EDITORIAL

The Molecular Lesion of Hereditary Spherocytosis (HS): A Continuing Enigma

By William N. Valentine

"The one fundamental variation from normal in congenital hemolytic icterus is the microspherocytosis. The anemia, jaundice, splenomegaly, reticulocytosis and increased fragility are all secondary to the globular form of the erythrocyte." R. L. Haden, Trans Assoc Am Physicians 49:308, 1934

Very few, if any, abnormalities of the human erythrocyte have been studied with such diligence as those associated with hereditary spherocytosis (HS). Not only has the small, round, densely staining spherocyte provided a long-recognized morphologic benchmark, but its inordinate susceptibility to lysis in hypotonic saline has been clearly documented for 75 years.1 Forty years ago, Haden (cited above) and Castle and Daland2 related the latter to loss of the usual discoidal form of the erythrocyte, and to a probable structural abnormality resulting in a decreased surface area/volume ratio. The unique role of the spleen in recognizing, sequestering, and finally destroying the abnormal cell, and the effect of splenectomy in correcting clinical manifestations were investigated in great detail in the 1940's and early 1950's.1-9 The assumption that a genetically determined, usually dominantly transmitted7,10 abnormality of the red cell membrane lies at the heart of the disorder has pervaded studies of its pathophysiology and, in the past 20 years, has fostered many investigations of red cell membrane structure and function. While these studies have added much to our understanding of red cell physiology, the secret of the ultimate molecular lesion still remains locked within the osmotically fragile spherocyte.

These investigations have defined a variety of abnormal properties in HS red cells. These cells are abnormally permeable to sodium and this "sodium leak"11,12 necessitates an increased glycolytic rate to support greater-than-normal adenosine triphosphate–adenosine triphosphatase (ATP–ATPase)-dependent pumping of sodium from the cell's interior.12-14 While total lipids have been reported to be relatively somewhat decreased both before15,16 and after7 splenectomy, and abnormalities of phospholipid turnover have been suggested,14 no consistently demonstrable data thus far support specific abnormalities in membrane lipid structure. However, under conditions of glucose deprivation, HS membrane lipid is abnormally unstable, and is released by a budding process, thus contributing to a still greater deficit in surface area.15,19,20
Other studies have focused on membrane proteins. The failure of lipid-free proteins extracted from HS membranes to increase in sedimentation on treatment with divalent cations has been attributed to inability to properly form aggregates or microfilaments. Vinblastine precipitates less protein from HS than normal membranes, and with intact cells induces spherocytosis. The agglutination of normal red cells washed in electrolyte-free media is reversed by low concentrations of Ca\(^{2+}\), whereas with HS cells this reversibility reportedly is achieved only at much higher concentrations of Ca\(^{2+}\). Red blood cell deformability has also been shown to be decreased when intramembrane Ca\(^{2+}\) is increased in normal red blood cells. The state and distribution of membrane Ca\(^{2+}\) in HS is, of course, difficult to evaluate other than in terms of simple quantification of Ca\(^{2+}\). In this regard, relative deficiency of Ca\(^{2+}\) dependent ATPase has been reported in HS. Finally, agents binding sulfhydryl groups produce sphering, and treated cells have many properties mimicking those of authentic HS cells.

In the present and previous issue of Blood, three investigations address themselves to the possibility that a defective state of membrane protein phosphorylation may be pathogenetic in HS. The postulate that a protein kinase-mediated phosphorylation affects the state and function of microfilamentous, perhaps contractile, membrane proteins is attractive. The attraction is potentiated by the fact that spectrin, a filamentous membrane protein, is a major substrate for phosphorylation by red blood cell protein kinase. Spectrin was first selectively solubilized from red cell membranes as a homogeneous protein in 1968. When treated with divalent cations and ATP, it aligns itself in long microfilaments, a “behaviour reminiscent of actin after its analogous extraction from skeletal muscle.” Actually spectrin is very likely complexed in vivo with erythrocyte actin and, while differences exist, has a number of properties closely resembling those of myosin. There is strong evidence that shape, motility, and contractibility of cells generally derive from structural elements such as microtubules and microfilaments. In addition, genetically determined HS in the mouse is characterized by a severe deficiency of spectrin in the erythrocytes. Greenquist and Shohet reported decreased phosphorylation of spectrin (averaging 50\%) in 22 of 25 patients with HS. Cyclic AMP (cAMP)-dependent phosphorylation of another protein was also deficient. Phosphorylation was greatly inhibited on heating to 50°C. The absolute level of phosphorylation was highly variable, but variation in simultaneously run normal samples was ±10\%. Dephosphorylation by protein phosphatase was normal in HS cells, Mg\(^{2+}\) dependent, inhibited by EDTA, and unaffected by 0.1–2.5 mM Ca\(^{2+}\). By the electrophoretic technique employed, HS and control membrane polypeptide patterns, as well as those in hereditary elliptocytosis, were identical, and spectrin band 2 was the principal site of labeling. The finding of defective protein phosphorylation was contrary to earlier observations of Zail and van den Hoek, but was in accordance with those of Matsumoto et al. Beutler et al., however, pointed out that in the studies of Greenquist and Shohet phosphorylation was assessed after 1 hr incubation. They agreed that net phosphorylation of HS membranes was moderately less than normal if measurements were made after 60 min. However, membrane phosphorylation was linear with time only for 5–10 min, and linear with membrane concentration...
only during short incubation times. During the linear period, protein kinase activity of HS membranes was normal. Net phosphorylation (Figs. 2a and 2b) appeared to nearly cease by 20 min, and, particularly with HS membranes, there was often net loss of label after that time. These differences in phosphorylation after 60 min when HS membranes were compared to normal membranes, and the fact that linearity of phosphorylation was preserved for a shorter period of time with HS than with normal cells, remain unexplained. The latter was not due to diminished protein kinase activity during the linear reaction period, nor to saturation of membrane phosphate-binding sites as incubation with ATP proceeded. Although adenosine diphosphate (ADP) had an inhibitory effect on protein phosphorylation, the decreased phosphorylation of HS membranes at 60 min could not be explained by increased ADP production, nor by greater than normal sensitivity to ADP inhibition. Neither was there evidence for either increased dephosphorylation by phosphatase or decreased stability of HS protein kinase. Other data support the observation that phosphorylation mediated by a certain protein kinase is linear for only brief periods of time.34,35

In contrast, Matsumoto et al.27 report that an average deficiency of phosphorylation of about 60%, was observed with HS membranes after only 10 min of incubation, during which near linearity with time was present. The deficient phosphorylation was uninfluenced by previous splenectomy, occurred with or without cAMP in the incubation mixture, and was more marked with red cells drained from splenic pulp during splenectomy. With heated normal red cells, osmotic fragility increased sharply, and membrane phosphorylation was sharply inhibited at the same temperature (between 48°C and 50°C) at which spectrin “melts” in a calorimeter. Red blood cell sphering after treatment with N-ethylmaleimide began at concentrations “exactly paralleling” those producing decreased membrane phosphorylation in ghosts. In this regard, disulfide bonds forming between thiols of protein subunits in myosin have been suggested as a mechanism for cation-induced aggregation of myosin subunits.36

It is clear that while these provocative observations serve as a potential baseline for continuing investigations, the definition of the molecular lesion in the hereditary spherocyte still remains unresolved. The protein kinase system in intact membranes is potentially one of great complexity (see Refs. 26–28 and their bibliographies). At this moment, it is not known whether one or more protein kinases play significant roles. Although the role of a cAMP-dependent kinase34,35,37,38 is clouded by the paucity of cAMP in the red cell, and by the fact that optimal conditions for its action appear to be very different from those pertaining in vivo,37 knowledge concerning cAMP-stimulated phosphorylation provides some insight into the possible complexities of protein kinase-mediated reactions. The chemical constitution of buffer, pH, ionic strength, Mg2+ concentrations, time and temperature are all of great importance in determining the data derived.35 The association of regulator and catalytic subunits is conditioned by a variety of factors, and the free and complexed forms of catalytic subunits even have radically different substrate specificities.35 Unfortunately, in membrane preparations, spatial relations of enzyme substrates, cofactors, and the enzyme itself are impossible to define.

There is difficulty in definitely determining whether differences between HS
and normal membranes are due to a fundamental disturbance in the protein kinase system, or are merely secondary to disturbed membrane relationships not fundamental to the system itself. In this regard, the parallelisms between membrane phosphorylation and sphering and its attendant osmotic phenomena—whether induced by heating or reagents binding sulfhydryl groups—while provocative, must be interpreted with great caution. Both heat and interference with ubiquitously distributed protein sulfhydryl groups have a very generalized potential for harm. In addition to the problems of evaluating membrane enzyme reactions contributed to by variations in the complex membrane organization, the possibility of proteolytic attack on enzyme subunits in the membrane is always present, difficult to evaluate, and potentially variable. Finally, the specificity of observed diminutions in membrane phosphorylating capacity in HS is challenged by the report of analogous, even more severe, aberrations in four cases of hemoglobin SS disease where the molecular lesion is known, and by the reported occurrence of similar aberrations in hereditary stomacytosis. While the present investigations give hope that the initially cited dictum of Haden may be refined and expanded to include a molecular lesion, additional studies are clearly necessary if this elusive goal is to be achieved.

REFERENCES

17. Cooper RA, Jandl JH: The role of mem-


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