DECREASED ACTIVITY OF THE IRON-CONTAINING ENZYME CATALASE IN HUMAN IRON-DEFICIENCY ANEMIA*

Şinasi Özsoylu, M.D**

Iron-deficiency anemia is the most common form of anemia in infancy, early childhood, pregnancy and in lactating women. Iron is essential in the biosynthesis of hemoglobin and myoglobin. It is also very important for cell metabolism in the electron transport mechanism which is handled by the iron-containing cytochrome system. Additional physiological roles of other iron-containing enzymes, such as peroxidase and catalase, are not clear. It has been frequently observed that during the treatment of severe iron-deficiency anemia, before a reticulocyte or hemoglobin response occurs, there is definite improvement in appetite and sleeping habits and a reduction in irritability.[1,2] Muco-epithelial and epithelial appendix changes in adults with iron-deficiency anemia cannot be explained on the basis of anemia alone. In view of the above observations, it was conjectured that decreased activity of iron-containing enzymes in iron-deficiency anemia was the cause, and activities of these enzymes have been measured in animals [4,5,6,7] and in humans. [8,9,10,11] Although decreased activity is known to occur in animals with iron-deficiency anemia, to date abnormalities have not been shown with any certainty in humans.

The catalase activity in erythrocytes was determined in 21 patients with iron-deficiency anemia at Children's Hospital Medical Center, Boston. All were found to be low. We continued determinations of serum iron, hemoglobin levels and erythrocyte catalase activities on 13 of the 21 patients. These returned to normal levels following treatment with oral ferrous sulfate.

---

* Portion of Docent Thesis to be presented in November, 1964. This study was done while working under Dr. Louis K. Diamond as a trainee and research fellow in research hematology at Children's Hospital Medical Center, Harvard Medical School, Boston, Massachusetts.

** Hematologist and associate in pediatrics, Hacettepe Medical Faculty, Ankara, Turkey.

Materials and Methods

Normal controls comprised medical and laboratory personnel whose hemoglobin levels, serum iron concentrations and serum iron-binding capacities were within normal limits, and some of the children who after treatment for their iron-deficiency anemia had shown a return to normal of their hemoglobin, serum iron and serum iron-binding capacities.

Iron-Deficiency Anemia Cases

Eighteen of the 21 cases of iron-deficiency anemia were children between 8 and 36 months of age. The remaining three patients were girls ages 8, 10 and 14 years with chronic mastoiditis, cirrhosis and periarteritis nodosa, respectively. All of the 21 patients had low hemoglobin and serum iron levels and the peripheral blood and bone marrow investigations yielded results of iron-deficiency anemia. All of the treated patients responded to iron administration.

Since the erythrocyte catalase activity was found to be low in cases of iron-deficiency anemia, studies of this catalase activity were made in other hypochromic microcytic anemias and in other hematologic disorders with low hemoglobin concentrations (i.e. thalassemia syndromes, anemia of infection, sickle cell anemia, aplastic anemia, spherocytosis and leukemia).

The erythrocyte catalase activity was measured by a modification of the method of Takahara, et al.[13] This modification consisted of the substitutions of 0.025 N potassium permanganate for 0.005 N, which was kept in a dark-colored bottle. Assays were performed on heparinized venous blood immediately or within seven hours on refrigerated samples. The hemoglobin and hematocrit levels were determined by conventional methods. Red cell counts were performed in quadruplicate on the Coulter counter (Model B). Fasting serum iron and serum iron-binding capacities were measured using the method of Schade and co-workers.[3]

The assay of erythrocyte catalase activity was performed as follows: 1 ml of whole blood was diluted with an approximate amount of distilled water at room temperature to make a final hemoglobin concentration of 0.14 gm/L, and mixed gently. One ml of this hemolysate was introduced with a blowout pipette immediately into a 50 ml beaker containing a 5 ml solution of 0.01 M. hydrogen peroxide

and 0.01 M. phosphate buffer, pH 6.8, which was kept refrigerated and was prewarmed to 37° C. in a constant temperature waterbath. After 15 seconds' incubation the reaction was stopped by the addition of 2 ml of 2 N sulfuric acid. A second beaker, to which the same amount of sulfuric acid was added before the diluted blood, was used to determine the initial hydrogen peroxide concentration. The hydrogen peroxide concentration in each beaker was determined by titration with potassium permanganate. These determinations were performed in duplicate. Under these assay conditions, the erythrocyte catalase activity is calculated from the equation: $K_{cat} = \frac{10^3}{15} \log \frac{Xo}{X}$

where Xo is the initial peroxide concentration expressed as milliliters of permanganate and X is the concentration after 15 seconds' incubation. After measuring the catalase activity in a hemoglobin solution of 0.14 gm/L concentration, the enzyme activity per 10^9 erythrocytes, expressed as units, was calculated by this equation: $\frac{K_{cat} \times b}{c}$

where K_{cat} = catalase activity of blood with a hemoglobin concentration of 0.14 gm/L, b = dilution factor (e.g., usually 400-1000) to make 0.14 gm/L hemoglobin concentration, and c = erythrocyte count in millions per cubic millimeter. In 15 duplicate determinations, the standard deviation of the difference between each pair was found to be 13 Units.

Results

In 33 normal controls, (15 adults and 18 children aged 10 months to 14 years) the catalase activity per 10^9 erythrocytes did not show any variation in respect to age or sex and the mean value was 936 Units (S.D. ± 81 Units) ranging between 800 and 1100 Units. One apparently normal adult, with hemoglobin and serum iron levels of 14 gms. % and 90 mcg. % respectively had a low erythrocyte catalase activity (645 Units) and will be discussed later. In eight cases, which were chosen as controls originally, although hemoglobin and catalase levels were normal, being respectively over 12 gms. % and 800 Units, serum iron levels were found to be low (10-56 mcg. %). In five children, in spite of low normal hemoglobin levels (10.75-11.3 gms. %) the catalase activity and serum iron levels were definitely low, being 530-709 Units and 10-46 mcg. % respectively.

When the erythrocyte catalase activity was measured in patients with iron-deficiency anemia and expressed in terms of 10^9 red blood cells, it was found to be low in all cases varying from 200 to 755 Units. The lowest value was found in a patient with cyanotic heart condition and iron-deficiency anemia. There was no correlation between the enzyme activity and the hemoglobin level in the individual cases. However, in some instances, the erythrocyte catalase activity was determined for the first time after these patients had been receiving iron treatment for several days or had been given transfusions because of the severity of their anemia. In Table 1, the erythrocyte catalase values of patients with iron-deficiency anemia and the follow-up of these cases during treatment with oral iron medication is shown. Figure 1 illustrates these changes in two patients.

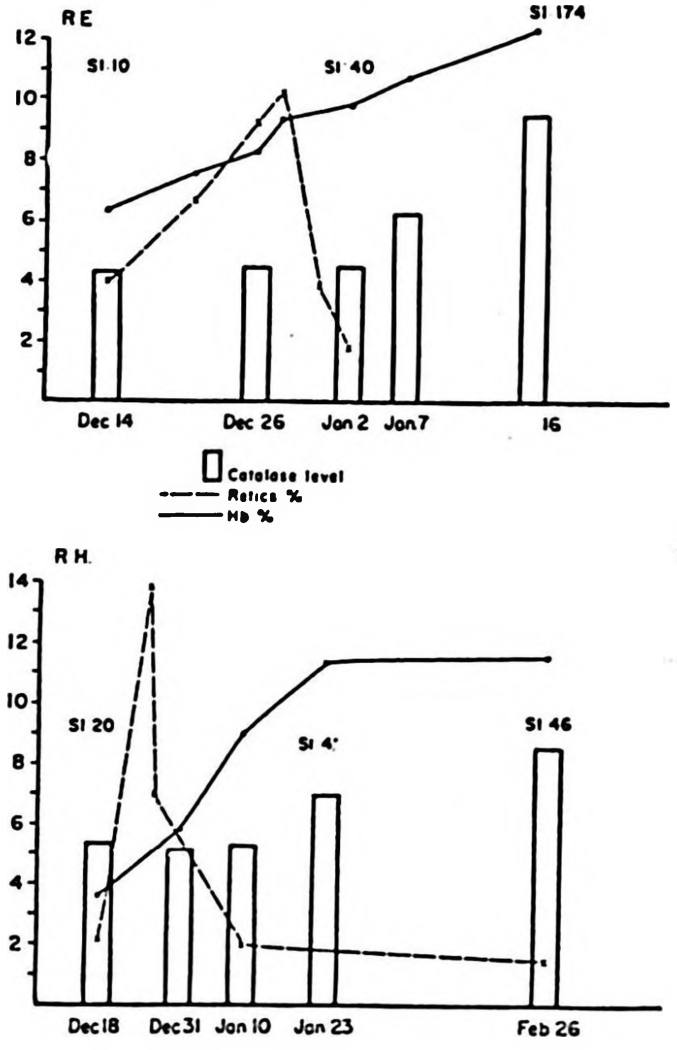

Figure 1. Changes of reticulocyte hemoglobin and erythrocyte catalase values in two patients during iron treatment.

In one case with a rare familial hypochromic microcytic anemia and normal serum copper (110 mg./100 ml %) and high serum iron

TABLE 1

THE CHANGES OF HB, SERUM IRON AND RED BLOOD CELL CATALASE VALUES IN IRON-DEFICIENCY ANEMIA CASES DURING FERROUS SULFATE TREATMENT.

					THERAPY											
		Initial Values			1–2 weeks			3–4 weeks			5–6 weeks			7–8 weeks		
Name	Age	S.I. γ %	Hgb. gm %	Catalase U.	S.I. γ %	Hgb. gm %	Catalase U.	S.I. γ %	Hgb. gm %r	Catalase U.	S.I. γ %	Hgb. gm %r	Catalase U.	S.I. γ %	Hgb. gm %r	Catalase U.
S.C.	10/12	22	5.45	348	—	10.8 (transfused)	612	—	——	—	74	13.2	825			
W.G.	10/12	10	4.75 (transfused)	650	—	7.5	502	—	——	—	—	12.2	1003	81	12.5	1003
F.S.	14/12	36	4.25	382	172 (?)	8.8	520	—	——	—	82	12.1	809			
D.C.	99/12	36	8.25	745	—	——	—	—	——	—	80	12.3	1100			
P.J.	19/12	48	9.5	556	—	11	565	68	12	670	—	12.3	760	—	12.5	825
A.P.	10/12	10	8.6 (transfused)	719	—	——	—	—	11.8	1100						
P.B.	13/12	30	6.75	502	—	——	—	—	——	—	71	11.2	802			
R.O.	10/12	46	7.4	442	—	9.9	620	—	11.6	811	↓					
G.S.	11/12	10	2.4	525	—	(transfused)	—	—	10.55	842						
R.H.	3	20	3.6	541	—	5.8	519	—	9	531	42	11.3	700	46	12	860
E.I.	19/12	46	11.2	714 (on treatment	—	——	—	228(?) (?)	13.75	1100						
R.E.	16/12	10	6.25	435	—	8.3	449	40	9.75	450	—	10.65	622	174	12.3	950
S.M.	11/12	58	11	735 (on treatment	—	——↓	—	—	11.7	852						

(220 mg./100 ml %) levels, radioactive iron studies had shown that he could not utilize iron for hemoglobin synthesis at a normal rate. The erythrocyte catalase activity was determined, and as expected, was found to be low (609 Units).*[33]

The erythrocyte catalase activity was assayed in seven patients with thalassemia major and eight with thalassemia minor. In all but two patients, who, it was demonstrated, were suffering from thalassemia minor *and* associated iron-deficiency anemia, the enzyme activity was within normal limits (808-1080 Units).**

Of five children with acute bacterial infections, three, with associated iron-deficiency anemia had low levels (435-620 Units). The other two did not have iron-deficiency anemia and their erythrocyte catalase activity was normal.

The activity of this enzyme was measured in hereditary spherocytosis (one case), acquired hemolytic anemia with spherocytosis (two cases), sickle cell anemia (two cases) and sickle cell trait (three cases). It was found normal in all. Seven cases of aplastic anemia were studied. In four, the enzyme activity was higher than normal (1265-1520 Units) and two showed signs of remission during testosterone and cortico-steroid therapy. In the remaining three cases of aplastic anemia, the erythrocyte catalase activity was normal (808-965 Units). In one case of chronic granulocytic leukemia (WBC: 598,000/cu.mm.) and two of acute leukemia (WBC: 38,400 and 192,000/cu.mm. respectively), the erythrocyte catalase activity was found to be normal.

Discussion

Catalase is an iron-containing enzyme and widely distributed in mammalian tissues but is most abundant in the liver, kidney and erythrocytes.[19] It constitutes 13.4 per cent of the non-hemoglobin protein of erythrocytes.[22] Catalase has been crystallized from bacteria,[18] beef liver,[16] human liver and human erythrocytes.[12] The prosthetic group of this enzyme, hematin, is identical to that found in methemoglobin, metmyoglobin and ferricytochrome, but apoenzyme and its mode of linkage to ferrihemin differs from the other ferriprotein complexes.[19] The species specificity of this enzyme is due to the composition of apoenzyme. Although the rate of synthesis and half-life

* In this case, it has been shown that he could not utilize iron for heme synthesis.

** Serum iron level in those two cases was found to be 28 and 30 microgram per cent.

of this enzyme in different organs are not the same,[17] the enzyme formed in different organs of the body is identical.[21] The molecular weight of erythrocyte catalase is 220.000, contains 4 hemin in one molecule, and its iron concentration is 0.01 per cent.[12] The protoporphyrin ring of catalase is apparently the same as that of hemoglobin and by degradation ultimately yields bilirubin.[20] Catalase is the most active enzyme ever known. One molecule of this enzyme is capable of splitting off 44,000 molecules of hydrogen peroxide per second at 0°C.[19] Physiologically, catalase may serve as a regulator of hydrogen peroxide concentration which is produced by aerobic dehydrogenase enzymes which transfer hydrogen directly into molecular oxygen. Catalase acts in two different ways: at low hydrogen peroxide concentration, the enzyme behaves as a peroxidase, while in high substrate concentrations, hydrogen peroxide is destroyed catalytically.[23] Although there is some evidence suggesting that the function of erythrocyte catalase is to protect hemoglobin from oxidation through hydrogen peroxide to methemoglobin, it has not been proven. The demonstration of the absence of catalase activity in erythrocytes and other tissues of an asymptomatic acatalassemic person may indicate that catalase is not an indispensable enzyme.

The red cell catalase activity in humans has been measured in different diseases.[9,10,13,22,29,30,32] It was also assayed in patients with iron-deficiency anemia by Beutler et al[8] and was not found to differ from those of the normal controls. However, this assay system differed in several respects from that of the present method in that a much longer incubation period, a lower hydrogen peroxide concentration and a greater dilution of the blood samples were used by these authors. Bonnichsen and co-workers[15] have emphasized the necessity of measuring the reaction rates after only seconds of incubation in order to avoid substrate depletion. Richardson and his collaborators[14] have shown that the optimal hydrogen peroxide concentrations for catalase assay are between 0.1 N and 1 N. The importance of the dilution factor for human blood was emphasized also by the same authors.

In our 21 cases of iron deficiency anemia, erythrocyte catalase activity was found to be low in all and returned to normal levels during treatment in all patients who were followed over a period of time.

The pattern of recovery seen in patients with iron-deficiency anemia showed that hemoglobin values were first to return to normal, followed in turn by normalization of erythrocyte catalase levels and finally by the serum iron concentration. It would appear that the

eight subjects previously described with low serum iron concentrations but normal hemoglobin and catalase levels showed the first evidence of iron deficiency. The other five persons with normal hemoglobin levels but low serum iron and erythrocyte catalase values could be considered to have more evidence of iron deficiency.

The plasma catalase activity was measured in two control cases (2.6 and 2.4 Units respectively). It was too low to interfere with erythrocyte catalase determinations. High reticulocyte counts, which were as high as 20 per cent and could be seen in the first few days following the commencement of iron therapy, did not produce any alterations in the catalase activity when compared with the original determinations. Although there is some controversy, Sass[28] has also shown recently that the reticulocyte catalase activity does not differ from that of erythrocytes. Since leukocytes contain mostly verdoperoxidase[19] and their catalase activity is low, they do not interfere with erythrocyte catalase assays. In one apparently healthy person with normal serum iron and hemoglobin concentrations, the erythrocyte catalase activity was found to be low (745 Units). His erythrocyte G-6PD activity was 182 Units which was within normal limits and his serum copper was 70 mcg. per cent which was slightly low. Since his family was not available, further studies could not be done. In iron and copper deficient rats, Schultze and Kuiken[5] have shown that liver and kidney catalase activity was low and that the normal catalase activity of these organs was re-established following the administration of whichever mineral was specifically lacking. In animals, it has been shown that starvation[27] and sedormid[17] decrease the liver catalase content without affecting the erythrocyte catalase activity;[22] in addition the liver catalase activity is influenced by thyroxine,[25] testosterone and corticosteroid administration,[26] and is inhibited by INH. This inhibitory effect can be reversed by Vitamin B_6.[24]

An inborn error of metabolism, acatalassemia, has been reported in humans[13] and in animals.[31] This is transmitted as an autosomal recessive disorder. A decrease in erythrocyte catalase activity of 40 per cent has been reported in G-6PD deficient Negroes.[22] However, the authors did not reproduce their own results later.

Although in animals the liver and kidney catalase activity is higher in males than females, neither Takahara et al[13] nor we found any sex or age difference in the erythrocyte catalase activity of humans.

The high erythrocyte catalase values in four cases of aplastic anemia could not be due solely to testosterone and corticosteroid

administration, since two of them had not received medication for more than three months. The normal erythrocyte catalase activity in thalassemia syndromes may indicate indirectly that heme synthesis is not impaired in these disorders.

We have found low erythrocyte catalase values only in cases of iron-deficiency anemia (with the exception of one healthy person whose low value is not readily explained although his serum copper level was slightly low). To our knowledge this is the first demonstration of decreased activity of an iron-containing enzyme in iron-deficiency anemia in humans. Although Jacobs[11] had shown by the staining method some decrease in cytochrome oxydase activity (which is an iron-containing enzyme) in the buccal epithelium in human beings, it was not consistent in all cases. Besides, the same result was obtained in one non-anemic person's buccal mucosa by assaying the activity. The assay of this enzyme is simple, quick and requires less blood than serum iron determinations. Because the erythrocyte catalase values were normal in anemias other than iron-deficiency anemia, determination of the activity of this enzyme appears to be a simple and rapid method of diagnosis and therapeutic evaluation in iron-deficiency anemia.

Summary

Erythrocyte catalase activity has been found to be decreased in iron-deficiency anemia. This is the first demonstration of deficiency of an iron-containing enzyme in iron-deficiency anemia in humans. It was measured by the method of Takahara et al and expressed as catalase activity per 10^9 red blood cells. In all treated patients, catalase values returned to normal after the anemia was corrected. In patients with anemia due to other causes, the erythrocyte catalase activities were normal.

Acknowledgement

I am indebted beyond words to Dr. L. K. Diamond for his encouragement and interest.

REFERENCES

1. Beutler, E.: Iron enzymes in iron deficiency. I. Cytochrome c, Ames. J. Med. Sc. **234**: 517, 1957.
2. Jennison, R.F. and Ellis, H.R.: Intramuscular iron. A clinical trial in pregnancy, Lancet **2**: 1245, 1954.
3. Schade, A.L., Oyama, J., Reinhard, R.W. and Miller , J.R.: Bound iron and unsaturated iron binding capacity of serum; rapid and reliable quantitative determination, Proc. Soc. Exp. Biol. and Med. **87**: 443, 1954.
4. Adams, D.H: The effect on mouse-liver catalase activity and blood hemoglobin level of a milk diet deficient in iron, copper, and manganese, Biochem. J. **54**: 328, 1953.
5. Schultze, M.E. and Kuiken, K.A.: The effect of deficiencies in copper and iron on the catalase activity of rat tissues. J. Biol. Chem. **137**: 727, 1941.
6. Lahey, M.E., Gubler, C.J., Chase, M.S., Cartwright, G.E. and Wintrobe, M.M.: Studies of copper metabolism. II. Hematologic manifestations of copper deficiency in swine, Blood, **7**: 1053, 1952.
7. Cohen, E. and Elvehjem, C.A.: The relation of iron and copper to the cytochrome and oxidase content of animal tissues, J. Biol. Chem. **107**: 97, 1934.
8. Beutler, E. and Blaisdell, R.K.: Iron enzymes in iron deficiency. II. Catalase in human erythrocytes, J. Clin. Invest. **37**: 833, 1958.
9. Krumbhaar, E.B. and Musser, J.H.: The catalase content of the blood in different types of anemia, J. Amer. Med. Ass. **75**: 104, 1920.
10. Jones, E.H. and McCance, R.A.: Enzyme activities in the blood of infants and adults, Biochem. J. **45**: 464, 1949.
11. Jacobs, A.: Iron containing enzymes in buccal epithelium, Lancet **2**: 1331, 1961.
12. Herbert, D. and Pinsent, J.: Crystalline human erythrocyte catalase, Biochem. J. **43**: 203, 1948.
13. Takahara, S., Hamilton, H.B., Neel, J.V., Kobana, Y., Ogura, Y. and Nishimura, E.T.: Hypocatalassemia: A new genetic carrier state. J. Clin. Invest. **39**: 610, 1960.
14. Richardson, M., Huddleson, I.F. and Bethea, R.: Study of catalase in erythrocytes and bacteria. I. Procedure for the determination of the catalase activity of erythrocytes, Archives of Biochemistry and Biophysics **42**: 114, 1953.
15. Bonnichsen, R.K., Chance, B. and Theorell, H.: Catalase activity, Acta Chemica Scandinavia **1**: 685, 1947.
16. Summer, J.B. and Dounce, A.L.: Crystalline catalase, J. Biol. Chem. **121**: 417, 1937.
17. Schmid, R., Figen, J.F. and Schwartz, S.: Experimental porphyria. IV. Studies of liver catalase and other heme enzymes in sedormid porphyria, J. biol. Chem. **217**: 263, 1955.
18. Herbert, D. and Pinsent, J.: Crystalline bacterial catalase, Biochem. J. **43**: 193, 1948.
19. White, Handler, Smith and Stetten: Principles of Biochemistry, second 2nd edition New York. Mc-Graw-Hill Book Co., 1959.
20. Lemberg, R., Norrie, M. and Legge, J.W.: Catalase, Nature, London **144**: 551, 1939.
21. Theorell, H., Beznak, M., Bonnichsen, R., Paul, K.G. and Akeson, A.: On the distribution of infected radioactive iron in guinea pigs and its rate of appearance in some hemoproteins and ferritins, Acta Chemica Scandinavia **5**: 445, 1951.
22. Tarlow, A., Kellermeyer, R.W.: The hemolytic effect of primaquine. XI. Decreased catalase activity in primaquine sensitive erythrocytes, J. Lab. Clin. Med. **58**: 204, 1961.
23. Wyngaarden, J.B. and Howell, R.R.: Acatalasia in Stanbury, J.B., Wyngaarden, J.B. and Frederickson, D.S. (eds.), The Metabolic Basis of Inherited Disease, New York, The Blakistan Div., McGraw-Hill Co., Inc., 1960, pp. 1398-1415.

24. Hawkins, J.E. and Steenken, W., Jr.: Inhibition of catalase activity by isoniazid and the effect of Vit. B_6, Proc. Soc. Exp. Biol. and Med. **112**: 30, 1963.
25. Gerken, G. and Kampschmidt, R.F.: Studies on the effect of thyroxine administration on catalase activity, Endocrinology **67**: 839, 1960.
26. Adams, D.H.: Hormonal factors influencing catalase activity in mice: testicular and adrenal factors, Biochem. J. **50** 486, 1952.
27. Miller, L.L.: Changes in rat liver enzyme activity with inanition, Fed. Proc. **6**: 279, 1947.
28. Sass, M.D.: Catalase activity in young red cells, Nature, London **197**: 502, 1963.
29. Anselmino, K.J. and Hoffmann, F.: Uber den Glutathion und Katalasegehalt des fetalen Blutes und seine Bedeutung für die Sauerstoff-versorgung der Frucht, Archiv für Gynakologie, **143**: 505, 1931.
30. Jones, P.E. H. and McCance, R.A.: Enzyme activities in the blood of infants and adults. Biochem. J. **45**: 464, 1949.
31. Allison, A.C., Rees, W. and Burn, G.P.: Genetically controlled differences in catalase activity of dog erythrocytes, Nature, London **180**: 649, 1957.
32. Paul, K.G. and Engstedt, L.M.: Normal and abnormal blood catalase activity in adults, Scandinavian J. Clin. and Lab. Invest. **10**: 26, 1958.
33. Özsoylu, Ş.: Eritrosit katalaz aktivitesinin demir noksanlığı anemisi teşhis ve tedavisindeki değeri. Docentship Thesis, 1964.