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Erythrocyte membrane lipid peroxidation in iron deficiency anemia

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Summary. Erythrocytes from normal subjects and from cases of iron deficiency anemia were exposed to hydrogen peroxide and the extent of membrane lipid peroxidation studied. Significantly less peroxidation was observed in intact anemic erythrocytes compared to normal. However, when isolated membrane lipids were subjected to peroxidation, there was no significant difference between the two groups. It is unlikely that lipid peroxidation per se plays a major role in the reported decrease in red cell life-span in iron deficiency.

Considerable evidence is available in the literature which suggests that the life-span of erythrocytes tends to decrease in iron deficiency anemia (IDA)[1-8]. Red cell fragmentation is commonly observed in experimental iron deficiency[9]. It has also been reported that hypochromic red cells survive normally in splenectomized but not in normal individuals[10,11]. However, the exact mechanisms underlying the decrease in the life-span of red cells are not known.

Peroxidation of membrane lipids leading to increased membrane rigidity has long been recognized as an important factor underlying the aging and destruction of red cells[12]. We have, therefore, made a comparative study of the susceptibility to peroxidation of membrane lipids of red cells from normal subjects and IDA patients, in an attempt to evaluate the possible role that oxidant injury might play in the reported shortening of the life-span of red cells in IDA.

Methods. The criteria employed in the selection of cases of IDA are detailed in a previous publication[13].

Red cells were routinely washed in normal saline and suspended in phosphate-saline medium (1 vol. of potassium phosphate buffer, 0.1 M, pH 7.3 mixed with 9 vol. of 0.15 M NaCl) for peroxidation studies. Intact red cells were exposed to H_2O_2 which was either added direct to the suspension medium or introduced by vapor diffusion from the center-well of a Warburg flask[14]. The resultant peroxidation of erythrocyte lipids was measured by estimating the malonyldialdehyde (MDA) formed, using the thiobarbituric acid (TBA) reaction described by Stocks and Dormandy[15].

Erythrocyte lipids were extracted and quantitated as follows: Saline-washed, packed red cells were lysed in 4 times their volume of distilled water. An aliquot of the lysate was used to determine the Hb content. The lipids in the remainder of the lysate were extracted as described by De Gier and Van Deenen[16]. An aliquot of the extract was used for estimation of lipid phosphorus according to Connerty et al.[17] and the relation between lipid phosphorus and Hb in the original lysate was established. Using this relation, the lipid phosphorus content of intact red cells in suspension was calculated from their Hb content.

Peroxidation of isolated lipids from intact red cells or from their ghosts was studied as described by Kurien and Iyer[14]. Red cell ghosts were prepared according to Dodge et al.[18]. Reduced glutathione (GSH) was estimated according to the DTNB colorimetric procedure of Beutler et al.[19].

Treatment of erythrocytes with N-ethylmaleimide (NEM) was carried out according to Morrel et al.[20].

Results and comment. The results of a comparative study of

Lipid peroxidation in normal and anemic erythrocytes (nmoles of MDA formed/mg lipid P)

Lipid source	Mode of addition of H_2O_2 Direct	p	Diffusion	p
A) Intact red cells				
1. Normal (10) *	1878 ± 234		1154 ± 165	
2. Anemia (10)	1988 ± 243	N S	838 ± 272	<0.01
3. Normal (NEM-treated) (4)			1726 ± 191	
4. Anemia (NEM-treated) (4)			1625 ± 266	N S
B) Isolated lipids				
From intact red cells				
5. Normal (5)	456 ± 63			
6. Anemia (5)	497 ± 79	N S		
From red cell ghosts				
7. Normal (5)	478 ± 61			
8. Anemia (5)	515 ± 59	N S		

Group A. 4 ml of a 2.5 % suspension of erythrocytes in phosphate buffered saline was mixed directly with H_2O_2 (10 mM final) and incubated at 37 °C for 1 h. In diffusion experiments, 4 ml of the suspension in the main chamber of a Warburg flask was exposed to H_2O_2 vapors distilling from 0.2 ml of 30 % H_2O_2 in the center-well for 6 h (oscillation 90/min). Other details of experiment were the same as described by Kurien and Iyer[14].
Group B. Emulsions of extracted lipids (0.03–0.04 mg lipid P) treated with H_2O_2, added direct (10 mM final) and incubated at 37 °C for 1 h in presence of Fe^{++} (100 μM final).
* Number of experiments in each group given in parenthesis;
N S, difference between the normal and anemia groups not significant.

the peroxidation of membrane lipids of erythrocytes from normal and anemic subjects are summarized in the table. In experiments in which intact red cells were exposed to very low concentrations of H_2O_2 introduced by vapor diffusion, MDA formation was significantly less in the case of anemic erythrocytes. This difference from normal erythrocytes was, however, nearly abolished on pretreatment of the cells with NEM. At higher concentrations of H_2O_2 added direct to the red cell suspension, MDA formation was higher in both normal and anemic erythrocytes but the difference between the 2 groups was not significant.

Again, lipids isolated from red cells or from red cell ghosts, from normal and anemic groups did not show any appreciable difference in the extent of peroxidation. In agreement with the observations of Stocks and Dormandy[15] isolated lipids yield much less MDA (per mg lipid P) compared to intact red cells.

From the foregoing results one finds that the susceptibility to peroxidation, of isolated lipids of red cell membranes, is not appreciably increased in IDA. In intact anemic erythrocytes, on the other hand, membrane lipids seem to be better protected at least at low concentrations of H_2O_2. It is possible that the protective agent is glutathione (GSH); two lines of evidence suggest this: a) pretreatment of red cells with NEM abolishes the observed difference in the peroxidation of lipids between the normal and anemia groups (table) and b) the red cell GSH levels are distinctly higher in IDA (2.47 ± 0.31 mmoles/l packed cells in IDA as against 1.73 ± 0.24 mmoles/l in normal erythrocytes in the subjects reported here). One would expect GSH to be even more effective in vivo in anemic erythrocytes on account of the higher activities of the enzymes glutathione reductase[21] and glutathione synthetase[22]. GSH presumably exercises its protective effect largely and directly through the GSH-peroxidase reaction. In addition, GSH has also been reported to be an essential requirement for a soluble, heat-labile inhibitor of lipid peroxidation distinct from GSH-peroxidase[23]. It would be interesting to know whether such an inhibitor is present in the erythrocytes and, if present, how its level is affected by iron deficiency.

In conclusion, the results presented here indicate that if the life-span of red cells is reduced at all in IDA, the reduction cannot be attributed to an increased susceptibility of membrane lipids per se to peroxidation.

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Skinned smooth muscle: Time course of force and ATPase activity during contraction cycle[1]

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Summary. The time course of ATPase activity and force has been determined during contraction and relaxation in skinned (hyperpermeable) anterior byssus retractor muscle, ABRM, of *Mytilus edulis* and compared with corresponding measurements on skinned taenia coli of guinea-pigs. Following a calcium-induced contraction, lowering the $[Ca^{++}]$ to 10^{-8} M rapidly reduces ATPase activity within 2 min to resting levels while force declines only to about 30–50% of maximal tension within the same time. Thus slow relaxation is due to a 'catch-like-state' which is common to different kinds of smooth muscles and can be reduced with cAMP in ABRM and by P_i in taenia coli.

After cessation of active contraction, certain types of molluscan smooth muscle[2] and vertebrate smooth muscle[3] relax extremely slowly while the rate of energy expenditure is rapidly reduced to resting levels. During slow relaxation, tension is not actively maintained; the muscle is said to be in catch or latch. The catch phenomenon may be demonstrated using skinned or demembranated fibers of the anterior byssus retractor muscle of *Mytilus*[4] in which the