Depletion of Plasma Hemopexin in Man by Hematin Injections* (33880)

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Hemopexin is a β_1-globulin that binds heme and is present in normal human plasma (1). Its level is reduced in certain patients with hemolytic disease (2–5), and in recent studies, its depletion was correlated with the degree of intravascular hemolysis or, more specifically, the plasma heme pigment level (4). From these latter studies it was postulated that plasma hemopexin is depleted in hemolytic disease due to its binding of heme which occurs when free hemoglobin exists in the plasma after haptoglobin depletion. There is no direct evidence in man for this hypothesis, but plasma hemopexin levels were diminished in rhesus monkeys following hematin injections (6). The present study was undertaken to determine the effect of repeated hematin injections on plasma hemopexin levels in man.

Materials and Methods. The subject was a healthy, hematologically-normal adult male. Recrystallized hemin[1] was sterilized in an autoclave and dissolved at a concentration of 10 mg/ml in sterile 0.1 *M* sodium carbonate just prior to use. This alkaline hematin solution was injected intravenously in a dose of approximately 1 mg of hemin/kg of body weight daily for 7 days.[2] Blood samples were drawn prior to each injection and then at intervals for a total period of 23 days. The blood, anticoagulated with 1-2 units of heparin per ml, was centrifuged immediately and the plasma frozen for later heme pigment studies. The methods of heme pigment quantification and minding have been described previously (4). They included measurement of total heme pigment levels by a benzidine method and separation and quantification of individual heme-proteins by electrophoresis on cellulose polyacetate, *o*-dianisidine staining, and densitometric scanning. Plasma hemopexin levels were assessed by an electrophoretic method and by immunodiffusion as previously described (4). Plasma bilirubin concentrations were determined by a modification of the method of Malloy and Evelyn (7).

Results. The results are depicted in Fig. 1. The plasma samples were obtained just prior to each dose of hematin or approximately 24 hr after each previous dose. Section A of Fig. 1 shows the gradual accumulation of heme in the plasma, most of which was bound to albumin. The difference between the values for methemalbumin and total heme pigment is the amount of hemopexin–heme plus any haptoglobin–hemoglobin present since these two heme-proteins were not separated by the electrophoretic techniques employed. After two hematin injections the difference was very small and well within the levels of haptoglobin–hemoglobin found in normal plasma in our laboratory, *i.e.*, under 5 mg/100 ml. In a plasma sample (not shown in Fig. 1) taken 15 min after the first dose of hematin, the total heme pigment level was 47.0 mg/100 ml of which only 26.3 mg/100 ml was methemalbumin. The remainder was hemopexin–heme plus any haptoglobin–hemoglobin present. Thus approximately 40% of the injected heme was bound to hemopexin initially. After the last hematin injection, plasma heme pigment levels returned slowly to normal, detectable plasma levels of methemalbumin persisting for 5 days.

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[1] Obtained from Eastman Organic Chemicals, Rochester, New York

[2] The seventh dose was recrystallized radioactive hematin prepared from the erythrocytes of a rabbit previously injected with ^{59}Fe. This was done as part of a study of heme clearance that will be reported.

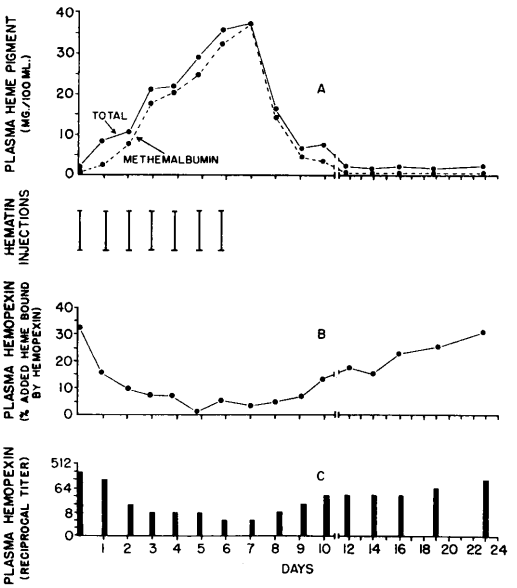

FIG. 1. Effects of repeated intravenous injections of hematin (approx 1 mg/kg of body wt) in a normal man: heme pigment concentrations are expressed in terms of oxyhemoglobin which was the standard used.

In Fig. 1B and C are shown the plasma hemopexin levels as measured semiquantitatively by the two methods described. Figure 1B depicts the percentage of added heme bound to hemopexin after incubation of plasma samples *in vitro* with amounts of heme that raised the plasma heme pigment level by 40–50 mg/100 ml (expressed as hemoglobin). Figure 1C shows the plasma hemopexin titer, that is the highest plasma dilution which produced a precipitin line after double diffusion in agar gel against a rabbit antiserum to human hemopexin.[3] By both methods a rapid decline in plasma hemopexin to very low levels is demonstrated with slow return to base-line values after hematin injections were discontinued. In normal subjects 13–40% of added heme is bound to hemopexin under these conditions, and normal plasma hemopexin titers are 1:128 or higher (4). The subject's normal plasma haptoglobin level (hemoglobin-binding capacity) was unchanged after hematin injection.

Plasma bilirubin determinations done on days 0, 1, 3, 5, 6, 7, and 8 showed no significant changes from base-line concentrations of total, conjugated, or unconjugated bilirubin.

Discussion. Estimates of the amount of hemopexin present in normal plasma have been in the range of 75–100 mg/100 ml (1, 3, 8). Assuming the lower concentration value, a molecular weight for hemopexin of 80,000 and a 1:1 molar binding ratio (1), normal plasma contains sufficient hemopexin to complex approximately 0.6 mg of heme/100 ml (or about 16 mg/100 ml if the heme is expressed as hemoglobin). The injections of 1 mg of hemin/kg of body weight in this study therefore provided several times as much heme as was necessary to bind all the plasma hemopexin. The amount actually complexed with hemopexin by electrophoresis of the plasma taken 15 min after the first injection was very close to the above value for saturation of hemopexin, so it can be assumed that all hemopexin was bound by the hematin injections employed.

The depletion of hemopexin following hematin injections in this study could have occurred either due to inhibition of synthesis or by enhancement of clearance of the protein from the plasma. The latter explanation seems more reasonable. Hemopexin depletion due to binding of heme may be analogous to the depletion of haptoglobin by binding hemoglobin. As the half-life of haptoglobin in the plasma is decreased when it complexes with hemoglobin (9), so the survival of hemopexin is probably foreshortened when it binds heme. As with haptoglobin this may be due to removal of the hemopexin–heme complex from the plasma as a unit, though this remains to be demonstrated directly. These observations lend credence to the hypothesis that hemopexin is depleted in hemolytic disease as a consequence of its binding of heme (4). The failure to eliminate plasma hemopexin completely in this study is most likely a reflection of the synthetic capacity for the protein, though the possibilities of recycling or mobilization from extravascular sites cannot be ruled out.

Summary. Repeated intravenous injections of hematin in a normal human subject produced depletion of the plasma heme-binding

[3] Obtained from Certified Blood Donor Service, Inc., Woodbury, N. Y.

β-globulin, hemopexin. The observations provide support for the hypothesis that hemopexin depletion in hemolytic disease is due to its binding of heme and consequent shortened survival in the circulation.

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1. Heide, K., Haupt, H., Störiko, K., and Schultze, H. E., Clin. Chim. Acta **10**, 460 (1964).
2. Muller-Eberhard, U. and Cleve, H., Nature **197**, 602 (1963).
3. Hanstein, A. and Muller-Eberhard, U., J. Lab. Clin. Med. **71**, 232 (1968).
4. Sears, D. A., J. Lab. Clin. Med. **71**, 484 (1968).
5. Muller-Eberhard, U., Javid, J., Liem, H. H., Hanstein, A., and Hanna, M., Blood **32**, 811 (1968).
6. Sears, D. A. and Huser, H-J., Proc. Soc. Exptl. Biol. Med. **121**, 111 (1966).
7. Malloy, H. T. and Evelyn, K. A., J. Biol. Chem. **119**, 481 (1937).
8. Augener, W. Protides Biol. Fluids **12**, 363 (1964).
9. Freeman, T., Protides Biol. Fluids **12**, 344 (1964).

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Effects of Intraluminal Pressure in the Colon on its Vascular Pressure-Flow Relationships* (33881)

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Distension of the small intestine has been shown to result in increased intraluminal pressure and resistance of blood flow (1). Welsh (2) concluded that mucosal blood flow in the colon is increased during periods of increased motility. Other studies showed that total colon blood flow is decreased during rhythmic contraction and by distension of the colon (3). Arterial pressure-flow studies have given evidence of autoregulation of blood flow in the small intestine (4) and colon (5). Elevation of venous pressure results in increased resistance in the intestine and the responses are abolished by the infusion of papaverine (5, 6). In the present paper data are given on the effects of distension of the colon on these vascular responses to changes in arterial or venous pressure.

Methods. Dogs weighing 20–25 kg were anesthetized with pentobarbital (30 mg/kg, iv). The colon was exposed and freed of its mesenteric attachments. Heparin (500 units /kg, iv) was given. Then a second dog to serve as a perfusion donor was anesthetized and heparinized. A femoral artery and vein were cannulated. The caudal mesenteric artery and colic vein of the exposed colon were then cannulated with PE tubing. The former was joined to the femoral artery cannula of the perfusion donor, the segment of gut quickly was excised and perfusion was started. Venous outflow was collected in a reservoir and returned by way of the femoral vein cannula. One-hole stoppers were tied into either end of the segment. Arterial perfusion, venous, and intraluminal pressures were recorded. Arterial inflow was measured with a Biotronex electromagnetic flowmeter. Arterial pressure was varied by means of an occlusive clamp on the perfusion tubing and venous pressure by changing the position of the outflow orifice. The preparations were distended with saline and intraluminal pressure was maintained at 50 mm Hg. Data are

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