# IRON ENZYMES IN IRON DEFICIENCY. II. CATALASE IN HUMAN ERYTHROCYTES

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In a previous paper we pointed out that while it was often assumed that the iron enzymes were "inviolate" in iron deficiency, this view was unsupported by any acceptable experimental evidence. Indeed, it was possible to demonstrate that in rats rendered iron deficient by a combination of bleeding and iron-poor diet, the cytochrome C content of the liver and kidneys was diminished markedly (1). As yet, these studies have not been extended to human subjects because of the difficulty in obtaining sufficiently large samples of cytochrome-containing tissues. However, another iron enzyme, the heme-protein complex catalase, is readily available for study in the red blood cells of patients, and it seems surprising that more attention has not been paid to this easily obtained and easily measured enzyme in the iron-deficiency state. A few measurements have been made of catalase activity in the organs of iron-deficient experimental animals and these have yielded contradictory results (2, 3). Studies in man have been limited in scope and have yielded difficult-to-interpret and inconclusive results. It is usually unclear whether the patients who were studied were iron-deficient or not (4–11). Furthermore, in nearly all instances the method of catalase estimation is clearly invalid; a catalase index was calculated by dividing the number of milligrams of peroxide destroyed in a fixed period of time by the red blood cell count. Since the destruction of $H_2O_2$ by catalase is a first order reaction, the quantity of $H_2O_2$ that has been decomposed at any given time is not a linear function of the amount of enzyme present. In addition, the incubation times used, up to two hours, were so long that virtually all the peroxide was destroyed, and the quantity of enzyme was no longer an important limiting factor in the reaction.

The study described in this paper was undertaken to clarify the effect of iron deficiency on catalase activity in the red blood cells of man.

## MATERIALS AND METHODS

*Patients.* All subjects studied were patients or laboratory personnel of The University of Chicago Clinics. The normal group was composed of five males and four females whose bone marrow showed the presence of adequate iron stores or who were regularly taking iron orally as prophylaxis against recurrence of iron-deficiency anemia. The diagnosis of iron-deficiency anemia in the other 11 subjects was based upon the usual clinical criteria.

*Blood samples.* Blood samples were obtained by venipuncture. Heparin was used as the anticoagulant. Red blood cell counts, hemoglobin determinations, and hematocrits were carried out on fresh blood samples, using conventional techniques. Freezing and storing did not influence catalase activity, and in a few instances, the samples were stored in the frozen state before the catalase activity was determined.

*Catalase activity determinations.* Catalase activity was measured according to the method of von Euler (12). Five ml. of 1:2,000 and 1:4,000 dilutions of whole blood in distilled water was added to flasks containing 45 ml. of 0.0078 N $H_2O_2$ in 0.03 M phosphate buffer at pH 6.8. All reagents were held at ice water temperature. The amount of peroxide remaining at three, six and nine minutes was measured by titration of an aliquot with permanganate after the reaction had been stopped with sulfuric acid. At the dilutions used, the reaction rate was found to be proportional to the amount of blood present. von Euler has employed the unit "Kat F" to designate the catalase activity per Gm. dry weight of enzyme preparation in this system. Since we were interested in the catalase activity as related, not to dry weight, but to volume of red cells, the number of red cells, and the hemoglobin content of the red cells, we have arbitrarily defined Kat $F_{(V)}$, Kat $F_{(RBC)}$ and Kat $F_{(Hb)}$ as the catalase activity of 1 ml. of red cells, $10^{10}$ red cells, and 1 Gm. hemoglobin, respectively, acting in a 50 ml. volume. Plasma is devoid of catalase activity and does not potentiate or inhibit the enzymatic activity of red cells. It is therefore ignored in these calculations. Base 10 logarithms have been used in determining the reaction constant.

## RESULTS

The catalase activity of red cells from normal and iron-deficient subjects is presented in Figures

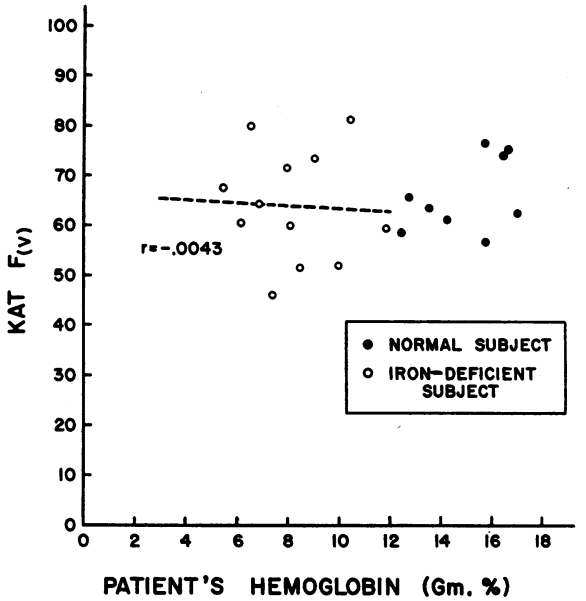


FIG. 1. THE RELATIONSHIP BETWEEN THE PATIENT'S HEMOGLOBIN LEVEL AND CATALASE ACTIVITY PER MILLILITER OF RED CELLS

1 through 3. It is apparent that the catalase activity per ml. of red cells was essentially the same in normal and iron-deficient subjects. This was true regardless of the severity of the anemia as indicated by the patient's hemoglobin level (correlation coefficient equals − 0.0043). The mean catalase activity of normal red cells was 65.9 ± 2.5 [1] Kat $F_{(V)}$ units while that of iron-deficient red cells was 64.0 ± 3.2 [1] Kat $F_{(V)}$ units. When expressed as catalase activity per $10^{10}$ red cells, however, the catalase activity of the red cells of the iron-deficient subjects was somewhat reduced. The degree of reduction of catalase activity was related to some extent to the severity of the iron deficiency as judged by the patient's hemoglobin level (correlation coefficient equals + 0.20). This is simply a reflection of the microcytosis of the red cells in iron deficiency. The mean catalase of normal red cells was 60.4 ± 2.1 [1] Kat $F_{(RBC)}$ units while that of iron-deficient cells was 54.8 ± 3.0 [1] Kat $F_{(RBC)}$ units. When the catalase activity was expressed as activity per Gm. of hemoglobin, a substantial increase in catalase activity in the iron-deficient as compared with the normal group was observed. The increase was inversely proportional to the hemoglobin level of the patient's blood (correlation coefficient equals − 0.60). This finding is a reflection of the reduction of hemoglobin concentration of the red cells which occurs in iron-deficiency anemia. The mean catalase activity of normal red cells was 198.2 ± 6.8 [1] Kat $F_{(Hb)}$ units while that of iron-deficient red cells was 241.4 ± 13.0 [1] Kat $F_{(Hb)}$ units.

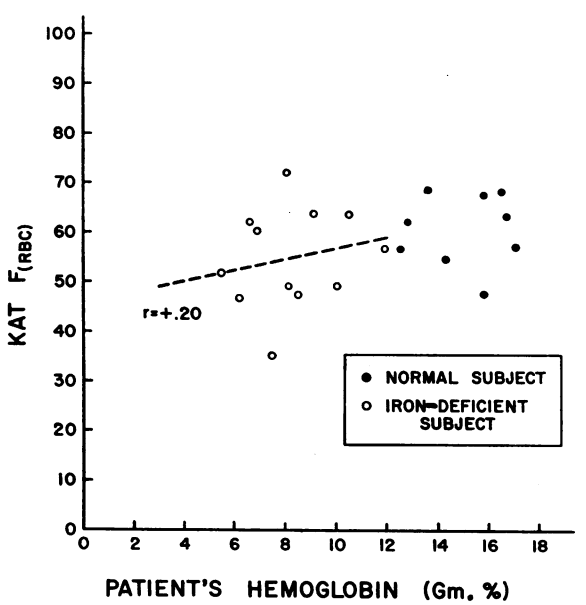


FIG. 2. THE RELATIONSHIP BETWEEN THE PATIENT'S HEMOGLOBIN LEVEL AND CATALASE ACTIVITY PER $10^{10}$ RBC

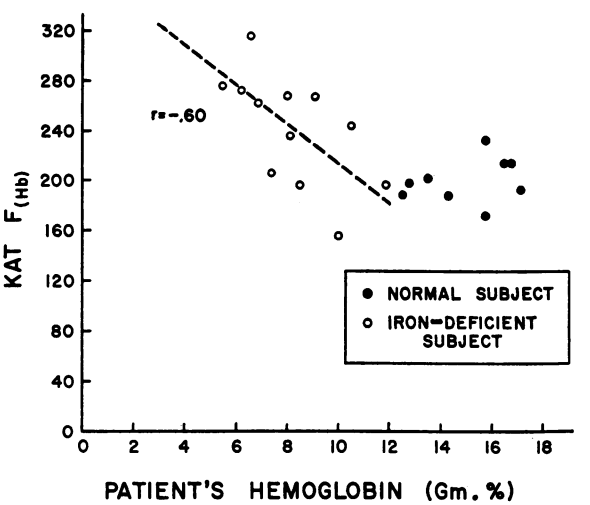


FIG. 3. THE RELATIONSHIP BETWEEN THE PATIENT'S HEMOGLOBIN LEVEL AND CATALASE ACTIVITY PER GM. OF HEMOGLOBIN

No significant sex difference in red cell catalase

[1] One standard error.

activity was observed in the small number of samples that was studied.

## DISCUSSION

It is apparent from these studies that even under conditions of severe iron need, the concentration of catalase in human red cells remains normal. If it is true, as has been suggested by Theorell, Beznak, Bonnichsen, Paul, and Akeson (13), that catalase and hemoglobin derive their heme groups from a common source, then it would appear that the catalase synthesizing mechanism has more success in competing for the limited amount of available heme than has the hemoglobin synthesizing mechanism. This would appear to be the reverse of the situation with cytochrome C, where it has been shown that the hemoglobin of iron-deficient animals returns to normal values while the cytochrome C content of liver and kidney is still very low (1).

## SUMMARY

The catalase activity of the red cells of 9 normal subjects and 11 subjects with iron-deficiency anemia has been determined. Iron deficiency produced no change in the catalase activity per milliliter of red cells. Therefore, there was a slight decrease in catalase activity per $10^{10}$ red cells and a well marked increase in catalase activity per Gm. of red cell hemoglobin, reflecting the microcytosis and hypochromasia which occur in iron-deficiency anemia. These findings indicate that the mechanism synthesizing red cell catalase is more successful in competing for a limited quantity of iron than is the hemoglobin synthesizing mechanism, when only a small quantity of iron is available.

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