Red Cell Membrane Stiffness in Iron Deficiency

By Ray Yip, Narla Mohandas, Margaret R. Clark, Sushil Jain, Stephen B. Shohet, and Peter R. Dallman

The purpose of this study was to characterize red blood cell (RBC) deformability by iron deficiency. We measured RBC deformability by ektacytometry, a laser diffraction method for determining the elongation of suspended red cells subjected to shear stress. Isotonic deformability of RBC from iron-deficient human subjects was consistently and significantly lower than that of normal controls. In groups of rats with severe and moderate dietary iron deficiency, RBC deformability was also reduced in proportion to the severity of iron deficiency. At any given shear stress value, deformability of resealed RBC ghosts from both iron-deficient humans and rats was lower than that of control ghosts. However, increase of applied shear stress resulted in progressive increase in ghost deformation, suggesting that ghost deformability was primarily limited by membrane stiffness rather than by reduced surface area-to-volume ratio. This was consistent with the finding that iron-deficient cells had a normal membrane surface area. In addition, the reduced mean corpuscular membrane surface area (MCHC) and buoyant density of the iron-deficient rat cells indicated that a high hemoglobin concentration was not responsible for impaired whole cell deformability. Biochemical studies of rat RBC showed increased membrane lipid and protein crosslinking and reduced intracellular cation content, findings that are consistent with in vivo peroxidative damage. RBC from iron-deficient rats incubated in vitro with hydrogen peroxide showed increased generation of malondialdehyde, an end-product of lipid peroxidation, compared to control RBC. Taken together, these findings suggest that peroxidation could contribute in part to increased membrane stiffness in iron-deficient RBC. This reduced membrane deformability may in turn contribute to impaired red cell survival in iron deficiency.

IN IRON DEFICIENCY, there is a reduction in red blood cell (RBC) lifespan that appears to result from premature RBC destruction in the spleen. In previous studies, decreased red cell deformability in iron deficiency was inferred from increased blood viscosity and the decreased ability of red cells to pass through small pores polycarbonate filters. Reduced surface-to-volume ratio was proposed as the primary cause of reduced deformability of iron-deficient cells. However, in addition to the ratio of membrane surface-to-cell volume ratio, RBC membrane viscoelasticity and intracellular viscosity also can have a major influence on red cell deformability. We have used a laser diffraction viscometer, the ektacytometer, to study the deformability properties of iron-deficient RBC. Our observations indicate that increased membrane stiffness, rather than reduced surface-to-volume ratio, is the major cause of the reduced cell deformability in iron-deficient RBC. In addition, biochemical studies suggest that oxidative membrane damage in iron-deficient RBC may be a cause of the increased membrane stiffness.

MATERIALS AND METHODS

Humans

Eleven adult patients who had microcytic anemia (mean cell volume [MCV] <70 fl and hemoglobin [Hb] <12 g), elevated erythrocyte protoporphyrin (>100 µg/dl RBC), and a low serum ferritin (<12 µg/liter) were considered to be iron deficient. Healthy control subjects were not anemic and had normal MCV, protoporphyrin, and serum ferritin values. All blood samples were collected in EDTA, and deformability studies were done within 8 hr after phlebotomy.

Animals

Male Sprague-Dawley rats were made severely or moderately iron deficient by providing diets containing 2 or 10 mg iron as ferric citrate/kg of diet, respectively, between 21 and 45 days of age. Control rats received the same diet supplemented with 50 mg iron/kg of diet. Samples of rat blood were collected in heparin from the abdominal aorta after pentobarbital anesthesia. This form of anesthesia was shown to have no effect on red cell deformability in pilot experiments.

Preparation of Resealed Ghosts

Fresh blood was washed 3 times in a solution containing 5 mM Tris-HCl (pH 7.4) and 140 mM NaCl (isotonic Tris-saline). Ghosts were then prepared by hypotonic lysis in 20 volumes of ice-cold 5 mM Tris-HCl, 7 mM NaCl (pH 7.4), and were washed once with the same lysing buffer. After centrifugation and removal of supernatant, the ghost pellet was resuspended in 10 volumes of isotonic Tris-saline and incubated at 37°C for 1 hr to promote resealing. The ghost preparation was concentrated by centrifugation for deformability measurements.

Deformability Measurements

Deformability of intact RBC and resealed erythrocyte ghosts was measured in suspended red cells or ghosts subjected to a controlled fluid shear stress. The shear stress varies directly with the viscosity of the suspending medium and with the rotation speed of the viscometer. Whole cell deformability measured by the ektacytometer is expressed as a deformability index (DI), which is equivalent to the ellipticity of maximally deformed red cells. DI values are from the Department of Pediatrics and Cancer Research Institute, University of California, San Francisco, CA. Supported in part by USPHS Grants AM13897, AM23077, AM26263, and HL20985. Submitted May 17, 1982; accepted January 10, 1983. Presented in part at the 32nd Annual Meeting for the American Society of Hematology, San Antonio, TX, December 7, 1981. This is publication no. 33 from the McMillan-Cargill Hematology Research Laboratory. Address reprint requests to Dr. Ray Yip, HSE-1434, University of California, San Francisco, San Francisco, CA 94143. © 1983 by Grune & Stratton, Inc.
reported either as the signal from the ektacytometer in volts or as a percent of the value obtained for the control samples. The details of this method have been described previously. Recent modification of the ektacytometer now extends the rotation speed of the viscometer from its previous limit of 100 rpm to 400 rpm. This gives a fourfold increase in applied shear stress for a given solution viscosity. In the interest of economy, polyvinylpyrrolidone (PVP), 360,000 mol wt (Sigma Chemical Co., St. Louis, MO), rather than dextran, was used in the current studies to adjust the viscosity of the suspending medium. For a given solution viscosity, cell deformability measured in PVP solutions was identical to that observed in dextran solutions. To study the effect of varying osmolarity on deformability of intact RBC, a continuous osmotic gradient of the PVP medium at 22 cp viscosity was used. The osmolarity of the PVP medium was varied linearly from 50 to 500 mosmole/kg and was continuously mixed with a concentrated RBC suspension. The final suspension was then passed through the ektacytometer. As previously shown, the curve showing the variation of DI with suspending medium osmolality can be analyzed to provide information about initial cell surface area and volume. Specifically, the DI reaches a minimum value in hypotonic medium at an osmotic strength corresponding to the point at which 50% of the cells hemolyze in an osmotic fragility assay. Computation of the cell volume at this point, resulting from osmotically induced swelling, permits estimation of the critical hemolytic volume and cell surface area. Previous work in this laboratory has verified that this estimate of cell surface area correlates with red cell cholesterol content. For resealed erythrocyte ghosts, Stractan (290 mosmole/kg, 22 cp viscosity, pH 7.4) was used as a suspending medium, because the difference in refractive index between the ghosts and the PVP medium was insufficient to produce a well defined diffraction pattern. All deformability measurements on intact RBC or resealed ghosts were performed in parallel with measurements on normal control samples.

**Mechanical Fragmentation Tests**

To obtain a measure of membrane resistance to shear-induced fragmentation, we performed a fragmentation test using the ektacytometer. Resealed ghosts were suspended in dextran solutions of 97 cp viscosity. Rotation of the ektacytometer chamber at a speed of 110 rpm generated a shear stress of 575 dyne/sq cm. Continuous application of this stress resulted in progressive fragmentation of the red cells into small undeformable spheres. This process was detected as a decrease in the DI, which was monitored as a function of time. The time required for the DI to fall to half of its maximum value was taken as a measure of the susceptibility of the ghosts to shear-induced fragmentation.

**Osmotic Fragility Test**

Osmotic fragility of RBC was determined according to the method of Parpart et al. The NaCl concentration at which 50% hemolysis occurred was used to compare iron-deficient and control samples.

**Density Gradient Separation of RBC**

Density gradients for separation of RBC were made with Stractan, purified according to the method of Corash et al.

**Other Analyses**

**Oxidative vulnerability.** RBC susceptibility to peroxidative damage by H$_2$O$_2$ was measured by the generation of malondialdehyde (MDA), an end-product of lipid peroxidation, using the method of Stocks and Dormandy.

**Measurement of spontaneously formed MDA in red cell membranes.** We analyzed lipid extracts of the red cells for the presence of a MDA-phospholipid complex that may be indicative of spontaneously occurring lipid peroxidation (e.g., without in vitro challenge by H$_2$O$_2$). Lipid extracts of red cells were prepared according to the method of Rose and Oklander, and phospholipid subclasses were separated by thin layer chromatography (TLC). The separated phospholipids were visualized with iodine vapor, scraped from the TLC plate, and analyzed for lipid phosphorus. The MDA–phospholipid complex that appeared between phosphatidylethanolamine and phosphatidylserine, and which was also analyzed for lipid phosphorus, was expressed as the percentage of total phospholipids. It has been previously shown that this MDA–phospholipid complex can be produced by the addition of MDA to normal RBC membranes and that it is present in untreated RBC from some individuals with sickle cell anemia. Lipid extracts were also used for determination of red cell cholesterol content.

**SDS-polyacrylamide gel electrophoresis (SDS-PAGE).** For electrophoretic analysis, ghosts were prepared without resealing. The solubilized ghosts were then fractionated on 6% gels according to the method of Fairbanks. Paired samples were run with and without dithiothreitol (DTT) to detect reducible protein crosslinking related to disulfide bonds.

To determine red cell Na and K, fresh blood samples were washed 3 times with Tris-buffered isotonic MgCl$_2$ (10 mM Tris-HCl, pH 7.4). The washed cells were lysed in a standard LiCl solution and analyzed for Na and K using a flame photometer (Instrumentation Laboratories, Lexington, MA).

Protoporphyrin in whole blood samples was analyzed by the hemofluorometer method (ZnP hematofluorometer, ESA, Bedford, MA).

Serum ferritin was measured with a radioimmunoassay kit obtained from Clinical Assays (Cambridge, MA).

**RESULTS**

**Human RBC Deformability Studies**

Hematologic data and erythrocyte protoporphyrin levels for iron-deficient subjects and normal controls are summarized in Table 1A. Iron-deficient subjects

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>Iron Deficiency (N = 11)</th>
<th>Controls (N = 11)</th>
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</thead>
<tbody>
<tr>
<td>(A) Hematologic and biochemical data</td>
<td></td>
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<tr>
<td>Hb (g/dl)</td>
<td>9.8*</td>
<td>14.1</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>64*</td>
<td>89</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>31.7*</td>
<td>33.8</td>
</tr>
<tr>
<td>Erythrocyte protoporphyrin (µg/dl RBC)</td>
<td>336*</td>
<td>48</td>
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<tr>
<td>(B) Deformability and shear-induced fragmentation data</td>
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<td></td>
</tr>
<tr>
<td>Intact RBC DI at 290 mosmole, 125 dynes/sq cm</td>
<td>89* (64–94)</td>
<td>100</td>
</tr>
<tr>
<td>Resealed ghost DI at 290 mosmole, 150 dynes/sq cm</td>
<td>90* (86–94)</td>
<td>100</td>
</tr>
<tr>
<td>T% for mechanical fragmentation test (sec)</td>
<td>161</td>
<td>152</td>
</tr>
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* $p<0.01$ in comparison with controls.

† Deformability index is expressed as mean percent of control value and range.
were significantly different from controls with respect to all parameters examined. Table 1B also summarizes the deformability and shear-induced fragmentation properties for intact iron-deficient RBC and ghost preparations. The ghost deformability of iron-deficient samples was reduced to the same extent as that of intact cells in isotonic medium, strongly suggesting that decreased membrane viscoelasticity was the reason for reduced deformability of whole blood samples, since this measurement is much less dependent on intracellular viscosity or surface-to-volume ratio. Fragmentation rates of the iron-deficient cells did not indicate any substantial alteration in shear sensitivity.

**Rat RBC Deformability Studies**

The hematologic and biochemical data in Table 2A show that the consequences of iron deficiency in the rat were generally similar to those in man. The major difference was that iron deficiency in rats was associated with a reticulocytosis and some nucleated RBC. These findings have been previously recognized in rats and other experimental animals with iron deficiency.23 The severely iron-deficient rats had more pronounced abnormalities than the moderately deficient group. Compared to the human subjects, the red cells from iron-deficient rats showed a more profound reduction of whole cell deformability in isotonic medium (Table 2). Furthermore, the degree of loss of deformability, as measured by the DI value, was correlated with the severity of iron deficiency. As in human iron deficiency, the deformability of iron-deficient rat RBC membrane preparations was also significantly reduced (Table 2), suggesting that increased membrane stiffness contributed to impaired whole cell deformability.

Further evidence of increased membrane stiffness was obtained from the variation in whole cell and ghost DI with varying applied shear stress. Iron-deficient rat red cells and resealed ghost preparations showed a decrease in DI that was particularly evident at low values of applied shear stress, a previously observed characteristic of cells with increased membrane rigidity.9 The DI versus shear stress curve for intact iron-deficient cells was always below that of normal cells for an applied shear stress range of 0–230 dynes/sq cm (23 cp suspending medium viscosity) (Fig. 1). However, an increase of the suspending medium viscosity to 90 cp (maximum shear stress 900 dynes/sq cm) raised the iron-deficient DI to the level attained by normal cells at 23 cp. Thus, to obtain equivalent deformation, an approximately fourfold increase in applied shear stress was required. The fact that the DI plateau could be raised by increasing suspension medium viscosity indicated that cell deformation was not limited by a deficiency of membrane surface area.

Ghost deformability measurements provided similar evidence for increased membrane rigidity of iron-deficient cells. The slope of the DI versus shear stress curve was markedly reduced, particularly in the low shear stress range (Fig. 2). However, the DI curves for iron-deficient ghosts were also brought closer to the control curve when the applied shear stress range was increased (Fig. 2). This again indicates a limitation of deformability primarily by membrane stiffness, rather than by a decrease in membrane surface area.

Further confirmation that the deformability of iron-

<table>
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<th>Table 2. Hematologic, Biochemical, and Red Cell Deformability Data of Severe and Moderate Iron-Deficient and Control Rats</th>
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<tr>
<td><strong>Study Groups</strong></td>
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<tr>
<td>Iron content diet (mg/kg)</td>
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<tr>
<td>(A) Hematologic data</td>
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<tr>
<td>Hb (g/dl)</td>
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<td>MCV (fl)</td>
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<td>MCHC (g/dl)</td>
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<tr>
<td>Reticulocytes (%)</td>
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<tr>
<td>(B) Biochemical data</td>
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<tr>
<td>Serum iron (μg/dl)</td>
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<tr>
<td>Transferrin saturation (%)</td>
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<tr>
<td>Erythrocyte protoporphyrin (μg/dl RBC)</td>
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<tr>
<td>(C) Deformability, shear-induced fragmentation and osmotic fragility data</td>
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<td>Intact RBC DI† at 290 mosmole</td>
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<td>Resealed ghost DI at 290 mosmole, 150 dyne/sq cm</td>
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<td>T½ mechanical fragility test (sec)</td>
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<td>Osmotic fragility test (% NaCl at 50% hemolysis)</td>
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* *p < 0.01 in comparison with controls.
† Deformability index is expressed as mean percent of control value and range.
‡ *p < 0.05 in comparison with controls.
deficient rat RBC was not constrained by reduced membrane surface area was obtained by direct measurement of red cell cholesterol content. As shown in Table 3A, although the volume of the iron-deficient cells was much smaller than that of control cells, there was no reduction in cholesterol content per cell. This implies that membrane surface area was not significantly reduced in iron-deficient rat RBC. In addition, the surface area of control and iron-deficient rat RBC was independently estimated by analysis of the intact cell Dl, with variation in the osmolality of the suspending medium, at a constant shear stress of 125 dynes/sq cm. As shown in Fig. 3, the cell deformability reached a minimum in hypotonic medium. The osmolality at which the minimum occurred was shown to correspond to the midpoint of the osmotic fragility curve, at which point the cells are swollen to a spherical configuration. The cell volume at this point represents the critical hemolytic volume, and it can be calculated from the isotonic volume on the basis of osmotic swelling. The membrane surface area should then be equal to the area of a sphere with that critical hemolytic volume. Such a calculation again showed no evidence for a reduction in membrane surface of the iron-deficient cells (Table 3B). The osmotic gradient Dl curves shown in Fig. 3 indicate that the minimum Dl for the iron-deficient cells occurred at a lower osmolality than that for the control cells. This change implies a decrease in osmotic fragility, which is in agreement with direct measurements of osmotic fragility, as summarized in Table 2C. This, in turn, implies an increase in the ratio of surface area to volume for the iron-deficient cells, which is consistent with their reduced MCV and normal surface area, as inferred by cholesterol content.

In addition to surface-to-volume ratio and membrane stiffness, whole cell deformability is also influenced by intracellular viscosity. Previous studies of normal cells with varying water contents showed that the primary determinant of intracellular viscosity
was the mean cell hemoglobin concentration. Thus, the reduction of MCHC in the iron-deficient red cells would seem to exclude the possibility that whole cell deformability was constrained by a high hemoglobin concentration. In addition to measuring MCHC, we also used Stractan gradients to analyze cell density distributions, since hemoglobin concentration is the major determinant of cell density. The density distribution of iron-deficient rat RBC showed an increase in the proportion of low density cells (Fig. 4), consistent with their lower MCHC. It would be expected that this reduction in MCHC would confer abnormally increased deformability in hypertonic medium. However, as shown in the osmotic gradient deformability curves in Fig. 3, the iron-deficient cells actually deformed less well than the control cells in the hypertonic range.

In addition, the isotonic deformability of intact RBC from each of five density gradient fractions showed that iron-deficient RBC had a significantly lower DI than control RBC collected from the same density region of the gradient. It was also noted that the top or low density fraction consisted primarily of reticulocytes and nucleated RBC and had a slightly higher RBC deformability than the denser fractions. Hence, the higher content of reticulocytes and nucleated RBC in iron-deficient rat samples cannot explain the reduced RBC deformability of the whole blood samples.

Biochemical Studies of Rat RBC Membranes

To assess the sensitivity of iron-deficient rat cells to peroxidative insult, we measured the amount of MDA
that resulted from membrane lipid peroxidation in the presence of added H₂O₂. The results of these experiments are summarized in Table 4A. The iron-deficient samples had a slightly, but significantly, increased susceptibility to peroxidation. Table 4B shows that lipid extracts from iron-deficient cells that were not treated with H₂O₂ contained more MDA—phospholipid complex than control cells. Previous work has suggested that the presence of this complex is indicative of in vivo peroxidation.¹⁸

RBC membrane preparations from fresh blood samples that had not been exposed to peroxidative challenge were analyzed on SDS-PAGE gels. Samples from iron-deficient rats contained a protein fraction that remained at the origin on the gels. This fraction accounted for approximately 2%—4% of total protein, compared to less than 0.5% in control samples, based on scanning of the stained gels (Table 4C). This presumably high molecular weight aggregated material was partially, but not entirely, reducible with DTT. This finding suggests that some of the aggregated material was crosslinked by disulfide bonds. The nonreducible portion may have been crosslinked by MDA-mediated reactions with amino groups. Other membrane protein bands in the iron-deficient samples appeared similar to those of the control samples.

The cation content of freshly collected rat RBC is summarized in Table 5. The iron-deficient samples had significantly higher intracellular sodium and lower potassium contents and a reduction in total cation content. These findings are consistent with a disturbance of cation transport or permeability, and possibly with cell dehydration. However, any dehydration that may have occurred was not associated with a higher than normal MCHC. In fact, measurements of MCHC and cell density distribution indicated that the majority of the iron-deficient cells had a subnormal MCHC.

**DISCUSSION**

In this study, RBC from both iron-deficient humans and rats were less deformable than controls. The decrease in deformability was more pronounced with increased severity of iron deficiency in the rat. These observations are in accord with previous reports of reduced filterability of RBC from iron-deficient rabbits and with greater than expected whole blood viscosity in patients who had both cyanotic heart disease and iron deficiency.²⁴ ¹ Tillman et al.⁷ have suggested that the reduced ability of iron-deficient RBC to pass through a polycarbonate filter and the observed increase in blood viscosity were due to the unfavorable surface-to-volume ratio of microcytes. In contrast, our direct measurements of red cell membrane deformability indicate that increased membrane rigidity (increased shear modulus) was the primary cause of reduced RBC deformability in iron deficiency. Moreover, the surface-to-volume ratio by direct measurement was actually increased and would be expected to result in increased, rather than decreased, deformability. Neither could the impaired deformability of these cells be explained on the basis of increased intracellular viscosity, because of their low MCHC. It should be noted that Klose et al.²⁵ recently measured the deformability of human iron-deficient RBC, both by viscometry and by measuring whole cell elongation by a method similar to ektacytometry. By exclusion, these authors also concluded that reduced membrane flexibility was the most likely cause of reduced whole cell deformability. The primary evidence for increased membrane stiffness in the present studies comes from direct measurements of deformability of membrane preparations. The large reduction of intracellular hemoglobin concentration during the preparation of
red cell ghosts minimizes the influence of intracellular viscosity on this measurement. Thus, any residual defect in deformability must be due either to membrane stiffness or to a lack of sufficient membrane surface to permit full cellular deformation. In the present study, a membrane surface deficiency could be ruled out on the basis of several observations. Measurements of red cell cholesterol content showed no membrane deficiency for the iron-deficient cells. This conclusion was also consistent with estimates of cell surface area based on the critical hemolytic volume, as derived from the osmolality at which the cells deformed minimally. In addition, when the applied shear stress was progressively increased, the deformability of iron-deficient RBC and ghosts approached that of the normal samples. This indicated that the poor deformability at lower shear stress was due to membrane rigidity and not to a lack of redundant surface area to accommodate cell deformation.

It might be questioned whether the small size of the iron-deficient cells could have caused an apparent reduction in deformability that was actually an artifact of the method used to measure deformability. Parallel studies of RBC of various sizes from different mammalian species have shown that the maximum attainable ektacytometric DI value is sensitive only to variations in cell surface area and not to variations in initial MCV. Because the iron-deficient cells had the same surface area as the normal cells, the reduction in maximum DI cannot be explained on the basis of reduced cell size.

Since the decreased viscoelasticity of iron-deficient RBC membranes suggested a significant alteration in the organization of the membrane, we studied the biochemical characteristics of the membrane lipid and protein of rat RBC. Several lines of evidence suggested that increased peroxidation may play a role in membrane damage in iron-deficient cells. First, in response to exposure to H$_2$O$_2$, the RBC from iron-deficient rats showed increased generation of MDA, a product of lipid peroxidation, in accord with previous observations. Furthermore, iron-deficient cells contained an MDA-phospholipid complex, which was suggestive of in vivo peroxidative damage. Additional support for in vivo peroxidative damage was provided by SDS-PAGE analysis of RBC membrane proteins. The presence of high molecular weight material in iron-deficient rat samples may represent crosslinked protein material that had resulted from a peroxidative process, since similar aggregates are produced when normal RBC are exposed to hydrogen peroxide or MDA. The partial reduction of this high molecular weight material with DTT suggested that some of the crosslinking may have represented disulfide bonding. In addition, the presence of nonreducible aggregates, as well as the MDA-phospholipid complex, suggests that MDA released during lipid peroxidation may directly contribute to membrane damage in iron-deficient cells. Although the quantities of in vivo crosslinked lipid and protein material observed in the iron-deficient rat RBC membrane were small, it is noteworthy that they developed spontaneously; a comparison with the larger amounts of crosslinked products induced in certain conditions with in vitro exposure to MDA or hydrogen peroxide is therefore inappropriate. Finally, the reduced intracellular potassium and increased sodium content of rat RBC were also consistent with in vivo peroxidative damage. Similar cation changes were previously reported in human iron deficiency and can be produced in normal RBC by peroxidative stress. Taken together, the constellation of biochemical changes in membrane lipid, membrane protein, and intracellular cations of iron-deficient cells associated with a reduction in cell deformability is reminiscent of normal cells subjected to in vitro peroxidation. It therefore seems that a peroxidative process may be responsible, in part, for the decreased membrane deformability of RBC in the iron-deficient rat. The consequent loss of whole cell deformability could be an explanation for the shortened survival of iron-deficient cells in rats and humans.

Although iron excess is well known to contribute to peroxidative damage of red cells, it is noteworthy that there appears to be a similar association with iron deficiency. Possible factors that may account for increased susceptibility to peroxidative damage in iron deficiency include either increases in intracellular oxidative compounds, e.g., protoporphyrin, or a decrease in intracellular scavengers for free radicals. Scavengers in normal cells include vitamin E, glutathione peroxidase, and catalase, all of which have previously been shown to be reduced in iron deficiency. Rachmilewitz et al. found evidence for increased peroxidative damage to the RBC membrane in thalassemia minor, a condition that resembles iron deficiency in being characterized by hypochromic RBC. Those authors speculate that the relatively low hemoglobin concentration in hypochromic cells may provide a greater opportunity for free radicals to react with the membrane rather than with hemoglobin. The same argument could be applied to iron-deficient red cells, in which the hemoglobin concentration is similar to that in thalassemia trait. However, the actual basis for the increased peroxidative activity in iron-deficient RBC, as well as other factors that can contribute to the increased membrane stiffness, requires further study.

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REFERENCES


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