Effects of Iron Deficiency on Blood Level of Sulfhydryl Compounds and on Structure of Gastric Mucosa in Rats

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The effect of iron deficiency on the sulfhydryl compounds in blood and on the structure of gastric mucosa of rats was experimentally studied. Iron-deficient rats exhibited lower values of sulfhydryl compounds of blood than those of iron supplemented controls. Significant degenerative changes were found in parietal cells of the stomach from iron deficient animals.

Ling and Chow\(^1\) reported that vitamin B\(_{12}\) deficiency in rats caused marked diminution of soluble sulfhydryl compounds, principally glutathione, in blood cells and that iron deficiency, unlike vitamin B\(_{12}\) deficiency, did not influence the levels of soluble sulfhydryl compounds. Johnston and Bloch\(^2\) demonstrated that the formation of glutathione in the liver was affected by supply of ATP. In rats with iron deficiency a decreased activity of many oxygen carrying enzymes and impaired synthesis of glutathione were reported by Cusack and Brown.\(^3\)

It has been indicated that achlorhydria and chronic gastritis are unusually frequent in patients with iron deficiency anemia. Although many writers notice the association of gastric abnormalities with iron deficiency in man, it is rather surprising that there have been no reports of functional or structural changes of the stomach in rat with iron deficiency except that of Valberg et al.,\(^4\) in which no structural changes were described in gastric mucosa from iron-deficient rats.

In this communication, the results of a study are presented of the levels of soluble sulfhydryl compounds in the blood of iron-deficient rats and those fed on iron supplemented diet. Also, the effect of iron deficiency on the osmotic fragility of erythrocytes and on epithelial cells of the stomach was reported.

**Experimental**

*Animals and diets.* Iron deficient basal diet contained either 6 or 18% of casein, soybean oil in 6%, salts (excluding iron) in 4%,\(^2\) cellulose in 2%, vitamin mixture\(^4\) in 1%, DL-methionine in 0.2% and glucose to make 100%. The corresponding control diet differed only in iron content. Four kinds of diet were given *ad libitum* to rats of 4 groups. Forty female weanling rats of the Wistar strain were divided into 4 groups of 10 each and were housed in individual cages made of stainless steel wire equipped with bottles of distilled water.

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Hemoglobin. The tail of the rat was warmed in water and wiped clean and dry; the dorsal vein was cut with a scalpel, and blood was drawn directly in a calibrated pipette. Hemoglobin concentration in blood was determined by the method of King.6

Sulfhydryl compound. Blood levels of sulfhydryl compounds were determined by the 5,5'-dithiobis 2-nitrobenzoic acid method of Ellman.7

Histological studies. Histological study was made of transverse sections from the glandular portion of the stomach. The specimens were fixed and the sections were stained by standard procedures with hematoxylin-eosin.

Osmotic fragility test. The liability to hemolysis was measured by the method of Prankerd8 with some modification. Blood cells were washed with 0.9% NaCl solution. After they were incubated for 24 hours in hypotonic solutions of NaCl, they were centrifuged and hemoglobin was determined in the supernatant.

RESULTS

Growth. The weight gain of animals of deficiency groups was apparently lower than that of those in supplemented groups both fed on 6 and 18% casein diet. The eyes of deficiency animals showed a transparent pallor and their incisor teeth were pearly white in color.

Hemoglobin. In 74 days hemoglobin concentration of deficiency rats fed on 6 or 18% casein diet dropped sharply and reached values of 3.1 or 4.1 g per 100 ml of flood, respectively (Table 1).

| TABLE 1. Effect of iron on hemoglobin, sulfhydryl compounds in blood and hypotonic fragility of erythrocyte |
|-------------|-----------------|-----------------|-----------------|-----------------|
| Day of exp. | 6% casein, + Fe | 6% casein, − Fe | 18% casein, + Fe | 18% casein, − Fe |
| 46          | 11.3±1.2        | 6.1±0.8         | 12.9±1.1        | 5.1±0.6         |
| 61          | 11.8±1.2        | 4.1±0.7         | 10.9±1.8        | 4.1±0.7         |
| 88          | 10.1±2.9        | 3.1±0.3         | 10.2±2.0        | 3.7±0.7         |
| 106         | 10.8±3.3        | 3.4±0.3         | 11.9±2.9        | 4.2±0.6         |
| Avg wt on 102 day, g | 176.4         | 164.3          | 205.7          | 184.8          |
| NaCl (%)    | 0.55            | 0.9±1.5        | 45.4±15.2       | 9.9±1.5        | 29.4±8.5     |
|             | 0.50            | 21.0±4.2       | 65.0±6.9       | 17.9±3.8       | 37.3±15.9   |
|             | 0.40            | 52.1±12.5     | 82.8±9.3       | 45.0±12.9     | 50.5±27.6   |

* Standard deviation of the mean.
† Figures represent percentage of cell hemolyzed.
Sulfhydryl compounds. Table 1 shows that the iron deficient rats, fed on 6 or 18% casein diet, had much lower sulfhydryl compounds in the blood than animals fed on iron supplemented diet. The sulfhydryl content seems to be roughly parallel to the content of hemoglobin.

Fig. 1. (A) Histologic findings of gastric mucous membrane of an iron supplemented rat. (18% casein).
(B) Histologic findings of gastric mucous membrane of an iron-deficient rat. Atrophy of parietal cells is seen. (18% casein). Hematoxylin-eosin stain. 10×40.
Osmotic fragility of erythrocyte. The data presented in the table show that the erythrocyte of the deficient animals was liable to hypotonic hemolysis when compared with that of control animals.

Gastric mucosa. Microscopic examination of the gastric mucosa of the deficiency groups both on 6 and 18% casein diet revealed degenerative changes in the tubular glands and especially in chief cells. As shown in Fig. 1, the gastric mucosa of the iron deficient group on 18% casein showed a change in parietal cells indicating a hypoactivity. On 6% casein and iron deficient diet the degenerative changes were complicated by the change caused by protein deficiency in addition to iron deficiency. Degenerative changes were seen in the stomach of rats fed protein deficient and iron supplemented diet.

DISCUSSION

Contrary to the findings of Ling and Chow\(^1\) we found a decreased amount of sulfhydryl compounds in blood of iron deficient rats irrespective of protein levels in the diet. The importance of soluble sulfhydryl compounds in relation to enzyme activity, cellular growth, detoxication, protection against radiation injuries, and oxidation-reduction in animal body has been recognized by numerous investigators and discussed by Barron.\(^9\) The formation of glutathione in the liver was said to be affected by the supply of ATP. So the present finding that the sulfhydryl compounds in blood have decreased in iron deficient animals is of interest.

Sulfhydryl compounds are known to be important for keeping stable the membrane permeability. Le Fevre\(^10\) found that the permeability of the red cell to glycerol was depressed on addition of mercuric salts, iodine, and p-chloromercuribenzoic acid; reversal of inhibition and protection was achieved on addition of cysteine or glutathione. Thus the increased hypotonic fragility of erythrocytes in iron deficient rats in the present study might be explained by the decrease of sulfhydryl compounds in the red cells.

The present authors observed a degenerative change in the glandular structure especially of parietal cells. Valberg et al.\(^4\) found no evidence of gastritis or gastric atrophy, and no change in the appearance of the parietal or chief cells in the deficient animal. The disparity is possibly due to the extent of severity of iron-deficiency. The mean hemoglobin concentration of the iron-deficient groups of their experiment remained between 6 and 9 g per 100 ml throughout 220 days of the experiment. In the present experiment the hemoglobin concentration decreased to 4 g per 100 ml after 61 days. In this connection, it is noteworthy that in the case of nicotinic acid deficiency in rats Koyanagi and Yamada\(^11\) found hyperplasia of gastric mucosa and an increase in the number of acid secreting cells after 5 months on deficient diet.
Acknowledgment

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References

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