BRIEF REPORT

Plasma Concentrations of Hemopexin, Haptoglobin and Heme in Patients with Various Hemolytic Diseases

By U. MULLER-EBERHARD, J. JAVID, H. H. LIEM, A. HANSTEIN and M. HANNA

CURRENT CONCEPTS regarding the disposal of plasma hemoglobin, derived from the intravascular breakdown of erythrocytes, are summarized in the scheme shown below. (Fig. 1) Hemoglobin (Hb), whether present as oxy-Hb or met-Hb, can interact with haptoglobin (Hp) to form a complex that is subsequently removed from the circulation. Hb in excess of that which can be accommodated by Hp in the plasma is also cleared from the circulation; only part of this uncomplexed Hb is found in the urine. Both Hb and the Hb-Hp complex are degraded in the reticuloendothelial system. In states of excessive hemolysis, part of the Hb dissociates into its components, heme and globin, and the heme complexes with both albumin (Alb) and hemopexin (Hx); globin, as well as Hb, is bound by Hp. Still unknown is how and where heme-Alb, heme-Hx and globin-Hp complexes are eliminated from the circulation.

The plasmas of patients with a variety of severe hematologic diseases have subnormal concentrations of both Hp and Hx. Under these conditions, substantial levels of heme-Alb may also be found. The present report correlates...
Fig. 1.—In vivo complex formations of hemoglobin or parts thereof (Hb-Hp, Heme-Alb and Heme-Hx); the globin-Hp complex has not been shown as yet in vivo.

Hx concentration, hemoglobin-binding capacity (an indirect measure of the concentration of Hp) and heme concentration in patients with various hemolytic anemias.

MATERIALS AND METHODS

Diagnosis for the 17 patients studied were: thalassemia major (3), sickle cell disease (5), sickle cell trait (3), sickle cell-Hb C disease (3) and spherocytosis (3). The 18 patients in whom only Hx and Hp levels were analyzed had paroxysmal nocturnal hemoglobinuria (PNH) (6), auto-immune hemolytic anemia (AHA) (4), myoglobinuria (1), erythropoietic protoporphyria (1), thalassemia-Hb E disease (1), sickle cell-Hb C disease (1), sickle cell disease (1) and spherocytosis (3). Only individuals over 3 years old were studied since Hp and Hx concentrations in children below one year of age are considerably lower than those of adults.

The plasmas were collected as described by Shinowara et al., and the Hx concentra-
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Fig. 3.—Haptoglobin and hemopexin concentrations for 31 determinations on 18 patients with hemolytic diseases. △, thalassemia major; ▲, sickle cell anemia; ◊, sickle cell trait; ■, sickle cell-hemoglobin C disease; ○, spherocytosis; □, paroxysmal nocturnal hemoglobinuria; ●, autoimmune hemolytic anemia; and ◆, erythropoietic protoporphyria; ◇, myoglobinuria; ○, thalassemia-hemoglobin E disease; □, pyruvate kinase deficiency and severe hemolytic anemia. The hatched area indicates the range of haptoglobin and hemopexin concentrations obtained for healthy adults.

... and the hemoglobin-binding capacity were determined either on fresh plasmas or on aliquots that had been deep-frozen immediately after separation of the cells. Only freshly-obtained samples were assayed for heme concentrations by a modification of the method described by Shinowara et al. utilizing as a standard the recovery of added amounts of heme to normal plasma; heme was determined by two independent technics.12-13

RESULTS

The relationship between Hx concentration and the level of heme in the plasmas is shown in Figure 2. Heme concentrations above 6.0 μg/ml. were invariably associated with very low Hx values. There was no similar relationship between Hp concentration and heme. Figure 3, however, shows a correlation between Hx and Hp levels. In almost all instances of hematologic disease, values of both were lower than those for normal individuals. It is thus evident that in the plasma of patients with sickle cell anemia, sickle cell-Hb C disease or thalassemia major, both proteins are usually equally depleted. On the other hand, patients with PNH, and especially those with AHA had markedly reduced concentrations of Hp while Hx levels were normal or moderately diminished. The three patients with AHA, whose Hp levels were below 140 μg./ml., had reticulocyte counts ranging from 23 to 70 per...
cent, and a strongly positive direct (3 patients) and indirect (2 patients) Coombs' reaction. Serial determinations of Hp and Hx were performed on one of the patients with hereditary spherocytosis before and after splenectomy. Before splenectomy, the Hx levels on each of two occasions were 400 \( \mu g./ml \) and the corresponding Hp values were 150 and 100 \( \mu g./ml \). On the fourth, eighth and twelfth day after splenectomy, the Hp level rose to 450, 700 and 600 \( \mu g./ml \) while the Hx concentrations remained relatively stable at 400, 500 and 400 \( \mu g./ml \).

**DISCUSSION**

Results obtained from this study demonstrate that high plasma concentrations of heme in patients with hemolytic anemia are invariably associated with low levels of Hx. When calculated on an equimolar ratio,\(^1\) a plasma Hx concentration of 770 \( \mu g./ml \) corresponds to a heme concentration of 6.3 \( \mu g./ml \). Therefore, assuming that all heme-Hx complex is removed from the circulation, Hx should be depleted when concentrations of heme exceed 6 \( \mu g./ml \). Complete disappearance of Hx was never observed, and this may be indicative of efficient recycling or a rapid de novo synthesis of this protein. The moderately reduced Hx levels in both PNH and AHA may also reflect a rapid synthesis of Hx in a situation where heme accumulation is not excessive.

In a similar study, Sears\(^2\) found that Hx was depleted only when Hp was absent and a substantial amount of heme was present. Similarly, no instance was encountered in the present investigation in which the Hx concentration was diminished without a simultaneous reduction of Hp level, although the converse was frequently observed. The consistent finding of only moderately reduced Hx levels, in spite of low concentrations of Hp in both PNH and AHA patients, is of interest although presently unexplained. The amount of intravascularly liberated Hb, which causes the depletion of Hp, may not be the only factor responsible for elevation of plasma heme levels and subsequent depletion of Hx. It can be concluded that the determination of plasma Hx levels appears to be a useful parameter in the investigation of hemolytic states since a depletion of this protein is a reflection of high concentrations of heme.

**SUMMARY**

Plasma concentrations of hemopexin, haptoglobin and heme were determined in patients with various hemolytic diseases. Diminished concentrations of hemopexin were found only when the concentrations of haptoglobin were decreased; the former were not in all instances lowered to the same extent as the haptoglobin levels. Invariably, high concentrations of heme were associated with low concentrations of hemopexin.
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ADDENDUM

In a patient with hemorrhagic pancreatitis, Sears encountered a situation of Hx depletion with normal Hp levels.

REFERENCES

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