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Catecholamines in adult iron deficiency patients[1]

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Summary. Patients with iron deficiency whether uncomplicated or associated with other types of anemias, had plasma catecholamine levels which were significantly increased above normal controls. Patients with a variety of other anemias had no significant increase in catecholamine levels. Plasma catecholamine levels in uncomplicated iron deficient patients approached normal values as early as 3 h following oral $FeSO_4$.

Catecholamines are thought to play a role in influencing human behavior, subsequently, any increase in catecholamines may contribute to determining behavioral symptoms such as are manifested by iron deficient patients, i.e., irritability, inattention, and decreased ability to concentrate.

The relationship between catecholamines and iron deficiency has recently been explored. Voorhess et al.[3] noted that urinary excretion or norepinephrine was elevated in children with iron deficiency.

Therefore, we felt it appropriate to investigate plasma catecholamine levels in adult patients with various types of anemias. In the present study, we report that patients with iron deficiency, whether uncomplicated or associated with other anemias, had plasma catecholamine levels that were significantly increased above normal controls. However, patients with a variety of other anemias had no significant increase in catecholamine levels.

Patients selected with iron deficiency were required to demonstrate morphological changes, i.e., hypochromasia, microcytosis, decreased serum iron, increased iron binding capacity, and absent bone marrow iron as determined by Prussian blue stain. Additionally, an increase in hematocrit following oral iron therapy was noted. Patients with iron deficiency co-existing with other diseases were diagnosed by the absence of bone marrow iron. Patients with other types of anemias, such as chronic simple anemia, hemolytic anemias, Thalassemia minor, had normal bone marrow iron stores. Epinephrine and norepinephrine were determined employing a fluorometric procedure by Haggendal[4] with certain modifications: 1. sodium thiosulfate was used as an antioxidant, and 2. dithiothreitol as a stabilizer. Plasma catecholamines were expressed as µg/l.

Table 1 demonstrated the serum iron, binding capacity and catecholamine levels in various anemias studied. Uncomplicated iron deficiency patients had a mean value of

Table 1. Serum iron, iron binding capacity, and plasma catecholamine values in anemia

Number	Patients and diseases	Catecholamines µg/l Norepinephrine	Epinephrine	Iron (µg/dl)	Total iron binding capacity (µg/dl)
10	Normal	0.5 ±0.5	0.3 ±2.0	120±60	340±80
14	Fe deficient	2.35±1.56	2.55±1.76	42±29	388±80
11	Fe deficient (with other disease)	3.80±2.21	2.80±1.97	54±44	299±72
13	Other anemias	1.53±1.36	0.91±1.00	82±41	297±70

Units expressed as ±1 SD.

Table 2. Plasma catecholamine levels before and after treatment with 600 mg of ferrous sulfate

Iron deficient patients	Catecholamines (before treatment) Norepinephrine (µg/l)	Epinephrine (µg/l)	Catecholamines (after treatment) Norepinephrine (µg/l)	Epinephrine (µg/l)
1	2.1	5.2	0.4	1.9*
2	5.1	3.3	1.5	0.7**
3	1.2	0.9	0.0	0.3***
4	2.3	1.6	0.6	0.2***
5	3.0	5.1	0.6	0.0***
6	1.9	1.1	0.8	0.3*

* 3 h post-treatment; ** 24 h post-treatment; *** 72 h post-treatment.

2.35±1.56 for norepinephrine and 2.5±1.76 for epinephrine. These values are significantly different from the normal with a $p < 0.0005$. Serum iron in this group of patients demonstrated a positive correlation co-efficient of −2.50 with the plasma catecholamines level. There is no correlation between the TIBC and plasma catecholamines. Patients with iron deficiency associated with other diseases, also showed a significant increase in catecholamine levels, 3.8±2.2 for norepinephrine and 2.80±1.9 for epinephrine, with a p value < 0.0005. However, there is no significant correlation co-efficient for the serum iron and catecholamine levels in this group of patients. Catecholamine levels for a heterogeneous group of anemias, non-iron deficient, were not significantly different from control values.

In 6 uncomplicated iron deficient patients following 600 mg of oral ferrosulfate, catecholamine levels were measured and described in table 2. Both levels of norepinephrine and epinephrine fell significantly and approached normal values in all patients studied within 3 days. In 1 patient a response was seen following 3 h after oral iron administration.

The present study demonstrated a significant increase in plasma epinephrine and norepinephrine levels in adult iron deficient patients whether or not their iron deficiency was complicated by other diseases. Furthermore, following 600 mg of oral iron, catecholamine levels promptly fell as early as 3 h following ingestion and returned to normal by 24 h. In contrast, normal catecholamine levels were determined in patients with other types of anemias. These findings are consistent with those reported by Voorhess et al.[3] who found increased urinary excretion of norepinephrine in iron deficient children. However, the present data does not support the hypothesis that elevated catecholamine levels in iron deficiency is due to a decrease in monoamine oxidase levels (MAO). Youdim[5] determined that iron was necessary for the operation of a functional monoamine oxidase and regenerated after 6 days of iron therapy[6]. Thus, it does not appear that the MAO level alone could explain the decrease in catecholamines seen in patients with iron deficiency anemia.

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Attempts to use carbodiimide (EDCI) to cross-link hemoglobin for transfusions

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Summary. The polymerization of hemoglobin for use as a blood substitute and an oxygen carrier would be of interest because high-mol.wt macromolecules would have a longer vascular retention time than the monomer. We found that the molecules resulting from the treatment of hemoglobin with ethyldimethylaminopropylcarbodiimide did not have a higher mol.wt than free hemoglobin and also had a dissociation curve resembling that of monomers, but seemed more stable.

The use of hemoglobin solutions as a blood substitute has been suggested frequently since the work of Rabiner[1]. Although it is known that free hemoglobin can transport and release oxygen, the low mol.wt and the poor viscosity of the solutions gives them a very short half-life. We have emphasized the care necessary for the storage of preparations free of methemoglobin[2] and demonstrated the changes the pigment undergoes in the transfusion apparatus, especially at interfaces[3]. We have also reported the value of providing a protein environment for hemoglobin and suggested that it could be associated or coupled with other macromolecules to improve its stability and vascular retention.

Attempts to polymerize hemoglobin for use in transfusions have so far been few and limited, since the only reagents tested so far have been diimidates[4,5], while the heme-oxygen relations were mentioned only in 1977 by Horowitz[5]. Therefore, we have tried to 'polymerize' hemoglobin using a carbodiimide and to study the properties of the product obtained: mol.wt as determined by gel-chromatography and analytical ultracentrifugation, viscosity, electrophoretic mobility, isoelectric point, stability, dissociation curve, p 50 and oxygen-binding capacity.

Materials and methods. 1. The hemoglobin solution was prepared according to a method derived from that of Rabiner[2], dialyzed, and kept at −20 °C. The 'polymerization' was performed with magnetic stirring for 3 h at 4 °C and at the pH of the solution (about 6.5), by the addition of powdered ethyldimethylaminopropylcarbodiimide (Fluka) in a 150- to 250-fold excess over the amount theoretically necessary to establish a single peptide bond. The reacted solution was then dialyzed against distilled water for 30 min at 4 °C.

2. The effect of the reagent on the hemoglobin was tested by gel-chromatography (7 runs on an ACA 44 LKB in a 90×2.6-cm column with 0.05 M Tris-HCl buffer, pH 8, flowing at 0.23 ml/min and at 4 °C; the OD was read at 280 nm) and analytical ultracentrifugation (at 20 °C in an

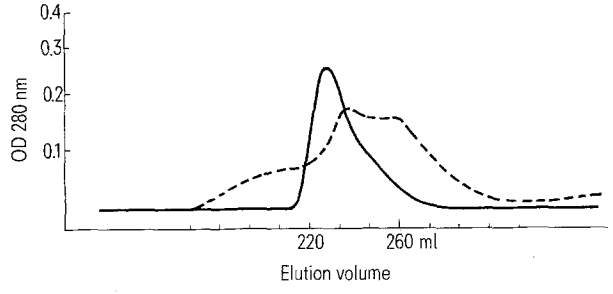

Fig. 1. Gel-chromatography on ACA 44 of 'free' hemoglobin (—) and after treatment with EDCI (– – –). OD at 280 nm is a function of elution volume of Hb in ml.