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Effect of Hemoglobin and Hematin on Plasma Clearance of Hemopexin, Photo-Inactivated Hemopexin and Albumin[1] (38575)

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Two serum proteins bind heme strongly; albumin, Kd $\sim 10^{-7}$ *M* (1), and hemopexin, Kd $\ll 10^{-8}$ *M* (2–4). During intravascular hemolysis or after intravenous heme injection, serum levels of hemopexin decrease (5–11). Heme–hemopexin, hemoglobin–haptoglobin and hemoglobin, but not methemalbumin, enter and are catabolized in the liver parenchymal cells (12–15). This communication describes the effect of intravenous administration of hematin,[4] hemoglobin or methemoglobin on the plasma half-clearance time ($T_{1/2}$) of albumin, hemopexin and photoinactivated hemopexin (16) in the rabbit.

Materials and Methods. Animals and proteins. Rabbit hemopexin was purified as previously reported (17) and rabbit albumin by zone electrophoresis (18). Both proteins were iodinated through the courtesy of Dr. F. J. Dixon, Jr. (19). The specific activity of ^{125}I-hemopexin and ^{131}I-albumin was 5–8 $\times$ 10^8 dpm/mg protein. New Zealand white rabbits, weighing 2.8–3.2 kg, were injected with 1.4–2.8 $\times$ 10^8 dpm. The rose bengal mediated photoinactivation of rabbit hemopexin is described elsewhere (16).

Heme and hemoglobin. Heme (Eastman Organic Chemicals) was dissolved and measured as reported (10). Hemoglobin and methemoglobin solutions were prepared as follows: Erythrocytes from heparinized rabbit blood were washed three times in cold physiological saline, pH 7.4. Red cells were then lysed by freezing at −20° for 24 hr, followed by thawing in a 37° waterbath. Chloroform was added to the lysate (1 part chloroform to 16 parts lysate) and the solution centrifuged at 600*g* for 45 min at 4°. The upper layer was decanted, recentrifuged at 21,000*g* for 60 min and filtered through a 0.22 μ millipore filter which resulted in sterile, stroma-free hemoglobin in a concentration of approximately 25–28 g/100 ml. Hemoglobin was stored in 5 ml aliquots at −80°; upon thawing, these solutions contained between 3 and 7% methemoglobin. Methemoglobin was formed by the addition of 200 μl of a 20% potassium ferricyanide solution to a 5 ml aliquot of hemoglobin solution. Excess ferricyanide was removed by dialysis against several changes of 0.9% sodium chloride over 24 hr. The resulting solution contained 100% methemoglobin. Hemoglobin and methemoglobin were determined by the method of Evelyn and Malloy (20). Plasma hemoglobin was determined by the method of Crosby and Furth (21). In each experiment, hematin (12.5 mg/kg body wt) or an equivalent amount of heme in the form of hemoglobin or methemoglobin was injected into a lateral ear vein, 48 hr after the administration of ^{125}I-hemopexin.

Determination of $T_{1/2}$. After injection of the isotope-labeled proteins, ten 1 ml serum samples were removed within 24 hr from the central ear artery cannulated with a 19-gauge scalp vein infusion set. The cannulus was then removed and subsequent samples were taken twice daily from the lateral ear vein. When heme was injected, hourly serum samples were drawn: One before and six after the heme injection. The hematocrit was determined with each bleeding to correct for serum dilution. Protein was precipitated with 95% ethanol, the supernatants were dis-

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[4] The terms, heme and hematin, are used interchangeably.

carded and the precipitates were counted in a dual-channel Nuclear Chicago Gamma Counter (Model 1185). Samples were counted to a minimum of 10^4 counts. The $T_{1/2}$ of the proteins was derived from the slope of a least squares fit of the final linear portion of the $\log_{10}$ (plasma radioactivity) vs time curve. In the 6 hr after heme injection, a least squares fit of the linear portion of the ^{125}I disappearance curve was used to calculate the $T_{1/2}$ of the heme–hemopexin complex. Values are expressed as mean ± SEM.

TABLE I. PLASMA HALF-CLEARANCE TIME OF ^{125}I-HEMOPEXIN IN RABBITS AFTER HEME INJECTION.[a]

No. of rabbits	Substance injected	$T_{1/2}$ Hemopexin (hr)[b]	$T_{1/2}$ Hemopexin (hr)[c]
4	—	—	34.0 ± 2.0
9	Hematin	7.4 ± 0.6	35.8 ± 2.5
7	Methemoglobin	6.9 ± 0.5	36.4 ± 2.1
9	Hemoglobin	7.6 ± 0.6	35.3 ± 1.2
Mean		7.2 ± 0.6	35.5 ± 1.9

[a] Heme (12.5 mg/kg body wt) was injected in the form of hematin, hemoglobin, or methemoglobin.

[b] $T_{1/2}$ calculated from the initial linear portion of the curve within 6 hr after heme injection.

[c] $T_{1/2}$ calculated from the final linear portion of the curve.

Results. The plasma half-clearance time of apo-rabbit hemopexin was 29–38 hr (Table I). Hematin, hemoglobin or methemoglobin were injected iv at 48 hr by which time intra-extravascular equilibration of ^{125}I-hemopexin and ^{131}I-albumin was complete. After administration of these heme compounds, 60–80% of the ^{125}I-hemopexin was removed with a $T_{1/2}$ of 4.9 to 11.1 hr. (Figure 1 and Table I.) During the first 6 hr, approximately 90% of the injected heme, hemoglobin or methemoglobin was removed from the circulation. The remaining ^{125}I-hemopexin was catabolized with a $T_{1/2}$ of 22–44 hr, similar to apo-hemopexin. A positive correlation (r = 0.654, n = 14, $P < 0.02$) was found between plasma levels of hemopexin and the rate of clearance of ^{125}I-hemopexin during the 6 hr after heme injection, i.e. the greater the concentration of hemopexin, the slower the rate of ^{125}I-hemopexin clearance.

^{131}I-albumin was catabolized with a $T_{1/2}$ of 94–110 hr (102.5 hr ± 2.0). The injection of heme, hemoglobin or methemoglobin did not affect the catabolism of ^{131}I-albumin (Figure 1, Panel A).

Injection of hematin caused only a minor change in the plasma disappearance curve of photo-inactivated ^{125}I-hemopexin (Figure 1, Panel B). This hemopexin, photo-oxidized

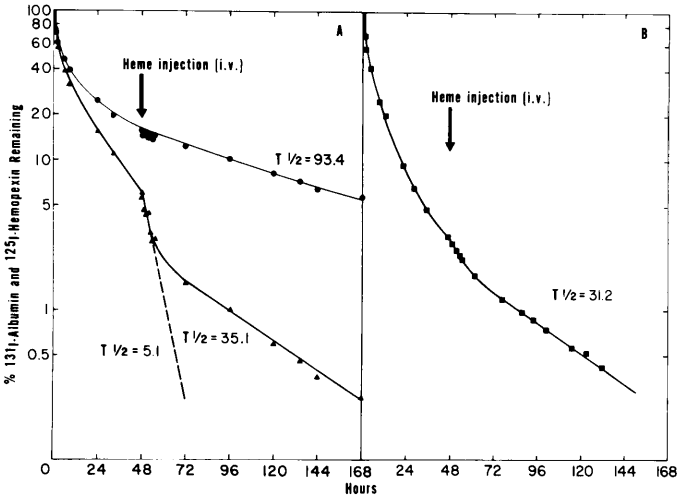

FIG. 1. Plasma clearance curves of ^{131}I-albumin and ^{125}I-hemopexin. The results of a typical experiment are shown. The effect of intravenous injection of hematin (12.5 mg/kg) on $T_{1/2}$ of albumin (●——●) and the $T_{1/2}$ of hemopexin (▲——▲) is depicted in Panel A, and on the $T_{1/2}$ of photo-inactivated hemopexin (■——■) in Panel B.

in the presence of rose bengal, had lost 80% of its heme-binding capacity.

Discussion. Previous data by Sears (5) and Wochner *et al.* (9) in man, and Lane *et al.* (11) in rabbits showed lowering of serum hemopexin concentrations for several hours after hematin injection. The serum levels began to rise only after all hematin was eliminated from the plasma (6, 9). Injection of hematin (12.5 mg/kg) 5 min after administration of the ^{125}I-hemopexin cleared 90–95% of the iodinated protein from the circulation in the first 24 hr. Within 12 hr, ca. 50% of the ^{125}I was excreted into the urine (11).

In the present study, we injected heme after equilibration of ^{125}I-hemopexin and ^{131}I-albumin with the body fluids to show that heme injection specifically affected the catabolism of native hemopexin. When heme was injected 48 hr after ^{125}I-hemopexin 60–80% of circulating ^{125}I-hemopexin was removed from the plasma within 6 hr. The $T_{1/2}$ of hemopexin in the absence of heme was somewhat longer (29–38 hr) than that previously reported (19–29 hr) (11, 22). Nevertheless, each protein preparation was catabolized at a consistent rate, i.e. the $T_{1/2}$ of apohemopexin before hematin injection did not differ from the $T_{1/2}$ of hemopexin following heme clearance (Table I). These experiments indicate that purification and iodination do not alter the biological function of hemopexin in heme disposal. The ^{131}I-albumin clearance rate was entirely independent of the injection of heme, which corroborates data suggesting that methemalbumin does not enter the liver (13, 15).

Hemoglobin or methemoglobin were as effective as hematin (11) in accelerating the catabolism of ^{125}I-hemopexin (Table). This finding reflects an efficient transfer *in vivo* of heme from circulating hemoglobin to hemopexin and is consistent with the transfer *in vitro* observed by Hrkal *et al.* (4).

The validity of these metabolic experiments was tested by selectively modifying the hemopexin molecule to destroy its ability to bind heme. Critical histidine residues of hemopexin were modified through photooxidation mediated by rose bengal (16). The photo-inactivated hemopexin was catabolized similarly to native apohemopexin, yet it no longer was affected by hematin injection (Panel B of Figure 1).

In conclusion, our results provide strong evidence that the effect of intravascular hemoglobin or heme on plasma hemopexin is to form heme–hemopexin complexes which are then cleared from the circulation.

Summary. The plasma half clearance time ($T_{1/2}$) of isotope-labeled rabbit hemopexin was 35.5 ± 1.9 hr in rabbits. After intra- and extra-vascular equilibration of ^{125}I-hemopexin and ^{131}I-albumin, injection of either hematin, hemoglobin or methemoglobin (12.5 mg of heme/kg body wt) resulted in the rapid removal of 60–80% of circulating hemopexin ($T_{1/2}$ = 7.2 ± 0.6 hr) but did not affect albumin catabolism.

After selective photo-inactivation of hemopexin, the $T_{1/2}$ of this hemopexin was comparable to that of the native molecule. However, its plasma disappearance curve was not appreciably affected by administration of hematin.

These findings demonstrate that hemopexin is cleared and catabolized at an enhanced rate during states of plasma heme load, and that modification of critical histidine residues of hemopexin eliminates its biological function in plasma heme disposal.

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