kinetics in the presence of the ionophore they found that the substance distributes homogeneously among all cells even at low concentration and redistributes between cells in a matter of seconds. This work further shows that Ca-buffering is also uniform in all cells and that, if there is a calcium compartment which exchanges faster than the main calcium pool, it must be small ( $\sim 1\%$ ).

### The calcium-pump

V. NIGGLI, E. SIGEL and E. CARAFOLI propose that the purified pump protein, reconstituted into artificial phospholipid ("asolectin") vesicles exchanges one  $\text{Ca}^{2+}$  ion for two protons (i.e. is not electrogenic) which seems to meet the most natural assumption, that the transport site after discharging  $\text{Ca}^{2+}$  moves back in the protonated (uncharged) form. This is not necessarily at variance with other observations showing that  $\text{Ca}^{2+}$  transport is accompanied by the movement of permeating anions (and is commuted to a Ca-cation exchange when they are absent) (WAISMAN et al., *JBC* 256, 415-424, 1981; ROSSI & SCHATZMANN, *J. Physiol.* 327, 1-15, 1982). A strong argument of NIGGLI et al. is that the observed appearance of extra protons (over and above those from ATP hydrolysis) in the medium is reduced by an uncoupler (the protonophore CCCP) which is unexpected if the appearance of protons were to reflect a redistribution according to the membrane potential set up by the pump.

G. GARDOS and B. SARKADI summarized experiments showing that the pump is activated either by calmodulin or by phosphatidylserine, phosphatidylinositides, lysophosphatidyl-

choline or oleic acid. The action of calmodulin is mimicked not only by such compounds but also by limited proteolysis, removing a 30 kD peptide (TAVERNA & HANAHAN, *Biochem. Biophys., Res. Comm.* 94, 652-659, 1980; SARKADI et al., *Cell Calcium* 1, 287-310, 1980; STIEGER & SCHATZMANN, *Cell Calcium* 2, 601-616, 1981; NIGGLI et al., *JBC* 256, 8588-8592, 1981). E. CARAFOLI, who further analysed the trypsin action, finds at very early stages of proteolysis a 90 kD fragment to which azido- $^{131}\text{I}$ -calmodulin still binds (and activates) and in later stages a 80 kD peptide which seems to be the calmodulin independent form. Thus the 30-40 kD peptide found previously (SARKADI et al., STIEGER & SCHATZMANN) seems not to be pertinent to the calmodulin sensitivity but a 10 kD peptide might. Mimicry of calmodulin by phosphatidylserine or oleic acid was also reported by K. GIETZEN, I. SADORF and H. BADER. SARKADI pointed out that Ca-activation (by calmodulin binding) of the pump is accompanied by phosphorylation-dephosphorylation of phosphatidylinositol. Since phosphatidylinositides also activate the pump he intimates a parallel role of these with calmodulin. ENYEDI et al. showed that  $\text{Mn}^{2+}$  instead of  $\text{Mg}^{2+}$  is particularly active in inducing phosphatidylinositol phosphorylation and might be a convenient tool to dissect this complicated interplay.

O. SCHARFF and B. FODER in elegant experiments determined separately the two rate constants of the reaction of Ca-calmodulin with the main pump protein.  $\text{Ca}^{2+}$  changes the two rate constants inversely with the result that the dissociation constant drops from 25  $\mu\text{M}$  to 2.5 nM when the  $\text{Ca}^{2+}$  concentration rises from 0.1  $\mu\text{M}$  to 20  $\mu\text{M}$ .

Transient phosphorylation-dephosphorylation of the main protein during a pump cycle is thought to be the normal pathway for ATP hydrolysis by the Ca-pump. A. ENYEDI et al. reported that high concentrations of  $\text{Mn}^{2+}$  or  $\text{Ni}^{2+}$  inhibit phosphorylation while  $\text{Co}^{2+}$  and  $\text{Fe}^{2+}$  block dephosphorylation. S. LUTERBACHER and H.J. SCHATZMANN presented evidence for  $\text{La}^{3+}$  to exert its inhibitory action on the system by interfering with the transition between the first ( $\text{E}_1 \sim \text{P}$ ) and the second ( $\text{E}_2 - \text{P}$ ) form of the phosphorylated intermediate. K. GIETZEN et al. studied the action of alleged "calmodulin inhibitors" such as penfluridol, trifluoperazine and calmidazolium and clearly showed that apart from having an effect on calmodulin (which is undisputed) they interact with the pump protein and therefore inhibit activity stimulated by procedures not involving calmodulin (see above).

H.U. WOLF et al. studied the Ca-ATPase from human red cells which does not bind to a calmodulin column. It is in all aspects very similar to the one which is retained but is, as expected, not activated by calmodulin. Its mol.wt. is 20-30 kD below that of the calmodulin sensitive protein. Interestingly, this truncated Ca-ATPase is inhibited by 8-azido-ATP (after photocoupling) whereas the classical enzyme is not.

D. MARETZKI and S. RAPOPORT find in isotonically prepared membranes of human red cells an EGTA-soluble protein of 120 kD mol.wt. made up of three subunits, one of which is calmodulin. This large protein increases the  $\text{Ca}^{2+}$  affinity of the pump, as calmodulin does, but it differs from calmodulin by decreasing the affinity for ATP (to a  $K_m^{\text{app}}$  of something like 0.7 mM).

#### The Ca-sensitive K-channel

On the basis of experiments involving binding studies of  $^3\text{H}$ -calmodulin to inside-out vesicles, L. PAPE and B.L. KRISTENSEN discussed the possibility that opening of the  $\text{Ca}^{2+}$ -sensitive K-channel is operated via calmodulin. J. GARCIA-SANCHO showed that the affinity for  $\text{Ca}^{2+}$  of this channel depends on the redox potential. Electron donors increase  $\text{Ca}^{2+}$  affinity and oxydized cytochrom c lowers it. At pH 7.5 the E6 of the pertinent membrane site seems to be 50 mV.

### TRANSPORT OF AMINO ACIDS AND SUGARS

#### Amino acids

A small group of communications focussed on the transport of amino acids and of sugars across the red cell membrane. ELLORY (Cambridge) reviewed the current knowledge on the complex and partially species - specific transport systems for amino acids. At least five different transport pathways for amino acids can be discriminated in human erythrocytes. Each one specifically mediates the transport of a small group of closely related amino acids. The transfer of amino acids is partially coupled to a simultaneous transmembrane movement of Na. Erythrocytes are well suited as model systems for quantitative studies of amino acid uptake. Thus, recent experiments have shown close correlations between the Na-dependent glutamate-transport in dog erythrocytes and the corresponding functionally very important transport system in nerve cells. In ganglionic cells, a rather difficult object for transport studies, glutamate serves as a precursor of the neurotransmitter  $\gamma$ -amino butyric acid.

#### Sugars

BRAHM (Copenhagen) compared the kinetic constants of glucose net efflux into a glucose-free medium and of glucose exchange diffusion in human red cells. He was able to measure these constants with high time resolution (msec) over a large temperature range (0-45°C). Differences in the kinetic constants for net and exchange fluxes in earlier experiments had suggested that

two different carrier systems might be involved in glucose transport. However, because of the high flux rates the earlier data had been obtained at lower than physiological temperatures. BRAHM observed similar kinetic constants for net and exchange fluxes at higher temperatures and therefore concluded that only a single carrier system for glucose exists in the red cell membrane.

By contrast to mammalian red cells, the transfer of sugars in fresh avian erythrocytes is not dominated by a specific saturable carrier-mediated flux. As shown by SIMONS (London) specific carrier transport for glucose is activated in pigeon red cells only after a drop in cellular ATP concentration. At normal ATP levels the transport of sugars is unspecific and not saturable. Surprisingly the transport rate in this unspecific system is linearly related to the intracellular free  $\text{Ca}^{2+}$  concentration.

## MEMBRANE STRUCTURE AND BIOCHEMISTRY

### Mechanical properties of the membrane and cell shape

The problem of the possible role of spectrin for the viscoelastic behaviour of the red cell membrane in shear and for maintaining the characteristic surplus of surface area were discussed by H. SCHMID-SCHÖNBEIN and R. GREBE. The authors propose - in contrast to the concept of a cytoskeletal network of spectrin - a high concentration of membrane-bound spectrin tetramers (dense periodical colloidal system, DPCS) at the inner surface showing thixotropic behaviour. The high negative charge of this DPCS is supposed to counteract the tendency of surface reduction. This concept of DPCS was further substantiated by the following posters: "Spectrin and red cell shape I: neuraminidase reverses exovesiculation in genetically spectrin deficient mouse red blood cells" (H. SCHMID-SCHÖNBEIN, H. HEIDTMANN, S. BERNSTEIN); "Spectrin and red cell shape II: neuraminidase treatment abolishes exovesiculation after spectrin denaturation of normal human RBC" (H. HEIDTMANN, R. GREBE and H. SCHMID-SCHÖNBEIN).

According to S. SVETINA and B. ZEKS the difference of surface area of the outer and inner lipid leaflet  $\Delta A$  is a critical parameter for the red cell shape ( $\frac{\Delta A}{A} \cdot 10^3 \approx 2-4$ ). The author calculated the leaflet area changes as a function of cation interaction with the charged polar headgroups. Basis of the calculation was the minimum electrostatic and elastic energy of the system.

I. SZASZ et al. dealt with the echinocyte shape change induced by  $\text{Ca}^{2+}$  and stressed that it probably involves many different factors and that a straightforward mechanism cannot be described at the present time.

#### Chemical architecture of the membrane

The transbilayer movement of PC, PE, PS and S was studied as a function of spectrin cross-linking by diamide by C.W.M. HAEST, J. ERUSALIMSKY, V. DRESSLER and G. PLASA. Also lysophospholipid flip-flop after incorporation from outside was used to measure the decrease of lipid asymmetry. As a result the spectrin cytoskeleton and its interaction with the internally located PS and PE is important for the maintenance of the asymmetric orientation of these two phospholipid classes. The externally oriented S is not affected by spectrin cross-linking and therefore does not seem to get access to the flip-flop sites. The work is important for a better understanding of the reason of the lipid asymmetry in red cell membranes.

In a part of their work on phospholipid transbilayer movement W. BERGMAN, C.W.M. HAEST and B. DEUTICKE especially studied the behaviour of lyso-PC and lyso-PS. Lyso-PC from human red cells equilibrates between the two leaflets with  $T_{1/2}$  of  $\sim 16$  h whereas lyso-PS flips about 5 times faster and becomes accumulated at the inner leaflet. As shown in previous presentations, the flip-flop rates become increased up to 200-fold after diamide treatment (cross-linking) of spectrin, and the accumulation of lyso-PS at the inner leaflet no longer occurs. Since the microviscosity of the membrane is not altered by this treatment, the authors interpret their data as a local perturbation of the lipid domain or of boundary lipids.

Upon introducing dimyristyl-PC (DMPC) into red cell membranes, M. WEITZ, O.J. BJERRUM, P. OTT and U. BRODBECK find a release of small vesicles lacking essentially the cytoskeletal protein components. Acetylcholinesterase and two other as yet unidentified glycoproteins were accumulated 2-7 times in these vesicles compared to band 3 protein and glycoprotein. The authors suggest that the redistribution of proteins in the vesicles reflects differences in their interactions with other membrane components and their relative mobility within the erythrocyte membrane.

Ankyrin is supposed to anchor the cytoskeletal spectrin 1 and 2 to band 3. However, on the basis of his data H.U. LUTZ could show that ankyrin is not necessarily distinct from spectrin, but rather originates from spectrin 2 by an exclusive cAMP-dependent phosphorylation of some specific domains which are different from the autophosphorylated sites.



B. DEUTICKE, B. POSER and P. LÜTKEMEIER showed that diamide treatment of red cells resulting in cross-linking of spectrin leads to an unspecific permeability increase for small non-electrolytes and monovalent ions. The authors suggest that aqueous pores are formed by this treatment, possibly fluctuating because the calculations result in 0.1 to 1 pore/cell formed. The mechanism is still unclear as well as the question whether such pores may naturally occur.

#### Membrane enzyme activities

Cell triggering or hormone signal transmittance through the plasma membrane may well be accomplished by  $\text{Ca}^{2+}$ -induced liberation of arachidonic acid as precursor for prostaglandin or HETE synthesis. M. HELLER, F. MESTEL, B. RINDLISBACHER, H. PORZIG and P. ZÄHLER showed that reticulocytes in presence of  $^{14}\text{C}$ -arachidonate incorporate this fatty acid into the membrane phospholipids. In presence of  $\text{Ca}^{2+}$  and Ionophore (A 23 187) most of the arachidonate is immediately processed to 15-HETE and 5-HETE suggesting an activation of the lipoxygenase or an inhibition of the acyl-transferase.  $\text{Ca}^{2+}$  therefore seems to be a potent regulator of the arachidonate metabolism.

Glycosylation of membrane proteins which takes place in endomembranes was studied by E.G. BERGER and F.J. HESFORD using galactose-deficient glycophorin and galactose transferase from human erythrocyte membranes as a model. It could be shown that the transferase is a strongly membrane-bound enzyme with an inaccessible cytoplasmatic-oriented active site. In Triton-X-100 the enzyme transfers galactose to galactose-deficient glycophorin in a 15-O-glycosidic position. Whether this enzyme exerts any function in mature erythrocytes or may be considered a remnant from membrane assembly is still unclear.

#### MATURATION-DEPENDENT FUNCTIONS IN RED CELLS

Two communications of the meeting focussed on characteristic changes in cellular metabolism and catecholamine responsiveness that occur during maturation and aging of mammalian red cells. Apparently, the creatine metabolism of red cells that was studied by RAPOPORT and SYLLM-RAPOPORT (East-Berlin) is a very sensitive indicator of red cell aging and elimination as well as of pathological disturbances of red cell maturation. The high level of cellular creatine in normal reticulocytes (10 mM) decreases to about 0.4 mM in mature red cells. Hypoxia and an increased rate of red cell formation result in the elevation of cellular creatine. In blood donors cellular creatine is frequently elevated for a period of up to 6 weeks.

Reticulocytes but not mature red cells contain the whole enzyme and receptor inventory of cells that are sensitive to  $\beta$ -adrenergic stimulation. PORZIG and coworkers (Bern) showed that rat reticulocytes - like other catecholamine-sensitive cells - desensitize towards  $\beta$ -adrenergic stimulation after chronic exposure to  $\beta$ -adrenergic agonists. This regulatory decrease in the cyclic 3'5'-AMP response and in  $\beta$ -adrenoceptor numbers suggests a functional role for the system in reticulocytes. However, specific cyclic AMP-dependent functions have not yet been described in reticulocytes. Therefore, the observation of a cAMP-dependent increase in reticulocyte K permeability by PORZIG et al. will perhaps help to define more clearly the functional importance of the  $\beta$ -adrenergic system in immature red cells.

### IN VITRO STUDIES ON MALARIA-INFECTED HUMAN RED CELLS

Two communications by CABANTCHIK and by GINSBURG (Jerusalem) were concerned with important new results on interaction of *P. falciparum* with human erythrocytes. Together with several coworkers the authors developed an in vitro culturing method for malaria parasites. They showed that the invasion of merozoites into red cells is probably preceded by the association of the parasite with a specific membrane receptor. The receptor is localized within the membrane glycoprotein glycophorin. The lectin "wheat germ agglutinin" that reacts specifically with glycophorin inhibits the invasion of red cells. These results explain earlier observations of a relative resistance against *P. falciparum* infection in persons with a genetic deficiency in glycophorin. During the intraerythrocytic development of *P. falciparum* the membrane permeability of the infected cells is drastically changed. The Jerusalem group was able to show that a new channel or pore-like structure is incorporated into the red cell membrane during the trophozoite stage. The parasite induced channel is rather non selective, discriminating as a molecular sieve merely according to size and conformation. The chemical nature of this structure is as yet unknown.

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