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**Research Article**

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# Exercise-induced Hemolysis in Xerocytosis

## ERYTHROCYTE DEHYDRATION AND SHEAR SENSITIVITY

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ABSTRACT A patient with xerocytosis was found to have swimming-induced intravascular hemolysis and shortening of erythrocyte life-span. In a microviscometer, xerocytes were more susceptible than normal erythrocytes to hemolysis by shear stress. Fractionation of normal and abnormal cells on discontinuous Stractan density gradients revealed that increasingly dehydrated cells were increasingly more shear sensitive. This sensitivity was partially corrected by rehydrating xerocytic erythrocytes by means of the cation-ionophore nystatin in a high potassium buffer. Conversely, normal erythrocytes were rendered shear sensitive by dehydrating them with nystatin in a low potassium buffer. This effect of dehydration was entirely reversible if normal cells were dehydrated for <4 h but was only partially reversed after more prolonged dehydration.

It is likely that dehydration of erythrocytes results in shear sensitivity primarily because of concentration of cell contents and reduced cellular deformability. With prolonged dehydration, secondary membrane changes may potentiate the primary effect. This increased shear sensitivity of dehydrated cells may explain atraumatic exercise-induced hemolysis in xerocytosis as cardiac output is shifted to vessels of exercising muscles with small diameters and high shear rates.

## INTRODUCTION

Exercise-induced hemolysis has been described after marching (1, 2), jogging (3–5), conga-drumming (6) and karate exercising (7). All of these activities entail repetitive impact of the hands or feet with an unyielding surface. Cushioning the blows by padding the hands (7) or feet (3, 8, 9) with soft rubber, or chang-

ing the running surface from asphalt to grass (3), eliminates the hemolysis, hereby implicating direct mechanical trauma to the erythrocytes as the cause of the hemolysis. Given a hard enough surface, and a long enough run, most individuals will develop some hemoglobinemia (4). There is some recent evidence that the most susceptible individuals may have an underlying membrane protein abnormality (10). Hemolysis has not, however, been described in atraumatic sports such as swimming or cycling (5). Here we describe atraumatic exercise-induced hemolysis in an individual with xerocytosis. We have examined the sensitivity of dehydrated erythrocytes to shear stress and have found it to be abnormal. This shear sensitivity of dehydrated erythrocytes may be important in relation to the acute nontraumatic exercise-induced hemolysis, and may also play a role in the chronic hemolysis of dehydrated erythrocyte syndromes.

## METHODS

*Case history.* The patient is a 21-yr-old world-class competitive freestyle swimmer with episodes of fatigue, jaundice, pallor, and darkened urine associated with periods of training over the past 12 yr. He was evaluated for this several years ago at which time he was found to have a transient low serum haptoglobin, hyperbilirubinemia, a moderate reticulocytosis, but no anemia. He is otherwise well, without significant medical illness, and is on no drugs.

Family members have not been studied, but give a positive history for jaundice and early cholecystectomy on the paternal side. Physical examination reveals a muscular healthy-looking young athlete with moderate scleral icterus. There is no hepatosplenomegaly, and no other abnormal physical findings are detectable.

*Routine hematologic studies.* Blood was drawn from this patient and from apparently healthy adult volunteers. Blood was collected in EDTA and processed within 2 h of drawing.

Hemoglobin, hematocrit, erythrocyte count, reticulocyte count, osmotic fragility, Coombs' test, sugar-water test, hemoglobin electrophoresis, hemoglobin heat stability, and Heinz body preparation were performed by established procedures (11). We calculated mean erythrocyte corpuscular

volume (MCV)[1] using the hematocrit measured in a micro-hematocrit centrifuge and an automated erythrocyte count (model S, Coulter Electronics, Inc., Hialeah, Fla.).

The cyanide-ascorbate test was done by the method of Jacob and Jandl (12). The erythrocyte enzymes hexokinase, phosphofructokinase, glucose phosphate isomerase, triose phosphate isomerase, phosphoglyceratekinase, pyruvate kinase, and glucose-6-phosphate dehydrogenase were assayed spectrophotometrically (13).

Intracellular sodium and potassium concentrations were determined as described by Glader et al. (14) with erythrocytes washed in isotonic magnesium chloride, hemolyzed in a lithium carbonate Unopette (Becton, Dickinson & Co., Rutherford, N. J.), and measured in a flame photometer. For estimation of net sodium and potassium fluxes, fresh cells were separated by centrifugation at room temperature for 5 min. They were washed three times and suspended at a 20% hematocrit in a Hepes-buffered salt (HBS) solution, pH 7.4, with the following composition: 5 mM glucose, 140 mM sodium chloride, 5 mM potassium chloride, 1 mM sodium phosphate, 1 mM magnesium chloride, 20 mM Hepes, and 1 mg/ml of bovine serum albumin. The suspensions were gently agitated in a 37°C water bath. Samples were removed hourly for 4 h for determination of the intracellular sodium concentration and the concentration of potassium in the suspending medium. These studies were done with and without the addition of 0.1 mM ouabain.

*Exercise studies.* To assess the effects of exercise on hemolysis, the patient had blood drawn before and after his routine training swim. The swimming session consisted of 2.5 h of 50-m laps done at various speeds and with varying intervals. There was no diving, butterfly stroke, or other drill involving impact on the water surface. Postswim studies were drawn immediately after the session. Plasma and urine hemoglobin was measured by the benzidine method of Crosby and Furth (15). Bilirubin and haptoglobin were done by standard techniques. Urine hemosiderin was determined by microscopic analysis of Prussian blue-stained urinary sediment.

*Effect of shear stress on patient and normal erythrocytes.* To measure their susceptibility to disruption by shearing forces, erythrocytes were exposed to various shear stresses and the resultant hemolysis was measured. A sample of blood to be tested was washed three times in HBS and concentrated to an hematocrit of 65–75. Viscosity of the suspension was manipulated by adding dextran (0–60%), with appropriate reduction of NaCl to give a final osmolality of 290. Total hemoglobin of the concentrated solution was measured with the aid of Drabkin's reagent. Shear stress was applied in a cone-plate microviscometer (Wells-Brookfield Co., Stoughton, Mass.) modified with the addition of a Luer port for introduction and withdrawal of sample, and a constant temperature waterjacket. A 0.5- or 1.0-ml portion of sample was introduced between the cone and the plate through the sample port. The motor was started, and the cone rotated at either 0.3, 0.5, 1.5, 3, 6, 12, 30, or 60 rpm. The sample was exposed to the rotating cone for 2 min. The sample was then carefully withdrawn from the viscometer and centrifuged in microhematocrit tubes in a microhematocrit centrifuge, and the supernate was separated and assayed for the presence of hemoglobin. The shear stress applied to the sample was calculated according to the following equation: shear stress (dyn/cm²) = viscosity (cp) × shear rate (s⁻¹). All experiments were done at 37°C.

*Density gradient fractionation of erythrocytes.* Discontinuous Stractan (St. Regis Paper Co., Tacoma, Wash.) gradients for separation of erythrocytes were prepared by a modification of a technique described by Corash et al. (16); 200 g of crude Stractan powder was dissolved in 100 ml of distilled water and passed over a Rexyn I-300 (Fisher Scientific Co., Fairlawn, N. J.) column several times until the osmolarity was <140 mosmol. It was then filtered through 0.45-μm filters (Millipore Corp., Bedford, Mass.), and lyophilized. Lyophilized Stractan (200 g) was then dissolved in 200 ml of distilled water and albumin was added to a final concentration of 3%. To 9 parts Stractan solution, 1 part 0.15 M potassium phosphate buffer, pH 7.4, was added. This solution was diluted 1:1 with buffered saline with glucose of the following composition: 8.1 g NaCl, 1.22 g Na₂HPO, 0.219 g Na₂H₂PO₄, 0.406 g MgCl₂·6H₂O, 2 g glucose. The buffered saline with glucose was dissolved in water (final volume, 1,000 ml) and the pH was adjusted to 7.4 with HCl.

Stock densities of 1.085, 1.090, 1.095, 1.10, and 1.12 were prepared by diluting the Stractan-buffered saline with glucose solution with buffered saline with glucose and were frozen for use in these experiments. Before using previously frozen material it was often necessary to readjust the pH to 7.4 with NaOH and the osmolarity to 300 mosmol with NaCl. The gradients were centrifuged at 4°C in a Beckman SW 40 swinging bucket rotor (Beckman Instruments, Inc., Fullerton, Calif.) at 37,000 rpm for 60 min without application of a brake. Populations of cells concentrated at the interfaces of the gradient were collected by slicing the gradient tube. Cells were freed of Stractan by washing four times in HBS.

*In vitro alteration of erythrocyte density.* Patient erythrocytes and dehydrated control cells were rehydrated using nystatin and a high potassium buffer by modification of the technique described by Cass and Dalmark (17). Cells were washed in HBS, then suspended at a 5% hematocrit at room temperature in the following medium: 150 mM KCl, 5 mM NaCl, 1 mM MgCl₂, 27 mM sucrose, 20 mM Hepes, 30 mg/ml nystatin, pH 7.4, for 30 min. The nystatin was then removed by three washes in HBS warmed to 37°C and the cells were resuspended in HBS at a 20% hematocrit and allowed to equilibrate for 30 min at 37°C.

Normal erythrocytes were artificially dehydrated with the same technique with a low potassium buffer: 85 mM KCl, 25 mM NaCl, 1 mM MgCl₂, 27 mM sucrose, 20 mM Hepes, 30 mg/ml nystatin, pH 7.4.

In some experiments dehydration of normal cells was maintained for as long as 24 h. In these experiments erythrocytes were initially dehydrated as above in a low potassium buffer in the presence of nystatin. The nystatin was removed after 30 min, and the cells were resuspended at an hematocrit of 20% in the low potassium buffer modified with the addition of 5 mM glucose. These cells were maintained at 37°C in a shaking water bath. The pH was readjusted to 7.4 every 8 h and glucose (5 μM) was added every 12 h. Before viscometry the cells were washed three times in HBS, resuspended in HBS at an hematocrit of 20%, and allowed to equilibrate for 30 min. A control population was exposed to the same nystatin treatment and prolonged incubation but using high potassium buffer only. ATP was assayed spectrophotometrically (13).

## RESULTS

*Clinical studies.* The hematologic values of the patient shown in Table I reveal a well-compensated hemolytic process associated with a mild macrocytosis,

TABLE I
## Hematologic Values of Patient

| | |
|---|---|
| Hematocrit, % | 50 |
| Hemoglobin, g | 17 |
| MCV, $\mu m^3$ | 102 |
| MCHC, % | 36 |
| Reticulocytes, % | 9 |
| Morphology | Normal, rare targets |
| Osmotic fragility | |
|     50% hemolysis | 0.065 M NaCl (C = 0.073 M NaCl)* |
|     Onset of hemolysis | 0.069 M NaCl (C = 0.080 M NaCl) |
|     Completion of hemolysis | 0.061 M NaCl (C = 0.070 M NaCl) |
| Intracellular cations, meq/liter | |
|     erythrocytes | Na = 14.3 (C = 9 ± 3) |
| | K = 76.7 (C = 98 ± 5) |
| | Na + K = 91.0 (C = 107 ± 5) |
| Net flux, meq/liter erythro- | |
|     cytes/h | |
|     Without ouabain | Na = 0.9  (C = 0.5) |
| | K = 1.4  (C = 0.6) |
|     With ouabain, 0.1 mM | Na = 4.1  (C = 2.1) |
| | K = 3.1  (C = 1.2) |
|     Active transport‡ | Na = 3.1  (C = 1.6) |
| | K = 1.7  (C = 0.6) |

* Values in parentheses refer to simultaneously run controls (C).
‡ Active transport was calculated from the difference in net fluxes in the presence and absence of ouabain. The values in the presence of ouabain are a measure of passive Na influx and passive K efflux.

increased mean corpuscular hemoglobin concentration (MCHC), and rare target cells. The hemoglobin electrophoresis and heat stability tests were normal. Cyanide-ascorbate, Heinz body, Coombs', and sugar-water tests were all negative. Assay of glycolytic and pentose-shunt enzymes listed in Methods were either normal or increased appropriately for this young population of erythrocytes.

Osmotic fragility was decreased with 50% hemolysis produced at 0.065 M NaCl (control = 0.073 M NaCl). As shown in Table I, intracellular cations were abnormal and characteristic of xerocytosis with an increased sodium, decreased potassium, and decreased total monovalent cation content. Cation fluxes were also abnormal and characteristic of xerocytosis with an increase in the passive permeabilities of both sodium (195%) and potassium (258%), but a relatively greater increase in potassium permeability.

*Pre- and postswim studies.* The pre- and postswim studies indicated that acute intravascular hemolysis occurred during the swimming session. There was an increase in free plasma hemoglobin (<5 mg/dl before, 45 mg/dl after) and a fall in haptoglobin concentration (25 mg/dl before, 6 mg/dl after). Despite the history of previous episodes of dark urine after swimming we did not detect urine hemoglobin on these occasions, as the renal threshold for hemoglobin was not ex-

ceeded. Chronic intravascular hemolysis was suggested by the presence of hemosiderin-laden tubular cells in the urinary sediment. One comparable swimmer was studied in the same fashion and had no free hemoglobin nor a drop in haptoglobin after a swimming session.

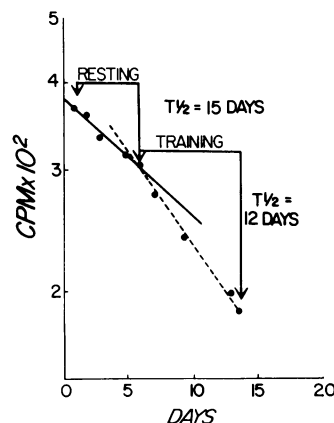*Erythrocyte survival.* Fig. 1 illustrates the effect of swimming on the autologous $^{51}$Cr-labeled erythro-



FIGURE 1  $^{51}$Cr erythrocyte survival of xerocytosis patient at rest (days 1–6) and during swim training (days 6–14). The difference in the slopes is significant ($P < 0.005$).
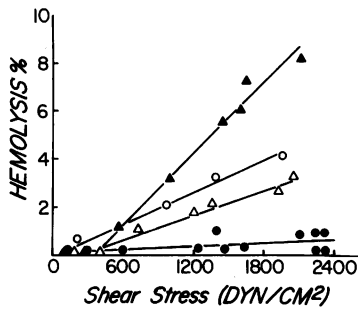
FIGURE 2 Hemolysis of erythrocytes exposed to different shear stresses for 2 min at 37°C in a microviscometer. ●, control erythrocytes; ▲, patient's xerocytic erythrocytes; ○, control erythrocytes after in vitro dehydration; △, xerocytic erythrocytes after in vitro rehydration.

cyte survival in the patient. On days 1–6 the patient was at rest and did not participate in any training sessions. The half-life of the cells during the rest period was short: 15.0 d (normal, 27–35 d). Later on day 6, the patient resumed a serious training schedule consisting of two nonstop 2.5-h swim sessions/d. His erythrocyte half-life fell to 12.1 d. The slopes of the survival curves were calculated by the method of least squares and were significantly different ($P < 0.005$). Correlation coefficients of the best-fit lines were 0.978 (resting) and 0.996 (training).

*Shear sensitivity of the patient's erythrocytes.* Different shear stresses were applied to samples of patient and control erythrocytes in a cone-plate microviscometer. The resulting hemolysis is an expression of the cells' sensitivity to the shear stress applied. Fig. 2 summarizes the data from two separate studies on the patient and two different controls. Each point represents hemolysis data from a separate aliquot exposed only to that shear stress. Control cells showed little or no hemolysis over the entire range of stresses studied, data comparable to previously reported work (18). These "shear-resistant" control cells are clearly

different from the "shear-sensitive" patient cells which exhibited increasing hemolysis over the range studied.

*Shear sensitivity of erythrocytes separated on Stractan gradients.* To assess the relative effect of dehydration on shear sensitivity, the patient's cells were separated on a discontinuous Stractan gradient. As shown in the upper portion of Table II, reticulocytes were concentrated in the least dense (top) layer. In more dense layers the cells progressively gained sodium and lost potassium. Potassium loss predominated, resulting in a net decrease in total cation content. This cation loss was associated with a shrinking MCV and increasing MCHC, both evidence of progressive intracellular dehydration.

The shear sensitivity of these increasingly dehydrated xerocytes is shown in the upper portion of Fig. 3. Although layers 1 and 2 are virtually identical, it is clear that layer 4, the most dense layer, is the most shear sensitive, and that layer 3 is intermediate. The relative shear resistance of layer 1 eliminates reticulocyte artifact as a cause of the patient's shear sensitivity.

Control erythrocytes, bottom portions of Table II and Fig. 3, showed a similar relationship between increasing dehydration and increasing shear sensitivity.

*Shear sensitivity of the patient's erythrocytes after in vitro rehydration.* The positive correlation between shear sensitivity and erythrocyte density suggests that dehydration may be an important factor in determining shear sensitivity. To test this hypothesis, we rehydrated a population of the patient's xerocytic erythrocytes and remeasured shear sensitivity. The characteristics of these rehydrated cells are shown in Table III (experiment 1). By exposing the patient's dehydrated, potassium-depleted cells to a high potassium buffer in the presence of the cation-ionophore nystatin, a new steady state was achieved. These cells accumulated potassium and total monovalent cations, swelled, and normalized their MCHC and osmotic fragility. Osmotic fragility profile showed a uniform population of normally hydrated cells without many

TABLE II
*Characteristics of Erythrocytes Separated on Stractan Density Gradients*

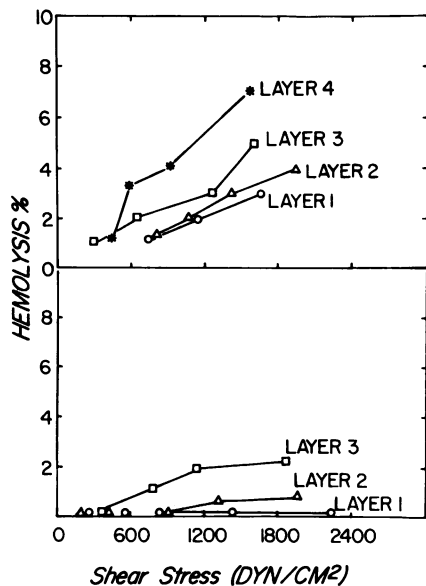| | Layer | Density | Reticulocytes | Na | K | Na + K | MCV | MCHC |
|---|---|---|---|---|---|---|---|---|
| | | g/ml | % | mEq/liter erythrocytes | | | μm³ | % |
| Xerocytes | 1 | 1.085 | 22 | 12 | 76 | 88 | 119 | 32 |
| | 2 | 1.090 | 5 | 20 | 67 | 87 | 100 | 35 |
| | 3 | 1.095 | 5 | 30 | 50 | 80 | 92 | 39 |
| | 4 | 1.10 | 3 | 35 | 40 | 75 | 90 | 40 |
| Control erythrocytes | 1 | 1.085 | 4 | 10 | 107 | 117 | 112 | 30 |
| | 2 | 1.090 | 0 | 11 | 85 | 96 | 95 | 32 |
| | 3 | 1.095 | 0 | 18 | 60 | 78 | 85 | 33 |
| | 4 | 1.010 | 0 | 22 | 50 | 72 | 81 | 35 |

FIGURE 3   Top panel: Hemolysis of layers 1 (O), 2 (△), 3 (□), and 4 (*) of xerocytic erythrocytes with increasing density separated by Stractan density gradient and exposed to various shear stresses for 2 min at 37°C in a microviscometer. Bottom panel: Hemolysis of layers 1 (O), 2 (△), and 3 (□) of control erythrocytes with increasing density separated by Stractan density gradient and exposed to various shear stresses for 2 min at 37°C in a microviscometer.

dehydrated or overhydrated cells. As shown in Fig. 2, these rehydrated xerocytes were less shear sensitive than the patient's unmodified cells, although they were still more shear sensitive than control erythrocytes (see below, on Reversibility of the dehydration effect).

Shear sensitivity of control erythrocytes after in vitro dehydration. Since rehydration of the patient's xerocytes decreased shear sensitivity, we studied the opposite situation, dehydration of control erythrocytes, to see whether shear sensitivity would be increased. Dehydrated control cells were prepared by exposing control cells to low potassium buffer in the presence of nystatin. These cells lost potassium, total cation, and volume, while MCHC and osmotic resistance increased. As anticipated, an increase in shear sensitivity was also seen (Fig. 2).

Reversibility of the dehydration effect. To study the reversibility of the dehydration-associated shear sensitivity, control erythrocytes were maintained in a dehydrated state for various periods of time after acute dehydration with nystatin and a low potassium buffer. At the end of these incubations some of the dehydrated cells were exposed to a shear stress of 2,300 dyn/cm² for 2 min at 37°C. At the same time another portion of the cells was rehydrated by exposure to nystatin in a high potassium buffer and then stressed at 2,300 dyn/cm². Osmotic fragility tests of the rehydrated erythrocytes showed that rehydration was complete, with normal mean osmotic fragility and onset and completion of hemolysis within 0.005 M of the mean. Control cells were exposed twice to nystatin and high potassium buffer for the same incubation periods (i.e., exposed to nystatin and the metabolic stresses of incubation without dehydration). Metabolically significant ATP depletion did not occur in any of the experimental groups since postincubation ATP levels were always >85% of the preincubation levels.

The results of these experiments are summarized in Fig. 4. As seen before, dehydrated cells were sensitive to shear stress, an effect which did not appear to be time-related. After dehydration and rehydration, shear sensitivity was restored to normal when dehydration was maintained for periods of ≤4 h. Between 7 and 24 h, however, there was measurable hemolysis even though rehydration was essentially complete and comparable to that observed at the earlier time points. Control erythrocytes exposed to nystatin and incubated under conditions of normal hydration did not demonstrate abnormal shear sensitivity. The data suggest that when dehydration is maintained for some time (in this case > 4 h) an irreversible "dehydration injury" is induced that increases only slightly with more prolonged dehydration.

TABLE III

Characteristics of Xerocytes and Control Erythrocytes after In Vitro Rehydration or Dehydration

| | Na | K | Na + K | MCV | MCHC | Mean osmotic fragility* |
|---|---|---|---|---|---|---|
| | meq/liter erythrocytes | | | μm³ | % | |
| Experiment 1 | | | | | | |
| Xerocytes | 20 | 70 | 90 | 100 | 35.5 | 0.068 |
| Rehydrated xerocytes | 18 | 118 | 136 | 104 | 33.2 | 0.073 |
| Experiment 2 | | | | | | |
| Control erythrocytes | 8 | 91 | 99 | 92 | 33.0 | 0.075 |
| Dehydrated control erythrocytes | 23 | 61 | 84 | 83 | 35.0 | 0.067 |

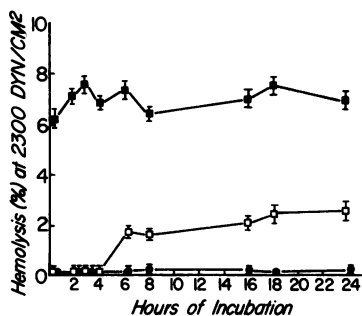* Molar concentration of NaCl required to produce 50% hemolysis.

FIGURE 4  Hemolysis of erythrocytes after various periods of incubation and exposure to a shear stress of 2,300 dyn/cm² for 2 min at 37°C in a microviscometer. ●, erythrocytes treated for 30 min with nystatin in a high potassium buffer, incubated in high potassium buffer for various periods of time, then treated again with nystatin in a high potassium buffer. □, erythrocytes treated for 30 min with nystatin in a low potassium buffer, incubated in a low potassium buffer for various periods of time, then treated again with nystatin in a high potassium buffer. ■, erythrocytes treated for 30 min with nystatin in a low potassium buffer, and incubated in a low potassium buffer for various periods of time.

## DISCUSSION

Hereditary xerocytosis (previously termed desiccytosis) is an autosomal dominant hemolytic anemia first described by Glader et al. (14). Several additional families have been reported (19–22). Our patient is typical in that he has a hemolytic anemia with osmotically resistant, low-potassium, high-sodium erythrocytes in which passive potassium efflux and net potassium loss exceed passive sodium influx and net sodium gain. This dehydration disorder represents one end of the spectrum of primary erythrocyte cation permeability abnormalities. The other end is represented by the hereditary stomatocytosis (or hydrocytosis) syndromes first identified by Lock et al. (23) and characterized by Zarkowski et al. (24) and others (25–27). These overhydrated, sodium-loaded erythrocytes have increased osmotic fragility owing to a defect in cation permeability in which sodium gain predominates over potassium loss. Between these extremes are numerous intermediate syndromes of varying clinical severity (28–34). The mechanism of hemolysis and primary molecular defect is not known for any of these disorders.

Nontraumatic exercise-induced hemolysis was documented in our patient with xerocytosis. Furthermore, there was a demonstrable exercise-associated decrease in ⁵¹Cr erythrocyte survival (Fig. 1). Previous reports of exercise-induced hemolysis have focused on individuals with presumably normal erythrocytes that were destroyed by direct mechanical trauma to the cells during exercise. Free-style swimming is considered an atraumatic sport and does not produce hemolysis of normal cells. Our patient's erythrocytes are dehydrated and susceptible to disruption by shear forces. There are important physiologic adjustments to prolonged strenuous exercise that may result in significant shear forces. These adjustments include increased stroke volume, increased heart rate, vasoconstriction of certain vascular beds, and redistribution of cardiac output to exercising muscles (35). At rest ~21% of cardiac output is directed to muscle. This figure increases to 48% with light exercise, to 71% with moderate exercise, and to 88% with vigorous exercise (35). The blood flow is directed to muscle arteries, arterioles, and capillaries that have narrower diameters and higher rates of shear than the main arteries and veins (36). Careful quantification of shear stresses in the circulation is a difficult task fraught with technical hazards, so that many authors provide figures that differ by orders of magnitude (37). It can be assumed, however, that shear stresses in the circulation are generally <2,000 dyn/cm², the lowest shear stress that will hemolyze normal erythrocytes (18). We found hereditary xerocytes to be sensitive to hemolysis at shear stresses that do not affect normal cells, and that could be present in certain small vessels during an exercise stress.

The role of dehydration in this shear sensitivity is suggested by the increasing shear sensitivity exhibited by increasingly dehydrated subpopulations of xerocytes and normal cells (Fig. 3). These data for xerocytes are supported by the data of Clark et al. (22), who show by ektacytometry decreasing deformability of xerocytes with increasing dehydration. Further evidence as to the importance of dehydration comes from the studies of artificially hydrated and dehydrated cells. Nystatin manipulation of xerocytic cells caused restoration of normal hydration as measured by MCHC and osmotic fragility (Table III). These restored cells had a partially restored stress-hemolysis curve (Fig. 2). Conversely, nystatin manipulation caused dehydration of normal erythrocytes with an increased MCHC and decreased osmotic fragility (Table III). These cells exhibited an abnormal shear sensitivity (Fig. 2).

The effect of dehydration on shear sensitivity is reversible by rehydration if the dehydration time is short (Fig. 4). Prolonged dehydration is associated with a lesion that is not reversed by rehydration (Fig. 4). The effect of prolonged dehydration may explain the only partial restoration of the shear sensitivity in the rehydrated xerocyte (Fig. 2). These data suggest that prolonged dehydration may have a permanent impact on the cell membrane and are consistent with the hypothesis of Sullivan et al. (38). In this respect it is interesting that hereditary xerocytes have a marked tendency to fragment during incubation, even in the absence of shear stress (21). Clark et al. (22) have different data showing that restoration of normal hydration to xerocytes restores cell deformability entirely

to normal. This apparent conflict with our results serves to show that the factors affecting cell deformability as measured at low shear stress with the ektacytometer differ from those affecting hemolysis at high shear stress.

In summary, dehydrated erythrocytes are more susceptible to hemolysis by shear stress than normal cells. This effect appears to result largely from concentration of cell contents, but may also include a somewhat smaller contribution from permanent membrane injury associated with prolonged dehydration. The exercise-induced hemolysis seen in xerocytosis may be a manifestation of shear sensitivity in exercising muscles. In a similar set of laboratory and clinical investigations in individuals with sickle cell anemia, exercise-induced hemolysis and shear sensitivity of the dehydrated sickle cells was also documented.[2] Less dramatic chronic intravascular hemolysis in erythrocyte dehydration syndromes may be the result of destruction of the most sensitive cells in high shear stress areas of the circulation.

## ACKNOWLEDGMENTS

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