CELLULAR AND MOLECULAR BIOLOGY OF NORMAL AND ABNORMAL ERYTHROID MEMBRANES

Organizers: Carl M. Cohen and Jiri Palek

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Cellular and Molecular Biology of Normal and Abnormal Erythroid Membranes

Genes for Erythroid Membrane Proteins-I

CD 001 MOLECULAR ANALYSIS OF SPECTRIN STRUCTURE AND BINDING, Daniel Branton, Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138

Spectrin's function in erythrocyte shape and membrane stability must depend on its length, its flexible structure, and its binding properties. To learn how these properties contribute to spectrin function, we are comparing and contrasting mammalian, avian and insect spectrins. DNA and predicted amino acid sequence comparisons emphasize some of the unique properties of different spectrins as well as the remarkably well conserved contour length, repeat structure, and binding interactions of spectrins that are as distantly related as man and fruit-fly. Given this conservation of properties, we have focussed our studies of spectrin on *Drosophila*, where analysis of function can utilize genetic as well as molecular and biochemical approaches. When spectrin cDNA is expressed in *E. coli*, the resultant fusion proteins appear to be normally folded, soluble molecules that retain many of their native binding properties. Using fusion proteins expressed from selected regions of *Drosophila* DNA, we have mapped calmodulin and interchain binding sites and have explored the extent to which these binding sites are conserved between distantly related species.

Genes for Erythroid Membrane Proteins-II

CD 002 CHARACTERISTICS OF A cDNA FOR HUMAN RED CELL ANKYRIN, Stephen Lambert, Jack W. Lawler, Hu Ilan Yu, David W. Speicher, Josef T. Prchal and Jiri Palek, Dept. of Biomedical Research, Division of Hematology/Oncology, St. Elizabeth's Hospital of Boston; Tufts University School of Medicine, Boston, MA; Wistar Institute, Philadelphia PA; University of Alabama Medical School, Birmingham, AL.

The major function of the peripheral protein ankyrin (M_r=210,000) is to couple the red cell membrane skeleton with the lipid bilayer through an interaction with spectrin and the integral protein band 3. Proteolytic domain mapping of the ankyrin molecule has shown it to be bipolar, the band 3 binding site being contained within a basic, proteolytically resistant domain, whereas the spectrin binding site is found within a neutral phosphorylated domain with a high degree of proteolytic sensitivity. To study the structure of ankyrin further, we have screened a human reticulocyte cDNA library (in λgt 11) with an anti-ankyrin antibody. The resulting 1.4 Kb clone has been verified by matching the derived amino acid sequence with 108 amino acids from 5 ankyrin proteolytic fragments. The clone is found to contain the amino terminus of the spectrin binding peptide and reflects the bipolarity of the ankyrin molecule. The first 150 residues are arranged in a series of highly conserved 33 amino acid repeats (homologies ranging from 27-45%) the sequence of which indicates a high degree of ordered secondary structure by predictive algorithms. However, these repeats are not conserved within the spectrin binding region of the clone and predicted secondary structure indicates a high content of β-turns. The use of this cDNA as a probe on a panel of cell-sorter separated human chromosomes allows assignation of the red cell ankyrin gene to chromosome 8. Northern blot analysis of mRNA from a human erythroleukemia cell line gives the size of the ankyrin message at 9 Kb. Southern blotting reveals the presence of only 1 ankyrin gene of at least 20 Kb.

Cellular and Molecular Biology of Normal and Abnormal Erythroid Membranes

Biogenesis of Mammalian Erythroid Membranes-I

CD 003 THE BIOGENESIS OF MEMBRANE SKELETON IN NORMAL AND ABNORMAL MAMMALIAN RED CELLS, Manjit Hanspal, Jatinder S. Hanspal and Jiri Palek, Dept. of Biomedical Research, St. Elizabeth's Hospital of Boston, Tufts University School of Medicine, Boston, MA 02135
The synthesis and assembly of membrane skeletal proteins has been studied extensively in chicken erythroid cells and to some extent in erythroleukemic lines, but relatively little is known about the biogenesis of membrane skeleton in mammalian rbc precursors. We have studied the synthesis, assembly and turnover of Sp, ankyrin and protein 4.1 in rat nucleated rbc precursors and reticulocytes, human nucleated rbc precursors and human reticulocytes. Furthermore, we evaluated the molecular basis of ankyrin and/or spectrin defeciencies in patients with HS and HPP. Pulse-labeling studies show that in both nucleated rbc precursors and early reticulocytes, α Sp is synthesized in 3-4 fold excess over β Sp, yet the two subunits are assembled in equimolar amounts on the membrane. Our results also show that Sp, ankyrin and protein 4.1 are synthesized in large excess than the amounts which get assembled on the membrane. The cytosolic pools of all three proteins are rapidly catabolized while their membrane-associated counterparts are stable. Since the synthesis and assembly of Sp and ankyrin in early reticulocytes is similar to that in their nucleated rbc precursors, we are employing the former system to study patients with different hemolytic anemias. In one such patient with severe atypical HS and whose rbc's contain half the normal amounts of Sp and ankyrin, we find that the synthesis of Sp is normal but its assembly on the membrane is markedly reduced due to the synthesis of unstable ankyrin. In an HPP characterized by mutant Sp $\alpha^{I/46}$ and a partial Sp deficiency, we find that the latter is due to increased susceptibility of the mutant Sp to degradation prior to its assembly on the membrane.

CD 004 ERYTHROID MEMBRANE AND CYTOSKELETAL DEVELOPMENT IN AN IN VITRO MODEL OF TERMINAL MAMMALIAN ERYTHROID DIFFERENTIATION, Mark J. Koury, Stephen T. Koury and Maurice C. Bondurant, Division of Hematology, Vanderbilt University and Veteran's Administration Medical Centers, Nashville, TN 37232. An in vitro model of terminal mammalian erythropoiesis has been developed. In this system, a homogeneous population of proerythroblasts isolated from the spleens of mice infected with the anemia-inducing strain of Friend virus (FVA cells) differentiates into reticulocytes in response to erythropoietin (EP). In vitro, FVA cells retain a maturation time course, an EP dose-responsiveness, and a sequence of specific differentiation events that are similar to uninfected, murine colony forming units-erythroid (CFU-E). α and β spectrin were synthesized in FVA cells both before and after their exposure to EP in culture. At all stages of FVA cell development, the majority of newly synthesized spectrins were found in the cytoplasm where they were rapidly degraded. Although twice as much newly synthesized α spectrin as β spectrin was found in the cytoplasm at both early and late periods of differentiation, α spectrin was degraded more rapidly. Although only minor proportions of the newly synthesized spectrins were membrane associated, these proportions increased in later stages of FVA cell maturation. These greater proportions of membrane-associated spectrin in later periods of FVA cell development coincided with the synthesis and accumulation of band 3 in the FVA cell membrane. Between 40 and 48 hours of culture with EP, the majority of FVA cells enucleated giving rise to reticulocytes and extruded nuclei. Each extruded nucleus was surrounded by a thin layer of cytoplasm and an intact plasma membrane. The distribution of spectrin, filamentous actin (F-actin), and microtubules (MTs) were monitored by immunofluorescence microscopy in differentiating FVA cells. Spectrin fluorescence was diffuse in all stages of maturation but had a prominent membrane association at the later stages. Spectrin segregated with the reticulocyte at enucleation. MTs formed prominent cages around the nuclei in early stages but during maturation their frequency declined such that by enucleation only diffuse fluorescence was found in the reticulocytes. F-actin had a diffuse cortical location in early phases of maturation, became patchy as the cells matured, and formed an annular structure between the nucleus and the forming reticulocyte during enucleation. This annular structure was similar to the F-actin structures found in cleavage furrows of mitotic cells. The patchy F-actin segregated with reticulocyte. Cytochalasin D reversibly blocked enucleation of FVA cells while colchicine, vinblastine, and taxol had no effect on enucleation. These results indicate that F-actin is essential for enucleation while neither the absence of MTs nor excessive numbers of MTs had an effect on enucleation.

Cellular and Molecular Biology of Normal and Abnormal Erythroid Membranes

Biogenesis of Mammalian Erythroid Membranes-II

CD 005 STRUCTURE OF THE ERYTHROPOIETIN RECEPTOR AND THE METABOLISM OF THE RECEPTOR IN ERYTHROPOIETIN RESPONSIVE ERYTHROID CELLS, Stephen T. Sawyer, Div. of Hematology, Dept. of Medicine, Vanderbilt Univ. School of Medicine, Nashville, TN 37232. A method of obtaining immature erythroid cells which respond to erythropoietin (EP) by undergoing almost complete late erythroid maturation including enucleation was developed in this laboratory. These cells are purified from the spleens of mice infected with the anemia strain of Friend virus (FVA cells). In addition to studies on the number of receptors for EP, the internalization and degradation of EP, and investigations into late erythroid maturation such as the changes in metabolism, iron gathering ability and induction of hemoglobin synthesis and erythroid specific proteins have been carried out during the progression of the FVA cells from proerythroblasts to reticulocytes. Autoradiography of bound ^{125}I-EP indicated that the receptors were lost by the entire population of cells during maturation. EP endocytosis was qualitatively the same at all maturation stages. EP receptors were also studied in two established cell lines which are dependent upon EP for proliferation and survival but do not differentiate in response to EP. Where FVA cells have both high and low affinity receptors for EP, these cells have more receptors (~4,000) which are only the low affinity type (Kd = 10^{-9}M). Exposure of these cells to EP leads to a 10-fold reduction of the number of EP receptors within 24 h. Withdrawal of the EP leads to a return of the high number of EP receptors within 3 to 6 h. This effect is due to the synthesis of new receptors and does not appear to due to recycling of occupied receptors. This suggests down regulation through destruction of receptors or the inhibition of receptor synthesis. Cross-linking of ^{125}I-EP to membranes from a variety of erythroid cells labels proteins of 100 kDa and 85 kDa (140 and 125 kDa bands). The relatedness of these two receptor proteins in FVA cells was investigated by proteolytic mapping using the Cleveland method. Identical lower molecular weight fragments were generated by protease treatment of the 140 kDa and 125 kDa ^{125}I-EP/receptor crosslinked complexes. This result suggests that the primary sequence of amino acids in the 100 kDa protein and 85 kDa protein of the EP receptor are very similar, if not identical. Digestion of cross-linked membranes with mixtures of glycanases which remove N-linked, O-linked sugars, and sialic acid showed no detectable carbohydrate on the receptor proteins. These results suggest that the two proteins of the EP receptor are the same gene product which are processed differently by the cell (other than glycosylation) or the products of closely related but distinct genes. A likely possibility is that the 100 kDa protein is cleaved by protease(s) to generate the 85 kDa protein either in the intact cell or during the binding and cross-linking processes.

Regulation of Membrane Protein Functions-I

CD 006 Erythrocyte Membrane Bound Protein Kinase is Regulated by Phosphatidylinositol-4,5-bisphosphate. R.A. Anderson, Department of Pharmacology, University of Wisconsin Medical School, Madison WI 53706 In the erythrocyte, a membrane-bound protein kinase has been shown to phosphorylate a number of membrane proteins modulating their function (Eder et.al., Biochemistry 25:1764, 1986). These include spectrin, ankyrin, band 3, proteins 4.1 and 4.9, the phosphatidylinositol-4-phosphate 5-kinase, and the kinase is autophosphorylated. Although this protein kinase appears important in regulation of membrane proteins, the mechanism of kinase regulation has not been elucidated. Here we report that the protein kinase binds to the membrane by an association with a minor membrane glycoprotein (a glycophorin). Binding to the glycophorin does not significantly modify protein kinase activity; however, upon binding the protein kinase activity is dramatically inhibited by normal membrane concentrations of phosphatidyl-inositol-4,5-bisphosphate (PIP_2). Other phospholipids or polyanions, such as inositol-1,4,5-trisphosphate or 2,3-diphosphoglycerate, do not inhibit membrane-bound kinase activity. By this mechanism, fluctuations in membrane PIP_2 could activate or inactivate the protein kinase. The effect of phosphorylation of protein 4.1 and the PIP kinase by the membrane-bound protein kinase will be discussed. Supported by NIH Grant # GM38906.

CD 007 PHOSPHORYLATION MEDIATED ASSOCIATIONS OF THE RED CELL MEMBRANE SKELETON, Carl M. Cohen, Yuri Danilov, Richard Fennel and Bipasha Gupta-Roy, Department of Biomedical Research, St. Elizabeth's Hospital, Boston, MA 02135

With the exception of actin, all of the proteins of the red cell membrane skeleton are phosphorylated, in some cases by multiple protein kinases. Previous evidence suggests that phosphorylation of band 4.1 by protein kinase C and cAMP dependent kinase markedly reduces its ability to bind to spectrin, and to promote spectrin binding to F-actin. This suggests that phosphorylation of band 4.1 in vivo may lead to a less stable or more flexible membrane skeletal network. The proteins of the membrane skeleton are bonded to the red cell membrane via several classes of associations. Of particular interest are the associations of band 4.1 with the integral membrane proteins band 3 and glycophorin. In order to determine whether these associations are affected by the state of band 4.1 phosphorylation we measured binding of band 4.1 to inside-out vesicles which had been stripped of all peripheral membrane proteins. We found that phosphorylation of band 4.1 by protein kinase C, but not cAMP dependent kinase, reduced its binding to inside-out vesicles by 65-70%. Similar studies done following digestion of inside-out vesicles with chymotrypsin suggest that phosphorylation specifically reduces the binding of band 4.1 to the cytoplasmic domain of band 3. We have now done preliminary studies of band 4.1 binding to the purified cytoplasmic domain of band 3 (CDB 3) and have shown that phosphorylation of band 4.1 by protein kinase C reduces its binding to CDB3 in solution. In order to correlate these observations with the behavior of the intact membrane skeletal network, we have tested the effect of phosphorylation on the resistance of membrane skeletons to chemical disruption. Preliminary results suggest that Triton shells prepared from ghosts which have been phosphorylated by protein kinase C are more susceptible than controls to disruption by 1-2 M Tris HCl. In aggregate our results show that phosphorylation of membrane skeletal components by protein kinase C, and possibly other kinases, results in destabilization of the entire skeletal network as well as its association with the cell membrane.

CD 008 **PROTEIN 4.1 AS A MYOSIN BINDING AND MODULATING PROTEIN: INSIGHTS INTO A NEW FUNCTIONAL CLASS OF PROTEINS.** Gary R. Pasternack, Richard H. Racusen, and Asoka I. Katumuluwa. Department of Pathology, the Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

The present studies provide evidence for a new protein 4.1 activity: protein 4.1 can bind to myosin, and, in so doing, can down-regulate myosin's actin-acitivated ATPase activity. Direct binding studies using monomeric rabbit skeletal muscle myosin immobilized on Sepharose beads via a thioether linkage demonstrate a dissociation constant of K_d = 1.2 x 10^{-7} M with a stoichiometry of 1 to 1. On myosin, the binding site localizes to the neck region, since heavy meromyosin competes for protein 4.1 binding with K_i = 4.5 x 10^{-8} M, while S1 competes much less efficiently. Proteolytic dissection of protein 4.1 localizes myosin binding to the same 10 kDa phosphorylated region responsible for spectrin-actin interactions. Similar binding is observed with erythrocyte and liver myosins. When protein 4.1 binds, actin-activated ATPase activity is inhibited non-competetively, with a K_i in good agreement with the binding parameters. The myosin binding activity of protein 4.1 parallels the myosin binding properties of a 38 kDa murine lymphoid nuclear phosphoprotein isolated from A_{20} cells which cross-reacts with protein 4.1, and, by peptide mapping, contains the 10 kDa region. Although A_{20} cells express no detectable amounts of more conventional 4.1 isoforms, plasmid pHE4.1-6 (gift of John Conboy) hybridizes at moderate stringency with a discrete, abundant A_{20} mRNA species; this plasmid contains a 1.3 kb insert which includes the 16 and 10 kDa regions. Eleven clones from an A_{20} cDNA library are presently being sequenced to establish the relationship of this second myosin-binding protein to protein 4.1.

Regulation of Membrane Protein Functions-II

CD 009 LOCALIZATION OF THE ANKYRIN-BINDING SITE ON BAND 3, Kevin A. Davies, Sam E. Lux* and Harvey F. Lodish: Whitehead Institute for Biomedical Research, Cambridge, MA 02142, and *Division of Hematology, Children's Hospital, Boston, MA 02115.

The interaction between ankyrin and the cytoplasmic domain of Band 3 (cdb3) helps anchor the erythrocyte plasma membrane to the underlying cytoskeleton. In order to define the site of the ankyrin-binding site (ABS) on cdb3, the binding of purified ankyrin to either full-length, truncated or mutagenized cdb3 is being studied using a non-denaturing gel shift assay. (Previous studies have suggested the ABS may be one of four stretches of amino acids that are conserved between human, mouse and chicken Band 3). When ankyrin is incubated for several hours at 0 C with purified, iodinated cdb3, prior to analysis on a gel, the resulting complex is clearly resolved from cdb3 incubated either alone, with heat-denatured ankyrin, or with excess unlabeled cdb3. This procedure has been adapted and repeated with a number of *in vitro* translated human Band 3 N-terminal peptides of varying lengths, using RNA synthesized from a human Band 3 cDNA template that encodes the entire cytoplasmic domain. Initial results show that certain cytoplasmic Band 3 peptides truncated at their C-terminus retain the ability to bind to ankyrin, indicating they still possess a functional ABS. The length of these peptides will be correlated with their ankyrin binding activity, resulting in a preliminary assignment for the binding domain. Further studies employing N-terminal deletions and site-directed mutagenesis will allow us to precisely define the ABS.

CD 010 PHOSPHORYLATION ABOLISHES THE INTERACTION PF PROTEIN 4.9 WITH HUMAN ERYTHROCYTE MEMBRANES, *Athar Husain-Chishti and Daniel Branton, Biological Laboratories, Harvard University, Cambridge, MA 02138, *Present Address: Department of Biomedical Research, St. Elizabeth's Hospital, Tufts University School of Medicine, Boston, MA 02135
Protein 4.9 along with spectrin, actin and protein 4.1 constitute the major components of human erythrocyte membrane skeleton. In vitro, protein 4.9 is shown to bundle actin and such bundling activity is completely dependent upon the phosphorylation of protein 4.9 by the cyclic AMP-dependent protein kinase (Husain-Chishti et al., Nature 344, 718-721, 1988). In order to investigate the possibility of an interaction between protein 4.9 and integral membrane proteins, we have used alkali stripped membrane vesicles which are completely devoid of peripheral membrane proteins including protein 4.9. Quantification by an immunoassay indicated binding stoichiometry of one protein 4.9 monomer to a band 3 tetramer. More interestingly, such binding was completely abolished upon phosphorylation of protein 4.9 by the cyclic AMP-dependent protein kinase but not with protein kinase C. A lack of significant binding of protein 4.9 to liposomes and a substantial (up to 75%) reduction of binding with trypsinized vesicles suggest that the cytoplasmic domains of band 3 and glycophorins are the potential acceptor sites. Experiments with the purified cytoplasmic domain of band 3 (43/41 kDa fragments) have failed to demonstrate any interaction with protein 4.9 and thus indirectly implicate glycophorins as putative binding sites. A definition of protein 4.9-membrane interactions would assist in understanding skeleton based membrane properties including ATP-dependent shape changes in human erythrocytes.

CD 011 REGULATION OF GLYCOLYSIS AND MEMBRANE-CYTOSKELETON ASSOCIATION BY THE CYTOPLASMIC DOMAIN OF BAND 3, Philip S. Low[1], Marietta Harrison[2], Robert L. Geahlen[2], Barry Willardson[1] and Bernard Thevenin[1], Departments of Chemistry[1] and Medicinal Chemistry and Pharmacognosy[2], Purdue University, W. Lafayette, IN, 47907 We wish to propose a new mechanism of metabolic regulation distinct from current mechanisms involving covalent or noncovalent modification of regulatory enzymes. This hypothesis, which has been most thoroughly investigated in red cells, involves the regulation of enzyme-membrane protein interactions by tyrosine phosphorylation of the enzyme's binding site on the membrane. In erythrocytes, band 3 binds and inhibits several glycolytic enzymes. These enzymes, which all have peculiar aqueous cavities extending through their central axes, may associate with the acidic N-terminus of band 3 like "donuts on a string". Tyrosine phosphorylation of band 3 in this region blocks this inhibitory interaction, resulting in a stimulation of glycolysis. While all factors which modulate the tyrosine kinase have not been investigated, several hormones/reagents which bind to the membrane and stimulate both the kinase and glyolysis have been found to activate a transmembrane NADH dehydrogenase. Furthermore, a potent inhibitor of the membrane-spanning dehydrogenase results in the simultaneous decrease in both kinase and glycolysis activities. These observations imply a functional linkage connecting hormone binding at the cell surface to stimulation of glycolysis via a transmembrane dehydrogenase and a cytoplasmic tyrosine kinase.

We have also been investigating the role of band 3 in controlling cytoskeleton-membrane interactions at the ankyrin binding site. The regions of band 3 involved in ankyrin binding have been identified and several physiological variables which can regulate the interaction have been characterized. These data, as well as their biological implications, will be discussed.

CD 012 LOCALIZATION OF THE ANKYRIN BINDING SITE ON ERYTHROCYTE BAND 3 CYTOPLASMIC DOMAIN. Barry M. Willardson, Bernard Thevenin and Philip S. Low, Department of Chemistry, Purdue University, West Lafayette, IN. 47907. Ankyrin binding to the cytoplasmic domain of band 3 and to spectrin tetramers represents the major linkage of the erythrocyte cytoskeleton to the plasma membrane. Identification of the site of ankyrin association on the highly elongated cytoplasmic domain of band 3 is of importance in understanding the regulation of this interaction. We have developed four monoclonal antibodies (mAb) and three polyclonal anti-peptide antibodies (pAb) to probe for the ankyrin binding site. Inhibition assays of the binding of ankyrin to KI-stripped, inside-out membrane vesicles (KI-IOVs) with these antibodies showed >90% inhibition with pAb to residues 191-204 and mAb mapping to the region 36-41Kda from the N-terminus, with 50% inhibition occurring at 1:1 molar ratio antibody to band 3. pAb to residues 1-14 and 143-155 did not inhibit at concentrations up to 50 fold molar excess. mAb to the region 41-43Kda from the N-terminus and a second mAb to the 36-41Kda region did not inhibit over the same concentration range. The antibodies that do inhibit ankyrin binding either contain cysteine 202 or are bordered by cysteine 317. These cysteines were shown to be in close proximity in the tertiary structure of the band 3 dimer by the observation that a native interchain disulfide can form between cysteine 202 of one strand of the dimer and cysteine 317 of the other strand. The data suggest that ankyrin binds to band 3 in a region near a cysteine cluster of residues 202 and 317. This cluster is in close proximity to a proposed regulatable hinge around which band 3 undergoes a conformational change which also effects ankyrin binding.

Cellular and Molecular Biology of Normal and Abnormal Erythroid Membranes

Skeletal Protein Mutations: Structure, Function and Expression

CD 013 SPECTRIN (Sp) αI VARIANTS IN HEREDITARY ELLIPTOCYTOSIS (HE) AND HEREDITARY PYROPOIKILOCYTOSIS (HPP): CLINICAL, RHEOLOGICAL AND BIOCHEMICAL EXPRESSIONS. P. Boivin, M.C. Lecomte, C. *Féo, M. Garbarz, H. Gautero, O. Bournier, C. Galand and D. Dhermy, INSERM U.160, Hôpital Beaujon, 92118 Clichy, *INSERM U299, Hôpital Bicêtre, 94275 Kremlin Bicêtre (France).

In attempt to better analyze the heterogeneity of HE and HPP, 83 HE and 5 HPP patients from 52 families were investigated. All these patients carried one of the four molecular α I Sp domain defects related to defective Sp self-association: $Sp\alpha^{I/78}$ (6 HE and 1 HPP/1 family); $Sp\alpha^{I/74}$ (10 HE and 4 HPP/4 families); $Sp\alpha^{I/65}$ (52 HE/34 families); $Sp\ \alpha^{I/46}$ (15 HE/2 families). In heterozygous patients, the expression of these four Sp variants could vary from 30 to 80 %, even in the same family. The Sp self-association defect (expressed as the percentage of Sp dimer in the membrane) was more pronounced in patients with the $Sp\ \alpha^{I/74}$ and $Sp\ \alpha^{I/78}$ variants and in these cases correlated with the amount of Sp variants. In contrast, a limited functional defect of Sp was found in the $Sp\alpha^{I/65}$ variants. The erythrocyte deformability, evaluated by ektacytometric studies, correlated with the amount of Sp variants. Transient poikilocytosis was observed in the majority of studied infants (<2 year-old), whatever the Sp variant. In adults, the most frequent variant, $Sp\alpha^{I/65}$, did not appear as severe as the other variants and it was never observed in HE with chronic hemolysis or in HPP. Even more, in two homozygous $Sp\alpha^{I/65}$ HE, clinical expression appeared less severe than that observed in one case of homozygous $Sp\alpha^{I/74}$ HE.

CD 014 MOLECULAR HETEROGENEITY OF α SPECTRIN MUTANTS IN HEREDITARY ELLIPTOCYTOSIS/PYROPOIKILOCYTOSIS. Theresa Coetzer, Jack Lawler, Petr Jarolim, K Sahr, B G Forget and Jiri Palek, Dept.of Biomedical Research, St. Elizabeth's Hospital, Tufts Univ. School of Medicine, Boston, MA 02135; Dept. Internal Medicine, Yale Univ. School of Medicine, New Haven, CT

The most common molecular defects associated with hereditary elliptocytosis and pyropoikilocytosis involve the N terminal α I domain of spectrin (Sp) representing the Sp heterodimer (SpD) self-association site. Homozygotes for the three most common α Sp mutants ($Sp\ \alpha^{I/74}$, $Sp\ \alpha^{I/46}$, $Sp\ \alpha^{I/65}$) differ strikingly in their clinical severity which correlates with the functional ability of the mutant SpD to self-associate into Sp tetramers (SpT). $Sp\ \alpha^{I/74}$ homozygotes are transfusion dependent and their SpD are incapable of self-association on the membrane, as well as in in vitro studies on inside out vesicles (IOV) and in solution. $Sp\ \alpha^{I/46}$ homozygotes have a milder hemolysis with ± 50% SpD in membrane extracts, but the $Sp\ \alpha^{I/46}$ D are unable to form SpT in vitro. $Sp\ \alpha^{I/65}$ homozygotes are clinically only mildly affected and the $Sp\ \alpha^{I/65}$ D partly retain the propensity to self-associate both on the membrane (± 26% SpD) and on IOV (± 50% of the control level). Amino acid sequence studies of tryptic peptides indicate that the cleavage site for $Sp\ \alpha^{I/74}$ involves lys 42 instead of arg 39. $Sp\ \alpha^{I/65}$ is cleaved at arg 131, and the primary structural defect is an insertion of leu at residue 150. The $Sp\ \alpha^{I/46}$ cleavage site is at lys 252 but sequencing up to residue 271 reveals no abnormalities. $Sp\ \alpha^{I/65}$ represents a homogeneous mutation that is predominantly present in the black population in which the same leu insertion has been detected in all subjects tested so far, whereas the $Sp\ \alpha^{I/74}$ mutation is heterogeneous and present in individuals of diverse racial backgrounds including caucasians, blacks and Melanesians. RFLP studies on $Sp\ \alpha^{I/74}$ kindred using α Sp probes suggest that, in some cases, the primary defect may reside in the β chain which, in turn, could influence the susceptibility of the α chain cleavage site to proteolytic digestion.

CD 015 THE DIVERSITY OF HEREDITARY ELLIPTOCYTOSIS IN NORTH AFRICA : PROTEIN ASPECTS AND MOLECULAR GENETICS OF SPECTRIN TUNIS (αI 35 Arg->Trp). L. Morlé, F. Morlé, N. Alloisio, A.-F. Roux, J. Godet, M. Garbarz, P. Boivin, R. Kastally and J. Delaunay, CNRS URA 73 and UM4, Université Claude-Bernard, Lyon, France ; INSERM U160, Clichy, France ; Hôpital Habib Thameur, Tunis, Tunisie.

A number of skeletal protein mutations yield hereditary elliptocytosis (HE). Depending on the molecular change, the condition varies in its clinical expression, mode of inheritance and ethnic distribution. North-African populations, which have undergone successive genetic admixtures, display a wide spectrum of elliptocytogenic mutations. Out of 12 North-African families studied with HE, the following conditions or variants were found: 4.1(-) HE (two families), Sp αI/65 HE (six families), and an unidentified lesion in one family. More specifically, spectrin Oran (Spall/21) is a variant of spectrin αII domain (Alloisio **et al.** Blood 1988 : 71, 1039) yielding severe HE in the homozygous state, but remaining asymptomatic and virtually undetectable in the heterozygous state. Another αII variant was recognized, with clinical and biochemical manifestations in the heterozygous state (unpublished). Both of these variants are characterized by an accelerated **in vitro** proteolysis of the αII domain. Spectrin Tunis generates a clinically asymptomatic elliptocytosis in the heterozygous state (Morlé **et al.** Blood 1988 : 71, 508). It is associated with a reduced spectrin dimer self-association and by the occurrence of a major 78 kDa fragment. This fragment reacts with a polyclonal anti- αI domain antibody kindly provided by Drs. J. Palek and J. Lawler. Aminoacid sequencing showed that the αI 78 kDa fragment results from an enhanced cleavage at lysyl residue 10. The abnormal proteolysis was still observed in isolated α-chains, ruling out the possibility that the primary change be located in the β-chain. Using a 13.0 kb genomic α-spectrin probe kindly provided by Dr. B.G. Forget (Linnenbach **et al.** Proc. Natl. Acad. Sci. USA 1986 : 83, 2387) and the **Xbal**, **PvuII** and **MspI** polymorphic sites detected with this probe, we deduced that the mutation is associated with the +-+ haplotype (in the above order). We synthesized 20mer oligonucleotides complementary to genomic DNA segments from introns 1 and 2, respectively. They were used for priming DNA amplification by the polymerase chain reaction. The amplified fragments were cloned in a M13 MP18 vector and sequenced. In two spectrin Tunis carriers, we found the C->T base substitution in the codon corresponding to position 35 of the αI domain (CCG ->TCG ; Arg->Trp). The mutation lies in the last part of the first long α-helix (residues 9-44) of the αI domain, while the disturbed site of proteolysis occurs where this helix starts.

CD 016 ABNORMAL SPECTRIN αII DOMAIN IN RECESSIVE SPHEROCYTOSIS, S.L. Marchesi, P.A. Agre, and D. W. Speicher, Yale University Medical School, New Haven, CT, John Hopkins Medical School, Baltimore, MD and Wistar Institute, Philadelphia, PA

We have studied erythrocyte spectrin from red cells of 117 members of 31 families in which one or more members (total of 46) express recessively inherited spherocytic hemolytic anemia (rHS). Peripheral smears of affected individuals from 24 families showed striking macro- and microspherocytosis; red cells of affected members of the remaining 9 families have mixed morphologies which include ovalo- and stomatocytes as well as spherocytes. In most cases, the presence of hemolytic anemia was recognized and treated (transfusion, splenectomy) in early childhood, a reflection of the severity of this disorder. Analysis of spectrin tryptic peptides by 2-dimensional (IEF/SDS) electrophoresis revealed an abnormal αII domain in 17 of the 31 families. In these cases, the parent (46 Kd) αII domain peptides and their largest cleavage product (35 Kd) show an acidic shift in isoelectric point which we call αII T46a and αII T35a. The position of αII T30 is normal. The inheritance pattern of spectrin "αIIa" is complex and varied; αII T46a and T35a appear to be present in the "homozygous" state in some cases of rHS within the 17 families, and to be "heterozygous" in others. αIIa typically occurs in the heterozygous state in one or both normal parents of affected individuals and in some of the unaffected siblings and offspring. In one family with HE, αIIa and a shortened α subunit (~235 Kd) segregated independently. αII peptides T46, T35 and T30 share the same N terminus. Thus the mutational event resulting in the isoelectric shift of T46 and T35 (but not T30) should reside in ~40 residues (5 kd) of the 9th repeat unit of the α subunit. Endoproteinase lysine C digestion of αII T35a produces peptides not present in αII T30a which, however, could not be distinguished by HPLC or by amino acid analysis from similar normal αII peptides. Immunopurification of αII and αIIa peptides from spectrin tryptic digests is now in progress in order to recover the presumed mutant 5 kd region for sequence analysis.

The Role of Lipids and Proteins in the Control of Red Cell Shape and Mechanical Properties

CD 017 MOLECULAR ANATOMY OF ERYTHROCYTE MEMBRANE SKELETONS IN HEALTH AND DISEASE. Shih-Chun Liu and Jiri Palek, St. Elizabeth's Hospital, Boston, MA 02135

The human erythrocyte is laminated by a dense submembrane network principally composed of spectrin, actin and protein 4.1. Previous studies of the extended skeletons revealed a primarily hexagonal lattice of junctional short F-actin complexes crosslinked by spectrin tetramers and medium size oligomers. In contrast, previous studies attempting to visualize the unstretched skeletons suffered from low resolution because of high protein density and high staining background. We have now improved the techniques to examine the skeleton in situ and estimate the proportion of the cytoplasmic surface covered by the skeletal proteins. Red cell ghosts fixed with 1% glutaraldehyde were sonicated to produce small openings allowing the visualization of the underlying skeleton. Upon negative staining with 1% uranyl acetate, the exposed cytoplasmic surface displayed a dense network and contained structures resembling those of spectrin and short F-actin filaments. Approximately 62 $\pm$ 3% of the surface was occupied by the membrane skeleton and its immediate associated proteins as measured by image reconstruction followed by video densitometry and image analysis. In a subsequent study of a patient with a severe hereditary spherocytosis (HS) associated with a known combined deficiency of spectrin (49% of the normal) and ankyrin (55% of normal), we have found that the cytoplasmic surface covered the by skeletal structures were markedly reduced (40 $\pm$ 5%). This decrease of HS skeleton density seems to underlie the destabilization of the lipid bilayer as indicated by the finding that HS ghosts released more spectrin-free vesicles than that of normal ghosts (16% in HS vs 3% in normal as measured by their acetylcholinesterase release) upon 0°C-isotonic incubation. We conclude that in normal red cell membranes, about 60% of the lipid bilayer surface is directly laminated by the membrane skeleton. In severe HS with spectrin and ankyrin deficiency, a considerably smaller surface area of the lipid bilayer membranes is laminated by the skeleton, leading to lipid bilayer destabilization.

CD 018 CONSEQUENCES OF STRUCTURAL ABNORMALITIES ON THE MECHANICAL PROPERTIES OF RED BLOOD CELL MEMBRANE. Richard E. Waugh, Department of Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Because of its role as a circulating corpuscle, the ability of the erythrocyte to deform yet maintain its physical integrity is an essential part of its physiological function. Because the interior of the normal red cell is liquid, the stability and deformability of the cell is governed by the properties of its membrane. Abnormalities in membrane mechanical function can result in hemolytic anemia, and a number of structural abnormalities in the membrane skeleton have been identified in patients with inherited hemolytic disorders. In an effort to understand the connection between the molecular lesion and its physiological effect, we have studied the mechanical properties of cells from patients having inherited, biochemically-identified, molecular abnormalities of the membrane skeleton. The abnormalities studied to date include partial deficiency in spectrin, abnormal or deficient protein 4.1, protein 4.2 deficiency, partial deficiency in high affinity ankyrin binding sites and reduced spectrin self-association. In the absence of an abnormality in spectrin self-association, we have observed a strong correlation between the degree of spectrin deficiency and the fractional reduction in membrane shear rigidity regardless of the presence of other abnormalities[1]. In contrast, membranes having abnormal spectrin self-association exhibit higher shear rigidity than normal. Cells from four patients from three different kindreds exhibit increases in membrane shear rigidity of 15.0-30.0 percent above normal. The mechanism that accounts for this increased rigidity remains obscure, but possible explanations include alterations in network topology or an increase in the intrinsic rigidity of the spectrin molecules. An increase in rigidity has also been observed in reticulocytes isolated from the rabbit. The mean rigidity of the reticulocyte population was 15-20 percent greater than the rigidity of mature cells, but there was a large variability among the reticulocytes, and individual cells exhibited rigidity as much as twice that of the mature population. This increased rigidity may be due to additional structural elements associated with the reticulocyte membrane that are lost during the late stages of maturation.

[1]Waugh, R.E. and P. Agre. 1988. J. Clin. Invest. 81:133-141.

Cellular and Molecular Biology of Normal and Abnormal Erythroid Membranes

Molecular Aspects of Red Cell Invasion by Malarial Parasites

CD 019 DISCRETE SITES OF SOLUTE PERMEATION INDUCED IN THE RED CELL MEMBRANE BY INTRACELLULAR MALARIA PARASITES: BIOPHYSICAL AND MOLECULAR DEFINITION, Z. Ioav Cabantchik, Josefine Silfen and Hava Glickstein, Department of Biological Chemistry, Hebrew University, Jerusalem, Israel 91904. So as to meet the increasing demand for import and export of nutrients and waste products, the intracellular malaria parasites produce profound changes in the permeability properties of the host (erythrocyte) membrane. Approximately 6 hrs after *Plasmodium falciparum* invades the human red cell, and a few hrs before any structural changes are detected in the red cell membrane, the latter becomes permeable to a discrete series of agents, which are otherwise impermeant or very poorly permeant to uninfected cells. These include polyols such as sorbitol and myoinositol, amino acids such as glutamine and histidine , a variety of anionic organic acids and trace metal ions. The permeation of these agents increases gradually (although not all in parallel) with intracellular parasite development, depends on *de novo* protein synthesis and can be blocked differentially with a series of bioflavonoid glycosides which also markedly decrease parasite growth. Most of the water soluble permeants appear to gain access into the parasitized cell by a pore-like pathway (anionic channel by patch clamp) while the more hydrophobic ones would favor partitioning into the parasite modified membrane. The pores are irreversibly blocked by covalent binding analogs of bioflavonoid-glycosides, the sites of binding are endofacial, and there are probably no more than a few hundred per cell. Specific anti-phlorizin antibodies are presently used for the identification of the putative membrane components associated with the parasite-induced alterations of permeations pathways in the host cell membrane. (Supported in part by AI-20342 and AID CDR C7-171).

CD 020 ERYTHROCYTE RECEPTORS FOR INVASION BY MALARIA PARASITES, Terence J. Hadley*, J. David Haynes†, Francis W. Klotz†, John P. Dalton**, Louis H. Miller**. *University of Louisville, Louisville, KY 40292, †Walter Reed Army Institute of Research, Washington, DC 20307 **National Institutes of Health, Bethesda, MD 20205

Plasmodium knowlesi (a monkey malaria) and Plasmodium vivax (a human malaria) invade all human erythrocytes except those that lack the Duffy blood group antigens (Fy^a, Fy^b, Fy^3). Also, antibodies against Duffy blood group antigens block invasion in vitro. Duffy blood group antigens are carried on a 35-43 kD glycoprotein identified by Western blotting with anti-Fy^a. Nichols et al.[1] produced a monoclonal antibody, anti-Fy^b, against the Duffy glycoprotein that reacts with human erythrocytes and new world monkey erythrocytes that can be invaded by Plasmodium vivax and Plasmodium knowlesi. This antibody does not react with erythrocytes from the new world monkey, Cebus apella. Cebus appella erythrocytes can not be invaded by Plasmodium knowlesi or Plasmodium vivax. Therefore, Fy^b may constitute a receptor site. We recently identified a Plasmodium knowlesi protein that binds to Duffy positive erythrocytes but not to Duffy negative erythrocytes. This protein binds to the Duffy area (34-43 kD) of Western blots. The 135 kD protein is possibly a parasite receptor that binds to the Duffy glycoprotein. Plasmodium falciparum, the human malaria that causes most malaria-related deaths, does not require Duffy blood group antigens for invasion. Experiments with variant erythrocytes (Tn, M^kM^k, En(a-) and glycophorin C-deficient erythrocytes) indicate that Plasmodium falciparum binds to sialic acid on glycophorin A and also to a trypsin-sensitive site that is not on glycophorin A, B or C. The trypsin-sensitive site has not yet been identified. A 170 kD protein of Plasmodium falciparum binds to human erythrocytes in a sialic acid dependent fashion. This 170 kD may be a parasite receptor. The 170 kD protein of Plasmodium falciparum and the 135 kD protein of Plasmodium knowlesi are now being further characterized.

[1]Nichols, M.E., Rubinstein, P., Barnwell, J., de Cordoba, S.R. and Rosenfield, R.E. (1987) A new human Duffy blood group specificity defined by a murine monoclonal antibody. Immunogenetics and association with susceptibility to Plasmodium vivax. J. Exp. Med. 166, 776-785.

CD 021 STRUCTURAL AND FUNCTIONAL ALTERATIONS OF THE ERYTHROCYTE MEMBRANE IN MALARIA, Shiroma M. Handunnetti and Russell J. Howard, Laboratory of Infectious Diseases, DNAX Research Institute, Palo Alto, CA 94304-1104. Blood stage malaria parasites subjugate the properties of the human erythrocyte to serve the needs of a rapidly proliferating intracellular organism. The parasite induces profound changes in erythrocyte shape, deformability, membrane lipid composition and fluidity, surface antigen expression, cell-adhesiveness and membrane transport/permselectivity. Some alterations reflect special metabolic needs of the parasite, others reflect elaborate mechanisms for parasite evasion of acquired and innate host immunity. A new cytoadherence property of human erythrocytes infected with the most lethal of the human malarias, Plasmodium falciparum, has been identified: mature infected cells adhere to uninfected erythrocytes, forming rosettes containing 2-7 uninfected cells around the central infected cell. Rosettes can be disrupted mechanically but rapidly reform. Sera from immune African adults also reverse rosettes and lead to agglutinates of infected cells alone. Rosetting depends not only on a malaria-induced change on infected cells, but also on properties of the uninfected cell. As might be expected, parasites which rosette strongly exhibit less in vitro cytoadherence to C32 melanoma cell monolayers (a model for in vivo adherence to endothelial cells). However, if rosettes are disrupted and infected cells rapidly purified, these infected cells adhere strongly to C32 cells. We suggest that in vivo rosettes could serve several functions. Rosettes may be more readily occluded in narrow capillaries than non-rosetted infected cells, actually leading to enhanced infected cell adherence to capillary endothelial cells after displacement of some/all of the uninfected cells in the rosette. Parasite proliferation may be increased if merozoites released from ruptured infected cells more efficiently invade the adjacent rosetted uninfected cells. The uninfected erythrocytes in rosettes may provide lipids and metabolites to the infected cells. Finally, infected erythrocytes in rosettes may not only be less accessible to host monocytes and other cells in the effector arm of host immunity, but may disguise the grossly altered infected cell within a coating of normal host erythrocytes.

Posters I

CD 100 STRUCTURE OF THE GENE FOR BRAIN α-SPECTRIN IN MOUSE AND MAN, Connie S. Birkenmeier, David K. Kerk and Jane E. Barker, The Jackson Laboratory, Bar Harbor, ME
The best characterized mammalian spectrins are erythroid α- and β-spectrin and brain α-spectrin. Genetic experiments show that separate genes code for these three spectrins. Sequence analysis of cDNA clones and of the proteins themselves verify their homology. We report an analysis of spectrin gene structure using DNA sequencing to study two human and two mouse genomic clones. The clones are overlapping and code for 30% of the protein. Our clones contain the sequence coding for the entire 3′ untranslated region of the mRNA. We have sequenced eight exons and nine splice junctions. The coding sequence is >95% homologous to the sequence of the rat brain α-spectrin cDNA clone of Leto, et al. (1). The intron-exon boundaries are strictly conserved between mouse and human. Six of the nine splice junctions fall at the end of a codon and three split a codon. The intron-exon structure of the gene does not show an obvious relationship to the 106 amino acid repeat of the protein (2). The repeat structure of the spectrin proteins is discontinuous at the c-terminus. We are currently sequencing our clones with emphasis on a comparison of the sequence coding for the c-terminal non-homologous regions of the mouse and human genes.
(1) Leto, et al. 1988. Mol. Cell. Biol. 8,1. (2) Speicher and Marchesi. 1984. Nat. 311,177.

CD 101 MOLECULAR ANALYSIS OF SPECTRIN INTERCHAIN BINDING SITES, Timothy J. Byers, Ronald Dubreuil, Lawrence S.B. Goldstein and Daniel Branton, Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138.
We have shown that *Drosophila* and mammalian spectrins are very similar by structural, biochemical and immunological criteria, including amino acid sequence similarity and conserved binding interactions between spectrin subunits of different species. The conserved interchain binding activity proved to be of practical value when we used chicken erythroid alpha spectrin as a probe to obtain *Drosophila* beta spectrin cDNA clones from an expression library. We are currently using purified spectrin chains and bacterially-expressed polypeptides coded for by *Drosophila* alpha and beta spectrin cDNA sequences to localize the interchain binding sites. Polypeptides expressed from progressively shortened alpha cDNA fragments were used in Western blot overlay assays to roughly localize the sites of subunit-subunit association in the C-terminal half of that molecule. Strong binding activity for both vertebrate and *Drosophila* beta spectrin probes was found in the C-terminal region of alpha spectrin that lacks the 106 amino acid repeat structure characteristic of most of the molecule. A fusion protein consisting entirely of repeat segments from the central region of the alpha chain did not show strong interchain binding activity. Comparisons of the level of binding activity between progressively shortened alpha polypeptides indicated that the C-terminal interchain interaction involves more than one discrete site. We have recently constructed progressive deletions of beta spectrin cDNA fragments for nucleotide sequencing and for the mapping of interchain binding sites on beta. Preliminary experiments demonstrate an interchain binding activity complementary to the C-terminus of the alpha chain in bacterially expressed polypeptides that contain sequences from a limited region near the N-terminus of beta.

CD 102 STRUCTURE AND ORGANISATION OF THE HUMAN ERYTHROCYTE ALPHA-SPECTRIN GENE, Peter Curtis, Leszek Kotula, Louise Showe, Wistar Institute, Philadephia Bernie Forget, Kenneth Sahr, Yale University School of Medicine, New Haven.
Spectrin is the predominant component of the erythrocyte membrane cytoskeleton, which forms a two dimensional protein lattice on the inner side of red blood cells. It consists of two polypeptides, alpha- and beta-subunits, which associate as heterodimers and interact through the beta-subunit with ankyrin and at the other end with band 4.1 and short actin filaments to produce a network. Defects in this structural network are considered to be the cause of red cell fragility in various forms of inherited hemolytic anemias.
Considerable progress has been made in determining the amino-acid sequence of human erythrocyte alpha- and beta-spectrin, from which a 106 amino-acid repeat unit was noted. The amino-acid sequence has now been completed, deduced from overlapping cDNA clones. The cDNA clones have been used to screen a human genomic lirary. Seven overlapping lambda phage were sufficient to cover the entire gene, which encompasses about 80 kilo base pairs.

CD 103 STRUCTURE AND CALMODULIN-BINDING ACTIVITY OF AN ALPHA-SPECTRIN FUSION PROTEIN, Ronald Dubreuil, Timothy J. Byers, Amy L. Sillman, Lawrence S.B. Goldstein and Daniel Branton, Department of Cellular and Developmental Biology, Harvard University, Cambridge MA 02138.

We recently described the isolation and expression of cDNA encoding part of the alpha subunit of *Drosophila* spectrin, as well as the calmodulin-binding activity of the bacterially-produced protein (fusion protein 9, Byers *et al.*, 1987, J. Cell Biol. 105:2103). We have sequenced alpha spectrin cDNAs spanning from segment one of the polypeptide through the carboxy terminus and find that the predicted amino acid sequence is strikingly similar to vertebrate spectrins. We expressed clone 9 in bacteria and the resulting soluble fusion protein (59 vector-encoded amino acids and 1276 residues of spectrin) was purified by gel filtration. EM of the rotary-shadowed fusion protein revealed molecules 54 nm (± 8 nm) in length which is 59% of the length of native alpha spectrin (Dubreuil *et al.*, 1987, J. Cell Biol. 105:2095). We estimate that fusion protein 9 represents 60% of the alpha coding sequence (assuming that the full length sequence begins with segment one). Thus, the observed length of the fusion protein is consistent with the current model for spectrin structure in which individual triple barrel segments independently contribute to the length of the molecule. We also used expression of cDNA fragments to map the position of a calmodulin binding site in fusion protein 9 by nitrocellulose blot overlays. Binding is dependent on a sequence that extends from the end of segment 13 to the end of segment 15. Segment 15 includes a region rich in basic residues that is remarkably similar in *Drosophila*, vertebrate erythroid and non-erythroid spectrins. Conflicting reports on the location of the vertebrate spectrin calmodulin-binding site raise the possibility that there may be multiple binding sites. We are using the fusion protein/overlay assay to ask whether there may be additional calmodulin binding sites on *Drosophila* spectrin. Supported by GM39686 and an ACS Postdoctoral Fellowship to R.D.

CD 104 GENOMIC ORGANIZATION OF HUMAN GLYCOPHORIN A AND B, IMPLICATION FOR GENE DUPLICATION AND A HOMOLOGOUS RECOMBINATION THROUGH ALU REPETITIVE SEQUENCES. Kudo, S., and Fukuda, M. La Jolla Cancer Research Foundation, La Jolla, California, USA 92037

Glycophorin A and B are members of a family of major sialoglycoproteins in human erythrocytes. Their expression is erythroid-specific and down-regulated in erythroleukemic cells by the addition of tumor-promoting phorbol esters. In order to understand how the expression of these glycoproteins are regulated, and how the two genes are related to each other, we have isolated genomic clones encoding glycophorin A and B by using our previously isolated cDNAs (Proc. Natl. Acad. Sci. USA, 84, 6735-6739, 1987) as probes.

In genomic sequences of about 40 Kb, seven exons (for A) and five exons (for B) could be identified. All of the intron-exon boundaries were sequenced by using synthetic oligonucleotides as primers. The results indicate that the genes for glycophorin A and B are organized into two parts. One part, which represents the 5'-three quarters of the total gene, is almost identical between glycophorin A and B genes. In this homologous sequence, however, the glycophorin B gene lacks one exon, due to a point mutation at 5'-end of a splicing site. This mutation made the sequence no longer available for splicing. This homologous domain is interrupted by Alu repetitive sequences, followed by sequences specific to glycophorin A or B at the 3'-side of the genes. These results indicate that glycophorin A and B genes were produced by duplication of the ancestor gene and a crossing-over between homologous repetitive Alu sequences during or after duplication. (Supported by grant CA 33000.)

CD 105 THE PRIMARY STRUCTURE OF THE CHICKEN BRAIN α-SPECTRIN. Wasenius V.-M., Saraste, M., Salven, P. and Lehto, V.-P. Departments of Pathology and Medical Chemistry, University of Helsinki, and Department of Pathology, University of Oulu, Oulu, Finland

The primary and domain structure of the chicken brain α-spectrin was determined based on the full-length cDNA sequence and partial genomic sequences. It comprised the entire coding frame, 5'and 3'untranslated sequences and terminated in the poly (A)-tail. The α-chain consists of 22 segments. 20 of these correspond to the repeat of the human erythrocyte spectrin, typically 106 amino acids long. The segments in the middle and at the C-terminus are excluded from the repetitive pattern. The highly repetitive structure probably accounts for the flexible, rod-like structure of spectrin. The anomalous domains outside the repeated structure evidently harbor some important functional features including: 1)homology with α-actinin and dystrophin, 2) two EF-hand structures close to the C-terminus that may be Ca^{2+}-binding sites, 3) a sequence that fulfills the criteria for calmodulin-binding, and 4) a domain that is homologous to the regulatory part of the src and src-like proteins and phospholipase C. Comparison of different spectrin sequences showed that α-spectrin was well conserved across the species boundaries from Xenopus to man and that the human erythrocyte α-spectrin is divergent from the other spectrins.

CD 106 ELLIPTOCYTOSIS ASSOCIATED WITH AN ABNORMAL α GLYCOPHORIN, Yi-Qing Lu, Jun-Fan Liu, Cheng-Han Huang, Olga O. Blumenfeld, Robert S. Schwartz, Christine Lawrence and Ronald L. Nagel. Division of Hematology and Biochemistry, Albert Einstein College of Medicine, Bronx, NY and the Department of Biochemistry, Bronx, NY, 10461 and Hunan Medical College, Changsha, Hunan, People's Republic of China.

Human erythrocyte membranes contain four main sialoglycoproteins, the α, β, δ, γ glycophorins (GPHs). Spectrin/protein 4.1/α-GPH (and probably other GPHs) interactions constitutes one of the anchoring sites of the cytoskeleton to the membrane, and its perturbation could lead to changes in shape and function. Protein 4.1 deficiency have been found in patients with hereditary elliptocytosis. We report here a patient with elliptocytosis exhibiting a hitherto undescribed abnormality of the α-GPH associated with apparent increase in proteolytic susceptibility of protein 4.1. The spectrin tetramer-dimer dissociation was found to be normal. The GPH profile in gradient gels had the normal bands but in addition, a clear-cut minor band migrating just behind the monomer of δ-GPH. This 24K GPH band stained when probed with a polyclonal antiserum to α-GPH and a antipeptide C serum, while δ-GPH did not. This immunoblotting behavior implies that the new band is structurally related to α-GPH but may exhibit length alteration to explain the different SDS-PAGE mobility. Autoradiography of the anti-protein 4.1 stained nitrocellulose transfer displayed increased intensity in the bands believed to be the proteolytic products of protein 4.1. Reassociation of purified protein 4.1 to 4.1 stripped membranes was normal suggesting that the binding site for protein 4.1 is available although the affinity constant is unknown. DNA mapping reveals no gross rearrangement or deletion of the α-GPH gene, hence the abnormality could be the result of a point mutation or a short deletion.

CD 107 TRUNCATED ß-12 SEGMENT AND POSSIBLE PEPTIDE POLYMORPHISM OF HUMAN ß-SPECTRIN, S.H. Yoon, C.G. Kentros and J.T. Prchal. Univ. Ala at Birmingham, AL. Erythroid spectrin is the fibre-like protein composed of two non-identical polypeptides, α and ß, which are twisted along each other into a heterodimer. At their head region spectrin heterodimers are self-associated to form tetramers and higher degree oligomers. Both spectrin subunits are composed of repetitive homologous non-identical 106 amino acid (aa) segments, α–spectrin of 20 and ß-spectrin of 18.

We screened two different human reticulocyte cDNA libraries with ß-spectrin specific polyclonal antibodies; over 20 authentic ß-spectrin isolates were identified and characterized that were either identical or similar to ß-spectrin cDNA clones previously identified in our laboratory(PNAS 84:7468, 1987). Subsequently, we screened these libraries with our original radiolabeled human ß-spectrin cDNA probe. Seven additional clones were identified; the largest had about 2.6kb insert that was characterized. Its deduced aa sequence corresponds to the ß-spectrin peptide starting from the ß-6 ß-7 boundary and extending to the ß-14 repetitive segment. At this time the nucleotide sequence confirmed in both orientations(1640bp) corresponds to the derived aa sequence of portion of ß-9 to ß-14 segments. It has complete homology with the shorter(826bp) nucleotide sequence we previously published as well as with partially known ß-12 aa sequence. Among these identified segments, #12 is seven aa shorter than the other repetitive segments. All these deduced aa exhibit strongly conserved consensus sequences. Recently, another laboratory described ß-spectrin cDNA isolated from human fetal liver cDNA library (Blood 72:328, 1988). Its nucleotide sequence partially overlaps with this as well as the sequence that we have already published. These overlapping sequences are homologous with the exception of codon #25 of ß-11 segment. Both our current and previously reported isolates have A in this position while the other investigators reported G in this position. This non-conservative codon discrepancy changes the code in this position from a histidine to an arginine. Thus, this discrepancy may represent an example of peptide polymorphism of human ß-spectrin.

In summary, we report derived aa sequences of largely previously unknown ß-12 to ß-14 segments of human ß-spectrin. The ß-12 segment is truncated to 99 aa, but the derived aa sequence of this fragment matches completely with the known 34 aa sequence. Additionally, comparision of the derived aa sequence with that reported by another laboratory reveals an example of possible protein polymorphism of the ß-11 segment of human ß-spectrin.

CD 108 EXON-INTRON ORGANIZATION OF THE DNA ENCODING THE α-I DOMAIN OF HUMAN SPECTRIN: APPLICATIONS TO THE STUDY OF MUTATIONS CAUSING HEREDITARY ELLIPTOCYTOSIS (HE), K.E. Sahr, T. Tobe, K. Laughinghouse, T.L. Coetzer, J. Palek, M. Garbarz, P. Boivin, S.L. Marchesi, V.T. Marchesi and B.G. Forget, Yale University School of Medicine, New Haven, CT, Tufts University School of Medicine, Boston, MA, INSERM U160, Clichy and Faculte X, Bichat, Paris, France.

We have determined the exon/intron organization and nucleotide sequence of overlapping human genomic clones that span approximately 17 kb and encode almost the entire α-I domain of the α-spectrin polypeptide. The 13 identified exons varied in size from 44 to 240 nucleotides; introns varied from 0.1 to 1.8 kb. There was no obvious relationship of exon size and organization to the repeat structure of the polypeptide. From this information, we used oligonucleotide primers and the PCR technique to amplify specific exons in DNA from individuals with three separate forms of H.E. (Marchesi et al J. Clin. Invest. 80:191, 1987): αI/68, αI/50a, and αI/50b. In 12 black individuals from 8 separate families, the αI/68 kb anomaly resulted from a duplication of the leucine codon in exon 5 that encodes residue 148: TTG-CTG to TTG-TTG-CTG. In two unrelated individuals the αI/50a anomaly was the result of different single base changes in exon 7: CTG to CCG (leucine to proline) at residue 254, and TCC to CGC (serine to proline) at residue 255. In a third αI/50a patient, the nucleotide sequence for residues 234 to 264 was found to be normal. This approach is also being used to study the mutation in exon 12 in patients with αI/50b H.E.

CD 109 ANALYSIS OF SPECTRIN CONFORMATION USING MULTIPLE APPROACHES, David W. Speicher and Kaye D. Speicher, Wistar Institute, Philadelphia, PA 19104.

It is now well known that red cell spectrin and spectrin-like proteins in other tissues are primarily comprised of homologous units of approximately 106 amino acid residues. The basic structural characteristics that make spectrin a long flexible rod and impart certain functional properties can be divided into three elements: a) the conformation of the basic unit, b) interactions between adjacent repeat units, and c) perturbations to the basic unit resulting from gaps or insertions. An earlier model (D.W. Speicher and V.T. Marchesi, [1984] Nature 311:177-180) defined the repeat unit as three antiparallel helices together with a short undefined connecting region. This simple theoretical model was not consistent with several properties of the molecule including locations of protease cleavage sites and the frequent occurrence of long range subtle conformational perturbations resulting from amino acid mutations. A new detailed model of spectrin conformation that addresses these concerns has been developed. This comprehensive model addresses all three basic structural characteristics described above. The new model has been constructed using a multifaceted computer prediction approach, written by us, which incorporates recently published concepts on end group analysis and loop structures. The model has been tested in several ways including: analysis of synthetic peptides, crosslinking studies, use of specific antibodies, and computer comparison of multiple data sets. Two of the most striking features of the model, relative to earlier models, are the presence of several nonhelical structures and the concept that adjacent units overlap in a functionally significant manner. The overlap region conducts conformational changes over long distances while changes in these regions probably modulate molecular flexibility. Finally, insertions and deletions in the overlap region may impart special properties to certain repeats; these repeats are usually located near the ends of the molecule where functionally critical binding sites are located.

CD 110 BIOSYNTHETIC ANOMALIES IN THE GLYCOPHORINS OF MURINE ERYTHROLEUKEMIA CELLS, Jeffrey B. Ulmer, Elizabeth D. Dolci and George E. Palade, Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06510.

Murine glycophorins are present in embryonic, fetal and adult erythroblasts, erythrocytes and in transformed erythroleukemia cells (MEL cells). However, there are major differences in the expression and biosynthesis of glycophorins in the transformed cells: (i) normal and transformed erythroblast glycophorins differ in structure, due at least in part to differences in their oligosaccharide side chains; (ii) MEL cells express extra forms of glycophorin; and (iii) some of the putative glycophorin precursors are inefficiently translocated across endoplasmic reticulum membranes and accumulate in the cytosol of MEL cells. The putative glycophorin precursors (both normal and transformed) undergo neither signal sequence cleavage nor N-linked glycosylation upon translocation. However, preliminary evidence suggests that they are modified by O-linked N-acetylglucosamine residues.

CD 111 cDNA CLONING AND SEQUENCE OF MURINE GLYCOPHORIN, Donella J. Wilson and Jose Planas, Division of Biomedical Sciences, Meharry Medical College, Nashville, TN. 37208. The three major sialoglycoproteins (SGP) in human RBC, glycophorins A, B, and C have recently been isolated and cloned by independent investigators. While the function of glycophorin in normal RBC has not yet been delineated, a portion of this transmembranous protein is believed to be involved in the malarial parasite recognition and/or invasion of erythrocytes by certain species of Plasmodia. This property of SGPs is a primary focus of this laboratory. To study the function of glycophorin-like proteins in the murine system, the use of lectin affinity chromatography, neuraminidase sensitivity, specialized staining and labeling properties was successfully employed to identify and isolate four prominent SGP bands from mouse red blood cells during SDS PAGE. Rabbit antibodies obtained from these SGP bands, in addition to a h-Glycophorin A clone (obtained from Siebert and Fukuda), were used consecutively as probes to identify two m-SGP cDNA clones (450 and 550 bp) from an anemic mouse spleen lambda gt11 library. These two clones were subcloned into M13, sequenced, and found to be non-overlapping sequences. The results of this sequence analysis in comparison to other glycophorin cDNA sequences will be presented.

CD 112 EXPRESSION OF THE RED CELL ERYTHROID β SPECTRIN GENE IN NONERYTHROID TISSUES: EVIDENCE FOR TISSUE-SPECIFIC DIFFERENTIAL PROCESSING OF 3' β SPECTRIN PRE-mRNA, John C. Winkelmann, F.F. Costa and Bernard G. Forget. Department of Medicine and Institute of Human Genetics, University of Minnesota, Minneapolis MN 55455; and Departments of Medicine and Human Genetics, Yale University School of Medicine, New Haven CT 06510
Spectrin is the most abundant molecular constituent of the erythrocyte membrane skeleton. It is a heterodimeric protein, with α and β subunits of 240 and 220 kDa, respectively. To study the tissue-specific expression of the erythroid β spectrin gene, we have isolated cDNA clones from a human fetal skeletal muscle cDNA library (generously provided by L. Kunkel) using a previously reported human erythroid β spectrin cDNA as a hybridization probe. Eight cDNAs have been characterized. Restriction map, nucleotide sequence and genomic DNA hybridization data show that erythroid and skeletal muscle cDNAs are identical over the majority of their lengths. Of particular interest is a sharp divergence of nucleotide sequence 300bp from the 3' end of the erythroid cDNA and 1kb from the 3' end of the muscle cDNA. There is a potential 5' (donor) splice site at the point of divergence. Sequence analysis of human genomic DNA, cloned from this region, reveals no intron at this location. A muscle-specific β spectrin cDNA fragment detects mRNA transcripts in rat brain and heart by northern blot hybridization. These data demonstrate that the "erythroid" β spectrin gene is expressed in other tissues; however, the C-terminus of red cell β spectrin, which is involved in spectrin self-association and phosphorylation, is replaced by a different amino acid sequence. The sequence has been derived from the cDNA. This probably results from tissue-specific differential processing of β spectrin pre-mRNA, involving alternative polyadenylation and selective use of a donor splice site within the erythroid β spectrin coding sequence.

Posters II

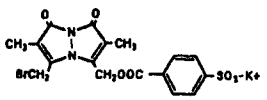
CD 200 BIOCHEMICAL CHARACTERIZATION OF THE ERYTHROCYTE Rh PROTEINS, Peter Agre and Barbara L. Smith, Departments of Medicine and Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD 21205
The erythrocyte Rh antigens contain an integral membrane protein of Mr 32,000 ("Rh polypeptide") which is the site of the antigenic polymorphism, and an associated protein of Mr 28,000 ("28kDa"). These proteins are linked to the membrane skeleton, exist in 60,000 and 140,000 copies per cell respectively, and were purified by hydroxylapatite chromatography. While Rh immunoreactivity is unique to human erythrocytes, proteins similar to the Rh polypeptide without the surface domain were isolated from erythrocytes from diverse mammalian species. It has previously been hypothesized that the Rh antigens contribute to the organization of the phospholipid bilayer, and the Rh polypeptide was found to be a major substrate of a novel acylation/deacylation mechanism associated with membranes of mature erythrocytes. 28kDa was also found in nonhuman erythrocyte membranes and in the apical brush borders of renal proximal convoluted tubules. A subpopulation of higher molecular weight 28kDa which is the N-glycosylated form of 28kDa exists in all membranes except those from rare phenotypes lacking all Rh immunoreactivity. The physiologic role of 28kDa is not known, however 28kDa contains a cytoplasmic domain of Mr > 10,000, which may provide a mechanism for linkage to the membrane skeleton. Its abundance in renal tubules suggests that 28kDa may provide structural support for ion transporters.

CD 201 UPTAKE OF LITHIUM INTO HUMAN ERYTHROCYTES BY NMR & AAS.
N.J. Birch and M.S. Hughes, *Biomedical Research Laboratory, Wolverhampton Polytechnic, Wolverhampton, England. WV1 1DJ*

Lithium is currently the most effective prophylactic agent in the control of bipolar affective disorders and has been for so for the past two decades. However, its mode of action remains unknown. The free passage of lithium into cells has been reported, the implicit suggestion is that it has a cytosolic site of action. Recent evidence from work on the gastrointestinal tract has suggested that there is a barrier to lithium import into cells. The present study investigates lithium uptake into human red blood cells. The intracellular lithium concentration of erythrocytes was analysed after loading with 40mM LiCl for 17 - 20 hours. Determinations were carried out of lithium in intracellular space, by 7-Li nuclear magnetic resonance (NMR) and extracellular fluid, by atomic absorption spectroscopy (AAS). A good correlation was observed between both methods using standard solutions. Non-invasive recordings were made by NMR using the hyperfine shift reagent dysprosium tripolyphosphate. Inter- and intraindividual differences between subjects were observed, although the differences were not statistically significant. Apparent internal lithium concentrations (NMR) were approximately 11 mmol/l, 28% of the external loading medium (AAS). We conclude that free intracellular lithium is lower than previously assumed in the aqueous environment of the cell. This may be due either to binding of lithium by internal surfaces of erythrocyte membranes or to restricted access of the ion to intracellular spaces as a result of the barrier properties of the erythroid membrane.

CD 202 FORMATION AND REGULATION OF THE PROPRANOLOL ACTIVATED POTASSIUM CHANNELS OF THE HUMAN ERYTHROCYTE, William D. Hobey, Department of Chemistry, Worcester Polytechnic Institute, Worcester, MA 01609. Activation of potassium efflux from human erythrocytes by propranolol is greatly accentuated by Ca^{2+}. However, different dependencies on Cl^- and ATP show propranolol activation to be distinct from the Gardos effect. Studies of permeability in the absence and presence of Ca^{2+} show two distinct induced pathways. The minor one is independent of Ca^{2+}, requires five propranolol molecules per channel, and saturates at higher drug concentrations. The major component requires two Ca^{2+} ions and seven propranolol molecules per channel, and is inhibited by excess propranolol. The latter also needs two Na^+ ions per channel, and is furosemide sensitive. The Na^+ are tightly bound ($K_{diss} = 0.43mM^2$). Quantitative studies of the inhibition of the induced flux by quinine confirm the existence of two distinct channels. Using drug uptake as a probe, we find Ca^{2+} removal, ATP depletion, or quinine addition affect the formation of the channel structure, while Na^+ and Cl^- are involved in its operation. Investigation of permeability changes induced by several analogs of propranolol indicate the presence of the naphthalene ring is necessary for its actvity. A model for activation wherein this ring system intercalates between α-helical segments of a transmembrane protein is proposed.

CD 203 A NEW BROMOBIMANE FLUORESCENT LABEL FOR ANION EXCHANGE PROTEINS, Edward M. Kosower*, Nechama S.Kosower**, Annette E.Radkowsky* & Judith Zipser**. *Biophysical Organic Chem. Unit, School of Chemistry, Faculty of Exact Sciences and **Department of Human Genetics, Sackler School of Medicine, Tel Aviv Univ., Tel Aviv 69978, Israel.
Bromobimanes are used for the fluorescent labeling of membrane and intracellular thiols under physiological conditions (Kosower and Kosower, Methods Enzymol. 143, 76, 1987). We describe here a new, negatively charged bromobimane, sulfobenzoyloxy-bromobimane (SBBr),



which selectively labels the anion exchange protein, Band 3, in intact erythrocytes(RBC) (pH 7.4,37°C,60min). In contrast, SBBr labels many proteins in RBC ghosts . The fluorescent product is stable and easily seen after electrophoretic separation of proteins from solubilized membranes(whole membranes or membranes stripped of peripheral proteins). Quantitation is carried out with solubilized membranes or by densitometry of the gel. Chloride ions diminish the rate of labeling. Diisothiocyanatostilbenedisulfonate (DIDS) (which binds to Band 3 and inhibits anion transport) inhibits labeling by SBBr. Substitution of Na^+ in the buffer by Li^+ or K^+ does not affect labeling. The major part of the label remains in the 60kDa fragment after chymotrypsin treatment of SBBr-labeled cells. SBBr allows the cross-linking of chymotrypsin fragments by DIDS. SBBr-binding sites may be different from those attacked by DIDS. SBBr is useful for studying band 3 and related proteins, including Cl^- channels.

CD 204 BAND 3 DEGRADATION BY CALPAIN IS ENHANCED IN ERYTHROCYTES OF OLD INDIVIDUALS, Nechama S.Kosower, Tova Glaser and Neta Ben-Meir, Department of Human Genetics, Sackler School of Medicine, Tel Aviv University, Israel.
Aging of erythrocytes (RBC) has been extensively studied. Little is known about RBC in relation to aging of the whole organism. We examined the sensitivity of Band 3 to calpain, a cytoplasmic, Ca^{++}-activated thiol protease. Band 3 degradation by calpain was enhanced in RBC membranes of old individuals (>70 years old) as compared to that of young ones (20-30 years old). Enhanced Band 3 proteolysis in old individuals was found in whole RBC ghosts as well as in membranes stripped of peripheral proteins by NaOH (which denatures Band 3 cytoplasmic domains). Thus, the protein itself was altered in aged individuals rather than the membrane organisation. Band 3 in membranes treated with acetic acid (which denatures membrane-spanning domains) was degraded more than base-treated Band 3, but without differences between young and old. Degradation was inhibited by the calpain inhibitor, calpastatin, and by EDTA, but not by the serine protease inhibitor, PMSF. DIDS (which binds to Band 3 and inhibits anion transport) enhanced the sensitivity of Band 3 to degradation by calpain, diminishing the difference between young and old. Alkylation of thiol groups by the fluorescent labeling agent, monobromobimane, did not affect degradation. In RBC ghosts prepared from chymotrypsin-treated RBC, the 65kDa fragment of Band 3 was further degraded by calpain. The calpain-sensitive site(s) seem to be in the cytoplasmic face of the membrane-spanning domain. The age-related enhanced degradation may be caused by modification(s) at these sites or be due to alterations in other Band 3 domains, and may be associated with the appearance of the 'senescent antigen'.

CD 205 NON-ERYTHROID BAND 3 GENE EXPRESSION IN UNINDUCED AND INDUCED K562 AND KMOE CELL LINES, Lisa D. Laury-Kleintop and Louise C. Showe, The Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104. A human cDNA homologous to the erythroid anion transporter, Band 3, has been sequenced in our laboratory. The Band 3-related message is present in non-erythroid cell types but is *not* present in reticulocytes suggesting that expression of the erythroid (HEB3) and non-erythroid (HKB3) genes might be mutually exclusive. We have now followed the expression of HKB3 and HEB3 during erythroid differentiation in erythroleukemic cell lines K562 and KMOE. Inductions were carried out over 4 days with either AraC, hemin, butyric acid, or hemin and AraC. DNA probes which distinguish between the two homologous genes were used on Northern blots to assay their respective mRNAs. The two cell lines responded similarly to each other with each inducer. The HKB3 message is quite stable, having a half-life greater than five hours in uninduced cells treated with Actinomycin D. After induction, as the levels of γ-globin (K562) or β-globin (KMOE) messages increased indicating erythroid differentiation, the amount of HKB3 message decreased from a high initial value to undetectable levels, suggesting complete shutoff of the gene. However, we have not detected any corresponding appearance of message from the erythroid gene indicating that the turnoff of HKB3 and the turn-on of HEB3 are separately controlled.

CD 206 CML ERYTHROCYTES ARE ENRICHED IN CA^{2+}-DEPENDENT MEMBRANE-BINDING PROTEINS, Robert A. Schlegel*, Patrick L. Williamson# and Michael Fujimagari*, *Department of Molecular and Cell Biology, Penn State University, University Park, PA 16802 and #Biology Department, Amherst College, Amherst, MA 01002.

Calcimedins are Ca^{2+}-dependent phospholipid-binding proteins discovered in a variety of cell types; their existence in erythrocytes has yet to be established. Using antibodies to the 35 and 67 kD species of calcimedins to probe blots of erythrocyte membrane proteins, we found reactivity at 67 and 48 kD in membranes isolated in the presence of Ca^{2+}, but no reactivity in membranes prepared in its absence. The 67 kD species was also seen in membranes of erythrocytes from patients with chronic myelogenous leukemia (CML), even when prepared in the absence of Ca^{2+}, as were two other species at 35 and 38 kD. When prepared in the presence of Ca^{2+}, CML membranes contained increased levels of these species, and reactivity at 48 kD as well. The 35, 38 and 67 kD species could be recovered when membranes were washed with EGTA, whereas the 48 kD species remained associated. When these three species were applied to an octyl sepharose column in the presence of Ca^{2+}, all were retained and could be subsequently removed using EGTA, indicating Ca^{2+}-dependent hydrophobic interactions. Not only is there an enrichment of Ca^{2+}- dependent membrane-binding proteins in the membranes of both CML erythrocytes and normal erythrocytes in which Ca^{2+} levels have been elevated, but as previously reported, normal transbilayer phospholipid asymmetry is disrupted in both cases, suggesting that abnormal calcimedin binding may be involved in this abnormal arrangement of phospholipids.

CD 207 COMPARATIVE STUDY OF ACTIVE SODIUM TRANSPORT IN RED BLOOD CELLS FROM HEALTHY DONORS AND PATIENTS WITH DIFFERENT FORMS OF HYPERTENSION BY 23-NA NMR, Sibeldina L.A., Knubovets T.L., Revazov A.V.*, Eichhoff U.**, Institute of Chemical Physics Acad.Sci.USSR, Moscow, 117977, *First Moscow Medical Institute, **Bruker Analytische Messtechnik GMBH, FRG. 23 Na NMR was used to measure the maximal rate of active Na+ efflux from human red blood cells at 37°C.Special conditions were applied to make active transport the only measurable process and increase its velocity to the maximal possible value:intracellular Na+ level was artificially increased and K+ level - decreased to eliminate these ions concentrations gradients on red blood cell membrane.The intracellular Na+ level 80 mM and more was shown to be sufficient to lead to maximum active Na+ efflux.Non-penetrating paramagnetic shift reagent - dysprosium (III) bis(tripolyphosphate) complex was added to the suspension medium to resolve the signals of intra- and extracellular Na+.The cells were proved to retain their physiological activity in the presence of this compound.Red blood cells from 20 healthy donors, 20 patients with essential hypertension and 20 persons with secondary hypertension (diastolic pressure 78.0 ± 1.2; 100.5 ± 1.8; 108.5 ± 1.8; systolic pressure 119.8 ± 1.2; 174.5 ± 3.0; 177.5± ± 3.6 respectively) were investigated. The maximal rate of active Na+ efflux from red blood cells was measured to be 10.5 ± 0.3; 9.0 ± 0.3; 10.3 ± 0.3 respectively in the case of healthy donors, patients with essential hypertension and persons with secondary hypertension. The decrease in maximal rate of active Na+ efflux for patients with essential hypertension appeared to be significant ($p < 0.005$, Student's test). The question arises whether this is a decrease in Na+,K+-ATPase activity itself or a reaction on the membrane structure distortion in case of essential hypertension.

CD 208 LOCALIZATION OF FORSSMAN GLYCOLIPID ON SHEEP ERYTHROCYTE MEMBRANES, Thomas W. Tillack, Margaretta Allietta, and William W. Young, Jr., Department of Pathology, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

The techniques of liquid helium fast-freezing and freeze-etch electron microscopy have been used to localize Forssman glycolipid on the surface of sheep erythrocyte membranes. Previous studies using slower freezing methods and ferritin-labeled antibodies on sheep erythrocyte ghosts had indicated a slightly clustered arrangement of Forssman glycolipids in ghost membranes (Biochim. Biophys. Acta (1983) 733:15). Intact IgG molecules with specificity for Forssman glycolipid were visualized directly on the membrane surfaces of unfixed and glutaraldehyde fixed erythrocytes. Unfixed erythrocytes labeled with anti-Forssman IgG showed marked aggregation of the immunoglobulin on the membrane surface, while fixed erythrocytes similarly labeled showed small clusters of antibody on the cell surfaces. Intramembranous particles in these preparations appeared randomly oriented. Fab fragments prepared from the anti-Forssman IgG could not be visualized on intact erythrocyte surfaces, probably due to shielding by overlying glycoproteins. Pronase treatment of glutaraldehyde fixed erythrocytes allowed visualization of the 5 nm Fab fragments, which were randomly oriented on the erythrocyte surface. We conclude that intact IgG causes aggregation of membrane glycolipids, even in fixed erythrocytes, and that Fab fragments provide a more accurate label for the localization of glycolipids in membranes.

CD 209 DENSITY ASSOCIATED LOSS OF Ca PUMP ACTIVITY IN RED BLOOD CELLS OF BEAGLES WITH HEMOLYTIC ANEMIA, Frank F. Vincenzi, Cynthia A. Pekow, Lillian Maggio-Price, William P. Hammond and Thomas R. Hinds, Departments of Pharmacology and Medicine and Division of Animal Medicine, University of Washington, Seattle, WA 98195 USA

Maggio-Price et al. (Am. J. Vet. Res. 49: 1020, 1988) recently reported on an hereditary nonspherocytic hemolytic anemia in Beagles. Anemic Beagles display reticulocytosis (11%) and red blood cells (RBCs) of anemic Beagles (^{51}Cr $t_{1/2}$ = 12 days) have decreased Ca pump activity compared to normal dog RBCs (^{51}Cr $t_{1/2}$ = 24). In the present experiments washed RBCs were selected as 'Top', 'Middle' and 'Bottom', (by inference younger to older, respectively) fractions following centrifugation in small glass tubes. Each fraction consisted of approximately 7% of the total packed cell volume. Ca pump activity was assayed in intact RBCs by measuring the Ca and A23187 induced loss of ATP (Hinds and Vincenzi, Proc. Soc. Exp Biol. Med. 181: 542, 1986). Results confirmed the lower than normal activity of the Ca pump in RBCs from anemic Beagles, and the pseudo-first-order loss of ATP under assay conditions. Data were expressed as the apparent rate constant for ATP loss (min^{-1}), or normalized to the value of the Top cells. Cells from normal Beagles (n=13) exhibited respective apparent rate constants of 0.153, 0.130 and 0.104, for Top, Middle and Bottom cells. By contrast, cells from anemic Beagles (n=11) exhibited respective apparent rate constants of 0.110, 0.047 and 0.058 for Top, Middle and Bottom cells. Results support the interpretation that, as with normal human RBCs (Vincenzi and Hinds, Blood Cells 14: 139, 1988), normal dog RBCs lose some Ca pump activity as cells age. Results also support the interpretation that RBCs in anemic Beagles lose activity of the Ca pump more rapidly and more completely. It is suggested, but remains to be established, that the loss of Ca pump activity is causally related to the hemolytic anemia. Supported in part by DHHS grants AM 18951, RR01203 and RR07019.

CD 210 MEMBRANE SURFACE MARKER EXPRESSION ON HUMAN ERYTHROID PRECURSOR CELLS STUDIED BY A TWO PHASE LIQUID CULTURE METHOD. Hideho Wada, Toshio Suda*, Yasusada Miura*, Eiji Kajii**, Shigenori Ikemoto** and Yoshihito Yawata. Div of Hematol, Dept of Med* and Dept of Human Biol**, Jichi Med School, and Div of Hematol, Dept of Med, Kawasaki Med School, JAPAN

The sequential expression of membrane surface markers, such as blood group antigens on various stages of human erythroblasts, was studied by the liquid culture system utilizing blood specimens from a healthy volunteer of A and MM blood type. The procedure was divided into two phases; (1) mononuclear cells were cultured in the presence of PHA-leukocyte conditioned medium for 7 days, and (2) after phagocytic cells were removed, recultures of the cells were performed in the hypoxic culture condition using the liquid culture system containing 30% fetal calf serum, 1% bovine serum albumin, 300 ug/ml iron-saturated transferrin and 2 U/ml recombinant erythropoietin. On days 0-3 of second phase of culture, more than 90% of cells were blastic cells. On day 4, orthochromatic erythroblasts developed and the number of cells increased until day 8, when denucleated cells were consistently recognized and the level of hemoglobin reached to nearly-normal level (17.5 $\mu g/10^6$ cells). Flow cytometric analysis revealed that glycophorin A-positive cells were around 90% on day 5 of culture, indicating that erythroid cells were selectively grown in this culture system. On day 3, cells reacting for monoclonal anti-M antibody developed and the number of positive cells increased as the erythroid cells were maturing. During this period, type N-positive cells were not detected. Regarding the expression of ABH, a small number of type H-positive cells were detected, inspite of very few type A-positive cells, on day 0 of culture. Blood type A-positive cells increased gradually as the erythroid cells were maturing.

CD 211 **CA++ ACCUMULATION AND LOSS IN INTERNAL VESICLES OF SICKLE ERYTHROCYTES**, Patrick Williamson, Estela Puchulu, Max Westerman, and Robert A Schlegel, Department of Biology, Amherst College, Amherst, MA 01002; Hematology/Oncology, Chicago Medical School, Mt Sinai Hospital, Chicago, IL 60608; Molecular and Cell Biology Program, Penn State University, University Park, PA 16802

Sickle cells contain internal vesicles accumulate Ca++, as judged by their labelling the Ca++ probe chlortetracycline (CTC). An antibody to the plasma membrane Ca-ATPase of human red cells was used to demonstrate the presence of this protein in the internal vesicles. The presence of the Ca pump explains how Ca++ accumulates within the vesicles, and suggests that they arise by endocytosis. The slow kinetics of accumulation, and a distinctive distribution within cells, argue that this endocytic process is different from normal, clathrin-mediated endocytosis, which also occurs in these cells. ATP-depletion of sickle cells results in loss of Ca++ from the vesicles, as judged by the disappearance of CTC staining without a corresponding loss of antibody staining. This loss can be inhibited by nitrendipine, suggesting the presence of a Ca channel in the vesicle membrane. The presence of both a pump and a channel in these vesicles suggests that the Ca which they contain is in dynamic equilibrium with the cytoplasm of the cell.

CD 212 STUDY OF THE EFFECT OF MILD TRYPTIC DIGESTION ON THE CALMODULIN STIMULATED CALCIUM TRANSPORT ENZYME OF THE INTACT HUMAN ERYTHROCYTE MEMBRANE. Yatian Zhang, Department of Psychiatry, College of Physicians & Surgeons of Columbia University, 722 West 168 Street, New York, NY 10032.
The calcium transport enzyme of erythrocyte membranes is considered to be activated by direct interaction with calmodulin. Using enzyme kinetic assays carried out with a programmable three axis automated liquid transfer apparatus, I have found that this enzyme (Calm-CaATPase), in contrast to a similar enzyme in membrane of red blood cells which transports sodium and potassium (Na,K-ATPase) is particularly sensitive to destruction by trypsin. In the present study I show that brief exposure of intact erythrocyte membranes to trypsin leads to a rapid loss of calmodulin activation. This was found to be the case in membrane preparations from erythrocytes of normal subjects as well as of patients with psychiatric illness. Similar results were obtained with erythrocyte membranes from patients with Duchenne and myotonic muscular dystrophies, diseases in which membrane abnormalities are suspected. Whether this effect is due to the destruction of the calmodulin binding site(s) or other changes in the microenvironment of the enzyme is at present not clear. The range of sensitivity of Calm-CaATPase, as well as the relative stability of Na,K-ATPase, differed between the normal subjects and the patients.

Posters III

CD 300 DEPOLYMERIZATION OF SPECTRIN BY MICROMOLAR CALCIUM. Joseph A. Babitch and Kong Chuang Fong. Chemistry Dept. and Chemistry of Behavior Program, Texas Christian Univ., P.O. Box 32908, Fort Worth, TX 76129
The shape, flexibility and deformability of erythrocytes is determined by the membrane cytoskeleton, a network composed primarily of spectrin lying under the membrane. The fragility of the erythrocyte membrane is increased by certain spectrin mutations and in some cases alterations of spectrin polymerization have been implicated. Increased concentrations of internal calcium ions also promote cell fragility, but the locus of this effect is unknown. Here we demonstrate that: (1) subunits of erythrocyte and brain spectrin can bind micromolar $^{45}Ca^{2+}$; (2) spectrin dimers bind five atoms of $^{45}Ca^{2+}$ with a K_d = 5μM; (3) binding of Ca^{2+} shifts the dimer-tetramer equilibrium in favor of dimers. Direct binding of calcium to spectrin may explain the effect of calcium on erythrocyte membrane stability. Because this spectrin lattice occurs under the plasma membrane of a variety of cells, these findings may help to understand other processes such as forms of exocytotic secretion which are accompanied by spectrin rearrangements.
Supported by NSF (BNS-8615582) and NIH (NS-26518).

CD 301 **Localization of a calmodulin-binding site on human erythrocyte spectrin. Lars Backman and Åsa Berglund, Department of Biochemistry, University of Umeå, S-901 87 Umeå, Sweden**

A common feature of all spectrins is their calmodulin-binding activity. Calmodulin binds readily and in a Ca^{2+}-dependent fashion to the 240 kDa subunit of spectrins [1] (spectrins are heterodimers composed of a common 240 kDa α-subunit and, depending on species and cell type, a β-subunit of 260, 235 or 220 kDa molecular weight). Although human erythrocyte spectrin (a 240/220 kDa heterodimer) binds calmodulin [2], the binding is much weaker and it is not clear to which of the subunits calmodulin binds. In 6 M urea, a 10 kDa fragment originating from the β-IV region (i.e. the tail region) of the 220 kDa subunit appears to contain the binding activity [3], whereas under non-denaturing conditions the predominant calmodulin-binding is associated with the 240 kDa subunit [4].

Using calmodulin-affinity chromatography, we have found that the 240 kDa subunit contains a Ca^{2+}-dependent calmodulin-binding activity. By tryptic digestion of isolated 240 kDa subunit followed by chromatography on the calmodulin-column, a major Ca^{2+}-dependent binding fragment of 30 kDa molecular weight was identified. Sequence analysis of the fragment showed that the N-terminal of this polypeptide starts at amino acid residue 253 of the 240 kDa subunit. This strongly implies that human erythrocyte spectrin binds calmodulin to a site in the head region (i.e. the part of spectrin that is involved in formation of tetramers).

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CD 302 DYNAMICS OF SEA URCHIN EGG SPECTRIN IN OOGENESIS AND EMBRYOGENESIS IS INDICATIVE OF ITS ROLE IN MEMBRANE BIOGENESIS, VESICLE TRAFFICKING, AND CELLULAR DIFFERENTIATION, Douglas J. Fishkind, Edward M. Bonder*, and David A. Begg, Harvard Medical School, Dept. of Anat. and Cell. Biol., Boston, MA, *Rutgers University, Dept. of Biol. Sci., Newark, NJ.
To investigate spectrin's (SP) role in sea urchin development we prepared monospecific antibodies to the egg isoform of SP. Using high-resolution fluorescence microscopy we have mapped the developmental expression, cellular distribution, and dynamic redistribution of SP in oogenesis and early embryogenesis. SP expression first increases in the ooplasm of early oogonia, whereupon it assembles onto newly synthesized organelles. E.M. immunolocalizations on membrane-cytoskeletal preparations of eggs show SP associated with the membranes of three organelles: 1) the plasma membrane (PM), 2) cortical granules, and 3) a population of cytoplasmic vesicles. Upon fertilization, the cortical granules fuse with the PM, and the second population of SP encoated vesicles reposition to the cell cortex. During early development when the embryo is undergoing a series of rapid cell divisions, the SP coated vesicles decrease in number, while intense SP staining appears along the newly formed apical/basolateral PM's of blastula epithelial cells. Since overall [SP]'s do not change during early development, the results suggest SP undergoes redistribution rather than new synthesis. In conclusion, this study supports two emerging concepts concerning SP's multifunctional role in differentiating cells: 1) SP-coats on the cytoplasmic surface of organelles represent membrane-cytoskeletal pre-assemblages that may provide important "cues" for vesicle addressing and trafficking to the cell surface, and 2) SP coated vesicles may serve as preprocessed, membrane-cytoskeletal precursors which are utilized during cell growth and differentiation.

CD 303 INHIBITION OF TROPOMYOSIN BINDING TO F-ACTIN BY TROPOMODULIN, A NEW TROPOMYOSIN-BINDING PROTEIN FROM THE HUMAN ERYTHROCYTE MEMBRANE, Velia M. Fowler, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037 We have discovered a new M, 43,000 non-actin tropomyosin-binding protein that we have named tropomodulin on the basis of its ability to inhibit tropomyosin binding to actin. This protein was initially identified by binding of ^{125}I-tropomyosin to nitrocellulose blots of erythrocyte membrane proteins separated by SDS-gel electrophoresis and was shown to be present in the membrane skeleton in a ratio of 1 for every 2 tropomyosin molecules (Fowler, V.M., 1987. J. Biol. Chem. 262:12792-12800). Tropomodulin is a potent uncompetitive inhibitor of erythrocyte tropomyosin binding to F-actin ($K_i \sim 0.8$ μM from Dixon plots), and is equally effective whether or not the tropomyosin is preincubated with the actin before addition of the protein. Unlike other proteins from non-muscle cells which influence tropomyosin binding to actin, tropomodulin itself does not bind to F-actin. We speculate that tropomodulin may act by blocking the ability of erythrocyte tropomyosin to self-associate head-to-tail, thereby weakening its binding to actin. This idea is suggested by the sequence of an internal 21 amino acid tryptic peptide from tropomodulin which reveals a striking homology with the C-terminal sequence of horse platelet tropomyosin. In muscle and some non-muscle tropomyosins, the C-terminal end of one tropomyosin molecule binds to the N-terminal end of another, mediating the head-to-tail self-association of the tropomyosin molecules along the actin filament. Erythrocyte tropomodulin is anticipated to be a representative of a new class of tropomyosin-binding proteins since immunoreactive polypeptides have been detected in a variety of non-erythroid cells and tissues, including skeletal muscle, brain, lens, neutrophils and endothelial cells. In erythrocytes, we propose that tropomodulin functions with tropomyosin to regulate the interaction of the membrane-associated actin filaments with spectrin or other erythrocyte actin-binding proteins and, thus, influences the assembly and organization of the erythrocyte membrane skeleton.

CD 304 CYCLIC AMP DEPENDENT PHOSPHORYLATION OF THE 22 x 8 PROTEIN OF HUMAN ERYTHROCYTE BAND 7 COMPLEX, Robert M. Johnson, Deborah Robertson, Fred Brohn, Ding Wang, Departments of Biochemistry and Pediatrics, Wayne State Medical School, Detroit, MI 48201. A polypeptide of the Band 7 complex of the red cell membrane is deleted in hereditary stomatocytosis, a genetic defect of the Na,K cotransport system (Lande et al, J. Clin. Invest. 70, 1273-1280, 1982). This protein, denoted as 22 X 8 because of its position on non-equilibrium pH gradient electrophoresis (NEPHGE) gels, is therefore implicated in Na,K co-transport. We have found that this protein is also a major substrate of the cAMP-dependent kinase of human red cells. Intact cells were incubated with ^{32}Pi to isotopic equilibrium and then briefly exposed to dibutyryl cAMP. Autoradiographs of membranes showed that the major substrates were bands 2.1, 4.1, 4.9, and 7. Band 7 is not phosphorylated in ghosts, implying that the cAMP-kinases involved are cytoplasmic. Band 7 contains at least four polypeptides that are not removed by extensive washing of the ghosts. To determine the Band 7 substrate of the cAMP-dependent kinase more precisely, we performed NEPHGE analysis of the membrane proteins from control and dibutyryl cAMP treated cells before and after detergent extraction. Coomassie blue staining revealed no differences between control and dibutyryl cAMP treated cells. In contrast, autoradiographs showed that the 22 x 8 component of band 7 is esentially unlabeled in controls but becomes highly labeled after cAMP treatment. Dibutyryl cAMP regulates Na,K co-transport in both avian and human cells, and the dibutyryl cAMP concentration dependence of 22 x 8 phosphorylation and cotransport inhibition are very similar. This suggests that phosphorylation of 22 x 8 may be involved in the regulation of erythrocyte cation transport.

CD 305 IDENTIFICATION OF MYOSIN LIGHT CHAIN KINASE (MLCK) IN NEONATAL RBC. S.L. Schrier & Hemant Thatte, Div. of Hematology, Stanford University School of Medicine, Stanford, CA 94305-5112. RBC undergo shape transformation under physiological and pathological conditions. It is assumed that viscoelastic properties of the membrane and its cytoskeleton modulate these structural changes. In platelets, contraction of actomyosin is regulated by the phosphorylation of myosin light chains by MLCK in the presence of Ca^{+2} and calmodulin (CAM). As in platelets, actin, myosin, tropomyosin, troponin I and CAM have been identified in RBC. A contractile system could affect cytoskeletal function but only if MLCK were present. Neonatal RBC were studied because of their elevated myosin content. Neonatal RBC were hemolyzed in 3mM EDTA, 2mM EGTA, 2mM DTT, 20μg/ml PMSF and 20mM Imidazole buffer, pH 7.7 and centrifuged. The supernatant was absorbed on DEAE-cellulose and adsorbed proteins were eluted by a 50-500mM NaCl linear gradient. Column fractions were resolved on SDS-PAGE and MLCK containing fractions were identified by Western blots using rabbit anti-turkey gizzard MLCK antibodies. Pooled concentrated MLCK fractions were assayed for enzyme activity using myosin light chains (19Kd) purified from neonatal RBC and γ^{32}P-ATP as substrate, in the presence of Ca^{+2} and CAM. MLCK fractions were then subjected to calmodulin-sepharose affinity column chromatography. MLCK was eluted with 0.2M NaCl in hemolysis buffer and migrated as single band on SDS-PAGE, exhibited a molecular weight of 110Kd, and reacted with rabbit anti-turkey gizzard MLCK antibodies. The enzyme had a specific activity of 2.78μM of phosphate transferred to the myosin light chains per mg of MLCK per min. The red cell enzyme was purified greater than 600 fold. Therefore, most of the components of a contractile system have been identified in neonatal RBC and could affect skeletal function.

CD 306 MODULATION OF ERYTHROCYTE MEMBRANE MATERIAL PROPERTIES BY Ca^{2+} and CALMODULIN, Yuichi Takakuwa, Teruo Ishibashi, Stephen B.Shohet*, and Narla Mohandas*, Department of Biochemistry, Hokkaido University School of Medicine, 060, Sapporo, Japan, and Cancer Research Institute, University of California San Francisco, CA 94132* It is well known that skeletal proteins of the red blood cell play an important role in maintaining membrane material properties such as stability and deformability. However, the regulatory mechanism of these properties have not been clearly defined. In order to test whether Ca^{2+} and calmodulin(CaM) might regulate membrane properties, using an ektacytometer we measured membrane stability and deformability of resealed ghosts which were prepared in the presence of varing concentrations of Ca^{2+} and CaM. In the presence of CaM, membrane stability decreased with increasing concentrations of Ca^{2+} (> 1uM). Moreover, these alterations were completely reversible. In the absence of CaM, an equivalent decrease in membrane stability was seen only when Ca^{2+} concentrations were two orders of magnitude higher. Examination of the effects of Ca^{2+} and CaM on various membrane interactions have enabled us to suggest that spectrin-protein 4.1-actin interaction may be one of the target for the effect of Ca^{2+} and CaM. In contrast to these changes in stability, membrane deformability decreased only at Ca^{2+} concentrations higher than 100uM, and CaM had no effect.

CD 307 PRESENCE OF ERYTHROID AND NONERYTHROID SPECTRIN TRANSCRIPTS IN HUMAN RETICULOCYTES, EYE LENS AND CEREBELLUM, S.H. Yoon, T. Papayannopoulou and J.T. Prchal. Univ Ala at Birmingham, AL and Univ of Washington, Seattle, WA. Spectrin is the major protein of the membrane skeleton which underlies the internal side of the erythrocyte membrane. Spectrin was initially thought to be unique to erythrocytes until recently when similar, but non-identical spectrin-like peptides have been identified in various non-erythroid cells suggesting the existence of a family of spectrin genes. Transcription of spectrin in human erythroid cells, eye lens and cerebellum were examined by direct hybridization with the known human α-and β-spectrin cDNA probes as well as human α-fodrin cDNA probe. Blots of poly(A)+ RNA from erythroid and several non-erythroid cells and total RNA from lens showed that β-spectrin cDNA probe hybridized to two distinct species of spectrin mRNA in human reticulocytes and eye lens. In human reticulocytes, the major 7.8kb band was approximately 7 times as abundant as the minor 10kb band. In human eye lens, two distinct bands of 8.6 and 7.4kb mRNA are present in some quantity. The human erythroleukemia cell lines, HEL 92.1.7, HEL R and K562 have the major 7.8kb β-spectrin mRNA but, in addition, have two smaller transcripts of approximately 4.5 and 3.4kb. The human cerebellum had a distinct 11kb mRNA transcript, in contrast to a major erythroid β-spectrin mRNA that has 7.8kb in size. A transcript of α-fodrin was found in the erythroleukemia line, but the size was distinct from that of β-spectrin. The α-fodrin mRNA was not detectable in human reticulocytes. The eye lens was also found to have 7.2kb α-fodrin transcript while cerebellum had 8.5kb α-fodrin mRNA transcript that was of the same size as that found in other non-erythroid tissues. In addition, human eye lens also had 7.5kb transcript of erythroid α-spectrin subunit. The abundance of erythroid and non-erythroid spectrin transcripts in lens was significantly greater than in any other tissues examined. In erythroid tissues the β-spectrin message was found to be the most abundant in human bone marrow. These data indicate that human reticulocytes and human lens have two distinct mRNAs for β-spectrin. The human erythroleukemia cells contain non-erythroid spectrin message in addition to multiple erythroid spectrin transcripts. Human eye lens has erythroid α and β-spectrin transcripts, as well as a unique α-fodrin transcript. In human eye lens the erythroid β-spectrin transcript is present in two distinct species different in size from the β-spectrin transcript found in erythroid cells. Human cerebellum has a unique single transcript of erythroid β-spectrin message as well as α-fodrin transcript of the same size as the other non-erythroid tissues.

Posters IV

CD 400 DEFICIENCY OF MEMBRANE PROTEIN BAND 4.2 IN RED CELLS OF TWO CASES OF CONGENITAL HEMOLYTIC ANEMIA (HS AND HE). Kazuyuki Ata, Akio Kanzaki and Yoshihito Yawata, Div of Hematol, Dept of Med, Kawasaki Med School, Kurashiki, JAPAN

A role of band 4.2 for red cell morphology and membrane stability was studied in 2 patients with membrane protein band 4.2 deficiency; case 1: common HE with severe uncompensated hemolysis, and case 2: classical HS of autosomal dominant inheritance with microspherocytosis. Both patients demonstrate (1) complete absence of band 4.2 on SDS-PAGE with no defect of other protein bands, (2) normal membrane lipid composition, (3) increased Na influx (2.15 mmoles/l RBC/hour in case 1 and 1.78 in case 2 (N:1.29±0.14), (4) spectrin studies: normal peptide mapping, polymerization and heat stability, (5) no release of ankyrin under the condition of low ionic strength as reported by Rubicki et al, (6) normal mechanical stability of Triton-shells, (7) moderately decreased red cell deformability in intact red cells examined by ektacytometry, and (8) increased stomatocytic changes superimposed onto original red cell morphology, compared to classical HS (n=25) and common HE (n=25) not accompanied with band 4.2 deficiency. It is interesting enough to know that complete deficiency of band 4.2 was observed at the different congenital membrane disorders, such as HS and HE, and that the deficiency appears to increase stomatocytic changes, and to reduce deformability in intact red cells.

CD 401 ACTIVATION OF A PLASMODIUM FALCIPARUM PROTEASE CORRELATED WITH MEROZOITE MATURATION AND ERYTHROCYTE INVASION, Catherine Braun-Breton and Luis Pereira da Silva, Institut Pasteur 75724 Paris Cedex 15 France

Merozoite invasion of erythrocytes appears to involve some serine protease activity, as serine protease inhibitors can block invasion *in vitro* (1,2,3). We have recently described a *P.falciparum* membrane antigen, p76, which has properties consistent with it being a serine protease (4). Interestingly, activation of the serine protease activity follows cleavage of the p76 glycosyl-phosphatidylinositol (GPI) membrane anchor by a phosphatidylinositol-specific phospholipase C (PI-PLC). *In vivo* solubilisation and activation of p76 occurs at or about the time of red blood cell invasion by the merozoite. Characteristics of 2 enzymes , the p76 serine protease and a *P.falciparum* anchor-degrading activity, involved in this biochemical cascade will be presented.

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2. Hadley,T. et al., Exp.Parasitol., **53**, 306-311 (1983)
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CD 402 SPECIFIC CHEMICAL MODULATION OF SKELETAL PROTEIN-PROTEIN INTERACTIONS LEADS TO SPECIFIC ALTERATIONS OF THE PHYSICAL STATE OF CELL-SURFACE SIALIC ACID: IMPLICATIONS TO TRANSMEMBRANE SIGNALING AND HEMOLYTIC ANEMIAS, D. Allan Butterfield, Department of Chemistry and Center of Membrane Sciences, University of Kentucky, Lexington, KY 40506

The skeletal network of proteins of human erythrocyte membranes is known to have several important cellular functions including control of cell shape, viscoelasticity, deformability, and attachment site(s) for certain transmembrane proteins such as Band 3 and glycophorin A. It is conceivable that the skeleton also may play in role in transmembrane signaling events, i.e., those processes by which a response on one side of the membrane is generated by a stimulus on the opposite side. If this is applicable to erythrocytes, then specific modulation of crucial protein-protein interactions in the membrane skeleton may effect the physical state of cell-surface sialic acid, 70% of which is on the external side of glycophorin A. Increased and decreased membrane skeletal protein-protein interactions were induced by two agents that bind specifically to the RBC membrane skeleton: spermine and hemin, respectively. The physical state of cell-surface sialic acid was monitored by electron spin resonance spin labels specifically and covalently bound to this carbohydrate. 1.2 mM spermine, a polyamine that is increased in certain cancers and in other traumatic cellular insults, is shown to increase skeletal-skeletal and skeletal-transmembrane interactions and to cause a 40% increase in motion of sialic acid ($P<0.001$, $N=6$). In contrast to spermine, hemin, a hemoglobin breakdown product that is elevated in aged red cells and in sickle cell disease and β-thalassemia, is reported to decrease skeletal protein-protein interactions by initially disrupting the spectrin-Band 4.1 interactions. A 60% decrease in sialic acid motion was found with 10 μM hemin addition to spin-labeled ghosts ($P<0.001$, $N=8$). These results demonstrate that specific skeletal protein alterations can affect the physical state of cell-surface carbohydrates and are consistent with a cardinal hypothesis of transmembrane signaling mechanisms. Similar studies in certain hemolytic anemias in which known skeletal protein alterations exist are in progress and may yield insight into molecular mechanisms by which altered skeletal proteins lead to altered cellular functions. Supported in part by a NSF grant (RII-86-10671).

CD 403 PURIFICATION AND CHARACTERIZATION OF ERYTHROCYTE MEMBRANE COMPONENTS CARRYING DUFFY BLOOD GROUP ANTIGENS. POSSIBLE RECEPTOR FOR P.KNOWLESI AND P.VIVAX MALARIA PARASITE. A.Chauhuri, V.Zbrzezna, C. Johnson, M.Nichols W.L.Marsh and A.O.Pogo. New York Blood Center, New York, N.Y. 10021. Duffy phenotypes Fy(a+b-), Fy(a-b+), Fy(a+b+) and Fy(a-b-) were purified and characterized by using a monoclonal antibody (mAb). Proteins that react with the mAb in immunoblots are pA and pB (> 100kDa) and pD (43-46kDa). Electro-eluted pD protein generates discrete bands of similar MW as pA and pB proteins. Electroeluted pA and pB proteins disaggregate yielding pD protein. Oligomers and monomers of pD protein are present in the three positive cells, and in negative cells in smaller amounts. pC (60kD),pE (35kD) and pF (28kD) proteins do not interact with mAb but copurified with pD protein. pC and pE proteins are present in the three positive cells and in minute amounts in negative cells. pF protein is present in positive cells only. By fingerprint analysis pD protein is different in Fy(a+b-) and Fy(a-b+) cells. Duffy phenotype is a multimeric red cell membrane protein composed of different subunits, of which only one, the polymorphic pD protein interacts with the mAb. Malaria parasite attaches to the red cell membrane of Duffy positive and negative cells but fails to penetrate the membrane of Duffy negative cells. It could be that a minimal quantity of Duffy proteins or lack of pF protein is the requirement for parasite invasion.

CD 404 THE RESA PROTEIN OF **PLASMODIUM FALCIPARUM** IS A PHOSPHORPROTEIN ASSOCIATED WITH THE ERYTHROCYTE MEMBRANE SKELETON, Michael Foley, Leecia J. Murray and Robin F. Anders, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria 3050, Australia.

The ring-infected erythrocyte surface antigen, is a M_r 155,000 malarial polypeptide that is released from merozoites and becomes associated with the erythrocyte membrane at the time of invasion. Inside out vesicles (IOVs) prepared from **P.falciparum** infected erythrocytes contain RESA and Triton X-100 extraction demonstrates that the majority of RESA molecules were Triton insoluble, indicating membrane skeleton association. When these IOVs were incubated with [γ^{32}P]ATP a M_r 155,000 could be seen labelled in IOVs from infected but not uninfected erythrocytes. Immunoprecipitation using rabbit antisera specific for RESA confirms that RESA was indeed a phosphoprotein. Phosphoamino acid analysis reveals that phosphoserine was the principal amino acid detected with small amounts of phosphothreonine but no phosphotyrosine was detected. Labelling of intact parasitised erythrocytes with inorganic P^{32} for several hours in culture revealed phosphorylated RESA in Triton insoluble extracts of infected but not uninfected cells. When this labelling procedure was repeated with parasites synchronised around the life-cycle it was demonstrated that RESA was phosphorylated only when it became associated with the erythrocyte skeleton and that although immunoblotting revealed the presence of RESA in mature parasites it was not phosphorylated. RESA molecules released into the culture supernatants by parasites can bind to inside out vesicles prepared from normal uninfected erythrocytes, and subsequent labelling with [γ^{32}P]ATP results in RESA becoming phosphorylated. The evidence suggests that RESA is phosphorylated by an erythrocyte membrane kinase and probably not by a parasite encoded enzyme.

CD 405 PLASMODIUM CHABAUDI ANTIGENS ASSOCIATED WITH THE RED CELL MEMBRANE.

C. Hamers-Casterman, R. Hamers, W. Deleersnijder and Sumalee Tungpradubkul, Vrije Universiteit Brussel, Instituut voor Moleculaire Biologie, Paardenstraat 65, 1640 Sint-Genesius-Rode, Belgium.

Several parasite encoded proteins are found associated with the red cell membrane of plasmodium chabaudi and can be defined by monoclonal antibodies.

One of these antigens is apparently one of the antigens involved in the very early immune responses of the host, IgM antibodies appearing three days after needle infection with 10^6 parasites. This antibodies detect the antigen as "tiny points" on the surface of the infected erythrocyte. An antigen displaying this tiny point "phenotype" can be copurified with the erythrocyte membranes but does not behave in Triton X 114 extraction as an integral membrane component. Partial sequence analysis, time of appearance and phenotype homology suggest that it is related to the P. falciparum 1-11 antigen, an antigen characterised by the largest repitope ever found.

CD 406 **A NEW CYTOADHERENCE PROPERTY OF MALARIA-INFECTED ERYTHROCYTES,** Shiroma M. Handunnetti, Aileen Gilladoga, and Russell J Howard, Laboratory of Infectious Diseases, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304. The asexual erythrocytic stages of malaria parasites alter the structural and fuctional properties of the host erythrocyte to serve the needs of a rapidly developing intracellular organism. Erythrocytes containing the more mature parasites acquire cytoadherence properties, leading to the attachment of infected erythrocytes to the vascular endothelium. Another cytoadherence property of malaria infected erythroytes has been identified: mature infected cells adhere to uninfected erythrocytes, forming rosettes containing 2-7 uninfected erythrocytes around a central infected cell. This new cytoadherence property was first described with a simian malaria parasite, Plasmodium fragile, which is sequestered in vivo in its natural host the toque monkey Macaca sinica. In P. fragile, rosetting in vitro and sequestration in vivo appear simultaneously as the parasite matures. Immune monkey serum reversed the cytoadherence of infected cells to uninfected cells (rosetting) and to vascular endothelium (sequestration).

Human erythrocytes infected with Plasmodium falciparum, the most lethal of the human malarias, also aquire the rosetting cytoadherence property. Rosettes are not formed when parasites are at the ring-stage; in contrast mature trophozoite/schizont infected erythrocytes form rosettes containing 3-7 uninfected cells. Rosettes can be disrupted mechanically but rapidly reform. Sera from immune African adults also reverse rosettes and leads to agglutination of infected cells alone. Rosetting depends not only on a malaria-induced change on infected cells, but also on properties of the uninfected cell. Parasites which rosette strongly exhibit less in vitro cytoadherence to C32 melanoma cells (an in vitro assay for in vivo adherence to endothelial cells). However, if rosettes are disrupted and infected cells rapidly purified, these infected cells adhere strongly to C32 cells. We suggest that in vivo rosettes could serve several functions. Rosettes may be more readily occluded in narrow capillaries leading to enhanced infected cell cytoadherence to capillary endothelial cells. Parasite proliferation may be incresased if merozoites released from ruptured schizonts more efficiently invade adjacent rosetted uninfected cells. Finally, infected erythrocytes in rosettes may not only be less accessible to monocytes and other cells in the effector arm of host immunity, but may disguise the grossly altered infected cell within a coating of normal host erythrocytes.

CD 407 SEVERE HEREDITARY ELLIPTOCYTOSIS IN TWO RELATED CAUCASIAN CHILDREN WITH A DECREASED AMOUNT OF SPECTRIN (Sp) α CHAIN, M.C. Lecomte, C. Féo*, H. Gautero, O. Bournier, C. Galand, P. Boivin, G. Tchernia** and D. Dhermy. INSERM U160 Hôpital Beaujon, 92118 Clichy,*INSERM U299 **Service d'Hématologie, Hôpital Bicêtre, 94275 Kremlin Bicêtre (France). The proposita, a 7-y old girl, experienced severe hemolytic elliptocytic anemia requiring blood transfusion until partial splenectomy was performed. Similar hemolytic disease was observed in her 5-y old full cousin. The proposita's mother was clinically normal and her blood smear displayed inconstant elliptocytosis. The maternal proposita's aunt who was asymptomatic had obvious elliptocytosis. The maternal proposita's grand-father was splenectomized with the diagnosis of congenital hemolytic anemia. Ektacytometric studies had revealed that erythrocyte deformability was decreased in both children and normal in their respective mothers. Erythrocyte thermal sensitivity was normal in each subject. Erythrocyte membrane mechanical stability was considerably reduced in the proposita. In the proposita and her full cousin membrane protein electrophoresis revealed a decrease in Sp α chain amount (30%). No Sp deficiency was observed in the respective mothers. The apparent self-association constant of Sp dimer in solution was decreased in both children as well as in their mothers, whereas the Sp dimer % in the membrane were at the upper limit of the normal range. All Sp tryptic digests were similar to the controls. Further investigations revealed an increased amount of ankyrin (30%) in the membrane of the proposita as demonstrated by membrane protein electrophoresis, immunoblotting technics and studies of Sp-ankyrin interactions. Other investigations concerning the synthesis and assembly of membrane proteins will be necessary to understand the physiopathology of this new membrane defect.

CD 408 DECREASED IN VIVO SURVIVAL OF OXIDATIVELY DAMAGED RED CELLS. J. McKenney, C.R. Valeri, N. Mohandas, N. Fortier, and L.M. Snyder, St. Vincent Hospital, Worcester, MA 01604, Naval Blood Research Lab, Boston, MA, University of CA, San Francisco, CA. In the present study we attempt to establish the consequence of in vitro H_2O_2 induced membrane damage as manifested by spectrin hemoglobin complex formation and decreased RBC deformability to in vivo survial of damaged RBCs using baboons. Baboon RBCs following exposure to 135-512uM H_2O_2 and reduction with DTE were infused into the animal and the % 24 hour survival and the $Cr^{51}T\frac{1}{2}$ were measured. In a dose dependent fashion a positive correlation was observed between in vitro membrane alterations and the 24 hour in vivo survival. 88% of control cells survived 24 hours in contrast to 79% following treatment with 315uM H_2O_2, 67% following 480uM H_2O_2 and 64% after 512uM. Pretreatment with CO increased survival from 67% to 82% following exposure to 480uM H_2O_2. The cells not removed from circulation in the first 24 hours had a normal $Cr^{51}T\frac{1}{2}$. These results suggest that a subpopulation of cells sensitive to oxidation are being removed during the first 24 hours. To identify this population, baboon RBCs were separated on Percoll Hypaque gradients. The top 10% (MCHC 32 gm/dl) and the bottom 10% (MCHC 37 gm/dl) RBCs were injected into the baboon either untreated or following exposure to 315uM H_2O_2. The untreated top (88%) and bottom (89%) show no difference in either the 24 hour survival or $Cr^{51}T\frac{1}{2}$. However, only the H_2O_2 treated bottom population showed a substantial decrease in 24 hour survival. These data support our hypothesis that during peroxidative membrane damage, Hb oxidation initiates a sequence of events resulting in skeletal changes which lead to membrane alterations and eventually to in vivo destruction, and that the dehydrated RBCs are most susceptible to H_2O_2 damage.

CD 409 ANKYRIN mRNA EXPRESSION IN NORMAL AND ANKYRIN DEFICIENT MICE, Luanne L. Peters, Robert A. White, Connie S. Birkenmeier and Jane E. Barker, The Jackson Laboratory, Bar Harbor, ME 04609

Mice homozygous for the normoblastosis (nb) mutation have severe hemolytic anemia. Reticulocytes of nb/nb mice have no detectable ankyrin (210 kD) (1). An immunologically cross reacting protein of a smaller size (150 kD) is present (2). We used a human erythroid ankyrin cDNA clone (3) to probe for ankyrin transcripts in total RNA from normal and nb/nb reticulocytes and bone marrow. Reticulocyte RNA from normal mice and bone marrow RNA from mk/mk (microcytic iron deficiency anemia) served as non-ankyrin deficient controls. An 8.4 kb ankyrin transcript was found in normal but not nb/nb reticulocytes. Two transcripts (9.0 and 8.4 kb) were found in mk/mk bone marrow RNA. A diffuse region ($\leq$ 9.0 kb) of faint hybridization was found in nb/nb bone marrow. The results suggest that although no ankyrin transcript is detected in nb/nb reticulocyte RNA, some ankyrin related transcription is occurring in nb/nb bone marrow. The 150 kD ankyrin related protein detected in nb/nb reticulocytes may originate from mRNA transcribed in bone marrow. Further experiments using more sensitive methods for RNA detection are planned. (1) Bodine, et al. 1984. Cell. 37,721. (2) White, et al. 1988. In preparation. (3) Kindly provided by Dr. Sam Lux, Dept. of Hematology Oncology, Children's Hospital, Boston, MA.

CD 410 CHARACTERIZATION OF A CYTOADHERENCE PROTEIN PRESENT ON THE SURFACE OF A KNOBBY LINE OF PLASMODIUM FALCIPARUM, E. Winograd and I. W. Sherman, Department of Biology, University of California, Riverside, CA 92521.

Infections with the human malaria Plasmodium falciparum are characterized by the retention of parasitized erythrocytes in tissue capillaries and venules. Erythrocytes containing trophozoites and schizonts attach to the endothelial cells which line these vessels by means of ultrastructurally identifiable excrescences present on the surface of the infected cell. Such excresences, commonly called knobs, are visible by means of scanning or transmission electronmicroscopy. The biochemical mechanisms responsible for erythrocyte adherence to the endothelial cell are still undefined. In an attempt to identify the cytoadhesive molecule on the surface of the infected red cell we have prepared monoclonal antibodies to knob-bearing erythrocytes. One of these, monoclonal antibody 4A3, is an IgM that reacts (by means of immunofluorescence) with the surface of erythrocytes bearing mature parasites of the knobby line, and does not react with knobless lines. By immunoelectronmicroscopy 4A3 was localized to the knob region; in an in vitro cytoadherence assay the antibody blocked binding of knob-bearing cells to formalin-fixed amelanotic melanoma cells. 4A3 was used to immunoprecipitate a protein from extracts of knobby erythrocytes which had been previously surface iodinated. The antigen recognized by 4A3 had an Mr of 85KDa. Peptide mapping suggests the 85 kDa antigen is homologous to band 3.

CD 411 COMBINATION OF HEREDITARY ELLIPTOCYTOSIS (HE) AND β-THALASSEMIA (β-Thal): CHANGES IN SPECTRIN, Sara Streichman, Ethy Herz and Ilana Tatarsky, Department of Hematology, Rambam Medical Center and Technion Faculty of Medicine, Haifa, Israel. The clinical and hematologic condition of a patient who inherited heterozygous HE and β-Thal was far more severe than expected with either of these diseases alone. The bizarre morphology of his RBCs and their extremely low MCV were very similar to those found in hereditary pyropoikilocytosis, but no significant thermal instability of the RBC membranes was observed. Nondenaturing (ND) gel electrophoresis of spectrin extracted at 0°C showed an extra band between the spectrin tetramers and dimers. Spectrin extracted at 37°C showed dimers only. Spectrin extracted at either temperature resolved to the normal α and β subunits on SDS-PAGE. Repeated ND gel electrophoresis of spectrin, done 2 years later when the clinical and hematologic condition of the patient was improved, showed that the previously observed extra band no longer appeared, but instead an increase in spectrin dimers was estimated. SDS-PAGE of the membranes showed that the amount of spectrin compared to band 3 was reduced in cells that were metabolically depleted during 48 h incubation at 37°C. In these cells the relative amount of band 4.5 was increased. We assume that this patient had an infantile type of HE which involves extreme instability of the skeleton. The increased instability of the skeleton eventually could lead to exposure of the spectrin molecules to oxidative damage known to occur in thalassemic RBCs. The consquences of such oxidation might have led to changes in spectrin associations. Abnormal associations of spectrin probably led to the appearance of the spectrin extra band found on ND gel electrophoresis and also to the tendency of the membranes to lose spectrin on metabolic depletion.

CD 412 **DIMINISHED QUANTITY OF THE RH ANTIGEN D ON RED CELLS OF PATIENTS WITH HEREDITARY SPHEROCYTOSIS (HS). Irma O. Szymanski, L. Michael Snyder, Departments of Pathology and Laboratory Medicine, University of Massachusetts Medical Center, Worcester, MA 01655 and St. Vincent Hospital, Worcester, MA 01604. Individuals with Rhnull and Rhmod red cell phenotypes (absent or decreased amounts of Rh antigens) exhibit similar clinical and laboratory findings as patients with hereditary spherocytosis (HS). This study was done to determine if HS red cells also have decreased amounts of Rh antigens. Initially we studied the red cells of five members of a family where two had HS. Using automated quantitative hemagglutination tests, we demonstrated that HS red cells agglutinated significantly less with Rh antisera of five different specificities than did normal red cells. The strength of other blood group antigens on HS red cells was estimated by manual titers and agglutination scores. There were no appreciable differences in the agglutination of HS and normal red cells with non-Rh antisera. The D antigen was measured on the red cells of 19 individuals with HS and their 11 normal relatives. On the average, HS red cells had 9,209 ± 4,084 D epitopes, whereas the normal individuals had 15,394 ± 5,763 D epitopes (p<0.01). Since the number of D epitopes is dependent on the Rh phenotype, we compared within the same phenotype the number of D antigen epitopes on normal and HS red cells. These analyses showed that HS red cells had about half of the normal number of D antigen epitopes. Our data indicate that the Rh antigen D is selectively decreased on HS red cells.**

CD 413 HEMATOLOGICAL AND MEMBRANE CHARACTERISTICS IN 33 PATIENTS WITH HEREDITARY STOMATOCYTOSIS. Yoshihito Yawata, Akio Kanzaki, Kazuyuki Ata and Hideho Wada, Div of Hematol, Dept of Med, Kawasaki Med School, Kurashiki, JAPAN

Contrary to commonly-held belief, substantial number of patients with hereditary stomatocytosis (HSt) does not show increased membrane permeability and abnormal cell hydration. Thus, hematological and membrane characteristics were reviewed in 33 patients of HSt studied at our institution. The type of the disease was categorized as based on Na influx: HSt with markedly increased Na influx (Type 1: > 5 mmoles/l RBC/hr), HSt with moderately increased Na influx (Type 2: 1.5-5), and HSt with not increased Na influx (Type 3: 1-1.5), compared to normal control (1.29±0.14). In Type 1 (n=7), RBC, MCV, MCHC, Na influx, Na efflux, [Na]RBC, [K]RBC was 312±85×10^4/μl, 120±9 fl, 29.3±1.8%, 8.2±3.6, 7.7±3.6, 45.4±22.7 mM, 40.4±12.5 mM, in Type 2 (n=15), 284.7, 97.9±21.8, 34.1±3.2, 2.2±0.8, 4.4±2.2, 13.1±6.1, 81.9±8.4, in Type 3 (n=10), 341.4±97.3, 92.3±11.3, 33.7±2.0, 1.24±0.2, 2.63±0.8, 12.8±6.6, 84.1±7.0; and in xerocytosis (n=1), 202, 86, 32.8, 1.92, 5.58, 5.34, 88.0, respectively. In these cases of various types of, HSt no substantial abnormalities were observed in lipid contents and phospholipid composition, in band 7 on SDS-PAGE, and in Na^+, K^+-ATPase activity. Other morphological features will be discussed. Concerning spectrins of these patients, dimer to tetramer conversion and two-dimensional mapping of extracted spectrins revealed no essential abnormalities.