THE RENAL HANDLING OF HEMOGLOBIN

I. Glomerular Filtration*,‡

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During a severe hemolytic process, circulating free hemoglobin readily passes through the renal glomeruli. Filtered hemoglobin is extensively taken up by the cells of the proximal tubule (1, 2). When the absorbtive capacity of these cells is exceeded, hemoglobinuria ensues.

The permeability of glomeruli, and presumably of other capillaries, for hemoglobin is considerably greater than for albumin, a protein of similar mol wt (3). Recent information concerning the structural and physical properties of the hemoglobin molecule offers a possible explanation. Hemoglobin is a tetramer consisting of two pairs of unlike chains ($\alpha_2\beta_2$). The molecule dissociates reversibly into two symmetrical dimers: $\alpha_2\beta_2 \rightleftharpoons 2\alpha\beta$. Under physiologic conditions of pH and ionic strength, a 100 mg/100 ml solution of human oxyhemoglobin is about one-fourth in the form of dimers, as calculated from a recently derived equilibrium constant (4). The reversible dissociation of hemoglobin into dimers, each with a mol wt of about 32,000, suggests the possibility that it is this form that so readily penetrates glomeruli.

The extent of dissociation is known to be affected by several conditions: (*a*) It is enhanced by dilution; (*b*) hemoglobins of various species differ widely in their tendency to form dimers (5, 6); (*c*) Simon and Konigsberg have recently studied the physical and chemical properties of hemoglobin that was reacted with the sulfhydryl reagent bis(*N*-maleimidomethyl) ether (BME) (7). One of BME's two imido groups binds covalently with each of hemoglobin's two reactive sulfhydryl groups, in a manner similar to *N*-ethylmaleimide (NEM) (Fig. 1). The remainder of the BME molecule interacts noncovalently with

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another part of the β-chain in such a way that dissociation of the tetramer into half molecules is markedly reduced, although the tertiary configuration of the tetramer is undisturbed.

The following experiments were designed to test the hypothesis that hemoglobin is filtered by the glomeruli primarily as the $\alpha\beta$-dimer. In addition, in vitro studies on BME hemoglobin were done in order to evaluate its possible usefulness as a plasma expander.

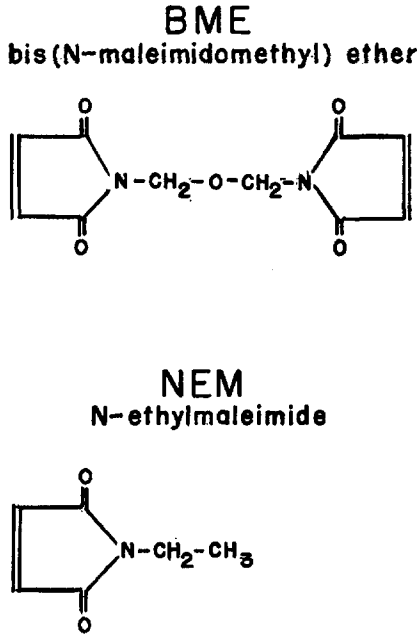

FIG. 1. Structural formulae of bis-(N-maleimidomethyl) ether (BME) and N-ethylmaleimide (NEM).

Methods and Materials

Male Sprague-Dawley rats weighing 250–350 g, New Zealand white rabbits, and mongrel dogs were used in these studies.

Preparation of Hemoglobin Solutions.—Rat, cat, and dog hemoglobins were labeled with ^{51}Cr by adding $Na^{51}CrO_4$[1] to freshly collected heparinized blood (1 μc/ml RBC) followed by 30 min of gentle shaking at room temperature. The labeled red cells were then washed 3 × with normal saline. Cells were lysed by adding two volumes of cold distilled water to packed red cells. Isotonicity was restored by the addition of one-fifth the water volume of 5.0% NaCl to cat and dog hemolysates. 7.5% $NaHCO_3$ was added to the rat hemolysate, since

[1] Abbott Radiopharmaceuticals Inc. Chicago, Ill.

others have shown rat hemoglobin is more soluble in alkaline solution (8). Each hemolysate was then centrifuged at 4°C for 30 min at 5000 *g*. The crystal clear supernatant was dialyzed overnight at 4°C against a mixture comprised of three parts normal saline and one part isotonic phosphate buffer, pH 7.4 (buffered saline). Dialysis effectively removed all unbound ^{51}Cr from the hemoglobin solution. This was demonstrated on several occasions by chromatographing an aliquot of the dialyzed ^{51}Cr hemoglobin on Sephadex G-25, which effectively separates the protein-bound and unbound ^{51}Cr (8). The specific activities of the hemoglobin solutions before and after chromatography were always identical.

^{59}Fe rabbit hemoglobin was prepared as follows. Reticulocytes were obtained by the removal of 30% of a rabbit's blood volume every 2nd day. 2 days after the third phlebotomy, the rabbit's blood contained 40–70% reticulocytes. The red cells were incubated with two volumes of heparinized plasma from an iron-deficient rabbit to which $^{59}FeCl_3$[2] and glucose (2.0 mg/ml) had been added. This mixture was incubated at 37°C with gentle shaking for 3 hr. After the red cells were washed 5 × with isotonic saline, hemoglobin was prepared as outlined above. ^{59}Fe rat hemoglobin was prepared as outlined by Keene and Jandl (9). BME hemoglobin was prepared by the addition of a 4 M excess of an aqueous solution of bis (*N*-maleimidomethyl) ether[3] to an aliquot of the labeled oxyhemoglobin (i.e., 1 mole BME/mole of hemoglobin heme). After the mixture stood for 4 hr at 4°C it was dialyzed against buffered saline to remove excess reagent. Titration of reactive sulfhydryl groups of hemoglobin solutions treated with BME, done by the method of Boyer (10), showed that the reaction had gone to completion. BME human hemoglobin was prepared as previously described (11).

Rat Experiments.—Intravenous injections were made into tail veins of rats lightly anesthetized with ether. Rats received 2.0 ml injections containing 65 mg of ^{59}Fe or ^{51}Cr rat oxyhemoglobin, either untreated or reacted with BME. This large dose of hemoglobin was greatly in excess of the 5–8 mg binding capacity of plasma haptoglobin. Periodic blood samples were collected with a 0.1 ml pipette after nicking the tip of the tail with a scalpel blade at a point several cm distal to the site of injection. In some experiments, animals were placed in separate metabolic cages permitting continous urine collection. 3–5 hr after injection, the animals were sacrificed. In some instances, organs were removed and radioactivity was measured, as described in detail elsewhere (9, 12). Liver and spleen radioactivity was corrected for the contribution from the activity of the plasma trapped in the organ at the time of sacrifice. Total urine activity was calculated from the cpm/ml and the measured urine volume. In one experiment, the hemoglobin concentration of rat urine was also measured by the benzidine method of Crosby and Furth (14). In another experiment, rats were nephrectomized under light ether anesthesia 18 hr before they were given hemoglobin injections.

Rabbit Experiments.—Rabbits were depleted of haptoglobin by two intravenous injections of unlabeled rabbit hemoglobin (50 mg/kg) 1 hr apart. 1 hr after the second dose, the animals were lightly anesthetized with sodium pentothal. A No. 22 polyethylene catheter was passed up from the lower abdominal aorta to the level of the renal arteries. The aorta was then ligated just distal to these vessels. The anterior mesenteric artery was also ligated. Rabbits so cannulated were injected rapidly with a 1 ml bolus of ^{59}Fe oxyhemoglobin of varying concentrations. 30 sec after the injection, the renal arteries were clamped and the aorta ligated proximal to the renal arteries. The kidneys were then perfused with 20 ml of isotonic saline and removed for counting. Following perfusion, renal vein blood generally had a packed cell volume of less than 2%. Urine flow persisted through the operative procedure, but no bladder urine activity could be detected. Both kidneys were sliced into four equal parts of approxi-

[2] Abbott Radiopharmaceuticals Inc. Chicago, Ill.

[3] A gift of Uniroyal Inc. Wayne, N. J.

mately 2 ml, each of which was counted separately in a well scintillation counter. One experiment employed haptoglobin-rich rabbit serum obtained from a rabbit 4 days after induction of a turpentine abscess (13). 1.0 mg of labeled hemoglobin was added per ml of serum, an amount insufficient to exceed the binding capacity of the serum.

Dog Experiment.—Two dogs were given an intravenous injection of 200 mg/kg of ^{51}Cr BME hemoglobin, while two others received untreated ^{51}Cr hemoglobin. Six blood samples were taken over an 8 hr period. No urine collection was made.

Miscellaneous Procedures.—After the addition of a known amount of unlabeled hemoglobin as carrier, ^{59}Fe hemin was crystallized from samples of plasma by the method of Labbe and Nishida (15). The purified hemin solution was then recounted. After correcting for incomplete recovery, the per cent of plasma activity present as ^{59}Fe heme could be calculated.

In order to test the extent of subunit dissociation of BME hemoglobin, a mixture containing 10 mg each of unlabeled rat carboxyhemoglobin and ^{59}Fe BME carboxyhemoglobin was chromatographed on a 50 × 2 cm column of Sephadex G-100[4] in 0.1 M phosphate buffer, pH 7.4. The total hemoglobin concentration of successive fractions was obtained from the absorbance of an appropriate dilution in Drabkin's solution measured at 540 mμ. The radioactivity of an aliquot of each fraction was also measured. From these data, the relative amounts of normal and BME hemoglobin in each fraction could be calculated as described in detail elsewhere (6).

Oxygen dissociation curves were done gasometrically on heparinized human whole blood and hemoglobin solutions in plasma by a modification of the method of Peters and Van Slyke (16), which is described in detail elsewhere (17).

The rate of auto-oxidation of solutions of untreated and BME hemoglobin in buffered saline was measured at 37°C. Except for the initial reaction with BME, both solutions were treated alike throughout their preparation. Ferrihemoglobin concentration was estimated by the procedure of Evelyn and Malloy (18).

Alkali denaturation of hemoglobins was tested by the method of Huisman and Meyering (19).

RESULTS

In Vivo Studies.—Following intravenous injection into rats, BME hemoglobin disappeared from the circulation much more slowly than untreated hemoglobin (Fig. 2). This was true whether hemoglobin was labeled with ^{59}Fe or ^{51}Cr. The plasma ^{59}Fe activity several hr after injection did not necessarily represent hemoglobin iron. However, crystallization of hemin in plasma withdrawn 5 hr after injection indicated that 88% of the plasma ^{59}Fe activity was heme iron. Thus there was little ^{59}Fe-transferrin activity in the plasma.

In one experiment, animals were sacrificed 3 hr after injection with ^{59}Fe-labeled BME and untreated hemoglobin, and the distribution of activity in various organs was measured (Table I). As expected, the plasma ^{59}Fe activity was considerably higher in the rats receiving BME hemoglobin. This difference was accounted for primarily in the lesser accumulation of ^{59}Fe in the kidneys and urine in the animals receiving BME hemoglobin. The total activity represented by the plasma, reticuloendothelial system, and urinary system in the group receiving BME hemoglobin averaged 54% of the injected dose, con-

[4] Pharmacia Fine Chemicals, Inc., Piscataway, N. J.

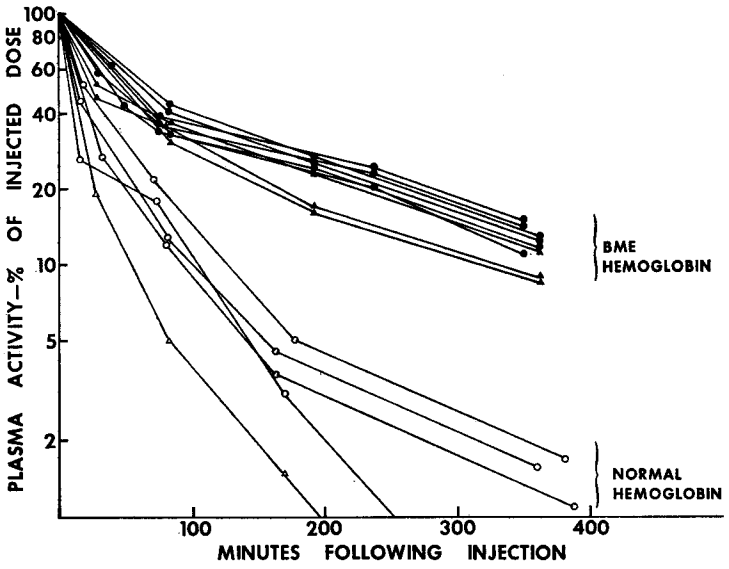

FIG. 2. Plasma disappearance of labeled rat hemoglobin. Rats were injected with 65 mg of either untreated ^{59}Fe hemoglobin (○), untreated ^{51}Cr hemoglobin (△), BME ^{59}Fe hemoglobin (●), or BME ^{51}Cr hemoglobin (▲).

TABLE I

Distribution of Radioactivity in Rats 3 Hr after Injection with 65 mg of ^{59}Fe Hemoglobin

^{59}Fe hemoglobin	Radioactivity						
	Renal			RE System			
	Plasma	Urine	Kidney	Liver	Marrow	Spleen	Total
	%	%	%	%	%	%	%
Untreated	5.2	33.8	18.4	13.6	8.9	1.2	81.2
	5.1	27.5	23.6	6.9	9.2	0.7	73.0
	4.6	40.3	23.6	6.1	6.5	1.4	82.5
BME	22.6	2.8	14.6	6.3	8.1	0.3	54.7
	20.3	2.7	14.4	6.1	7.1	0.3	50.9
	24.7	2.8	15.3	7.1	7.5	0.3	57.7

siderably less than the 79% found in the group which received untreated hemoglobin. The activity not accounted for by these organs presumably represented hemoglobin which permeated the general capillary bed. Because untreated hemoglobin was so rapidly trapped by the kidneys, after injection there was less circulating hemoglobin available for transendothelial escape.

The plasma disappearance of ^{59}Fe-labeled BME and untreated hemoglobin

was measured in nephrectomized rats (Fig. 3). The rates of decline in radioactivity in both groups were similar to the plasma disappearance of BME hemoglobin in intact rats (Fig. 2), confirming that the prolonged plasma survival of BME hemoglobin was due primarily to its minimal renal excretion.

In a parallel experiment, rats were injected with 65 mg of either ^{51}Cr dog hemoglobin or ^{51}Cr cat hemoglobin, and then sacrificed 3 hr later. (It was not practical to prepare ^{59}Fe-labeled cat and dog hemoglobins). The rationale of this experiment lies in the fact that cat and dog hemoglobins differ greatly in their degree of subunit dissociation. Under similar conditions cat hemoglobin splits into dimers much more readily than hemoglobin of the dog or other animals tested (6). The urinary excretion of cat hemoglobin greatly exceeded

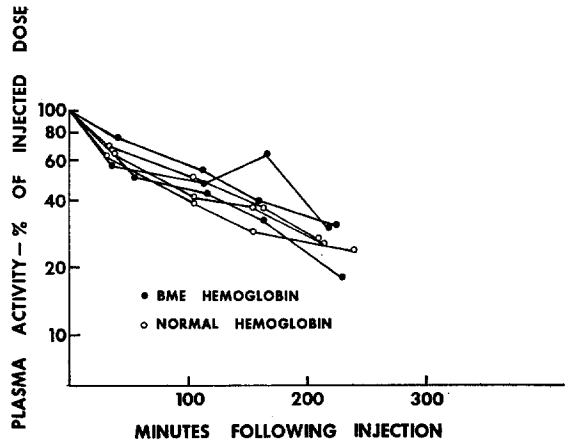

FIG. 3. Plasma disappearance of labeled hemoglobin in nephrectomized rats. Animals were injected with 65 mg of either untreated ^{59}Fe hemoglobin (○) or BME ^{59}Fe hemoglobin (●).

that of dog hemoglobin (Table II). The data shown in the right-hand column represent hemoglobin concentrations determined by a benzidine method (13), which is preferable to a direct spectrophotometric method since urinary hemoglobin is a mixture of various forms (oxyhemoglobin, ferrihemoglobin, parahematins) which have approximately equal peroxidase activities. Urine ^{51}Cr activities paralleled these measurements but were somewhat higher, especially with dog hemoglobin. Perhaps during the 3 hr collection period, some ^{51}Cr was eluted from the injected hemoglobin and excreted. Kidney activity was similar in the two groups, averaging 9%, but again, how much of this activity represents unbound ^{51}Cr, and how much was eluted from absorbed labeled hemoglobin are open to question.

In order to study the effect of varying hemoglobin concentration on glomerular filtration, the renal uptake of ^{59}Fe rabbit hemoglobin was measured 30 sec after rapid injection at the level of the renal arteries. This approach al-

lowed a rough approximation of the hemoglobin clearance during one circulation through the kidneys. The uptake by other organs (such as the reticuloendothelial system) was minimized. Since perfusion of the kidneys presumably removed most of the intravascular hemoglobin, and since no activity ever appeared in the urine, the renal activity represented primarily hemoglobin that was filtered and absorbed. As shown in Fig. 4, the per cent of the hemoglobin

TABLE II

Urinary Excretion 3 Hr after Injection of 65 mg of ^{51}Cr Labeled Cat or Dog Hemoglobin into Rats

	Urinary hemoglobin	
	^{51}Cr activity	Peroxidase activity
	%	%
Cat hemoglobin	43	36
	37	36
	18	22
	49	54
	45	39
	33	26
	43	35
Mean ± 1 SD	38.3 ± 11.1	35.4 ± 10.1
Dog hemoglobin	25	22
	32	17
	31	30
	27	16
	27	23
	26	21
	28	20
	30	14
Mean ± 1 SD	28.3 ± 2.5	20.4 ± 5.0
P	0.05	0.01

taken up by the kidneys decreased with increasing concentrations of infused hemoglobin. When glomerular filtration was prevented by binding ^{59}Fe rabbit hemoglobin to rabbit haptoglobin (100 mg/100 ml), only 1.3% of the injected activity was detected in the kidneys (average of three experiments).

Dogs were given 200 mg/kg of either ^{51}Cr-labeled BME hemoglobin or untreated hemoglobin (Fig. 5). As in the rat, BME hemoglobin had a much more prolonged plasma survival than untreated hemoglobin. The two dogs appeared to tolerate BME hemoglobin well. Complete blood counts and blood urea nitro-

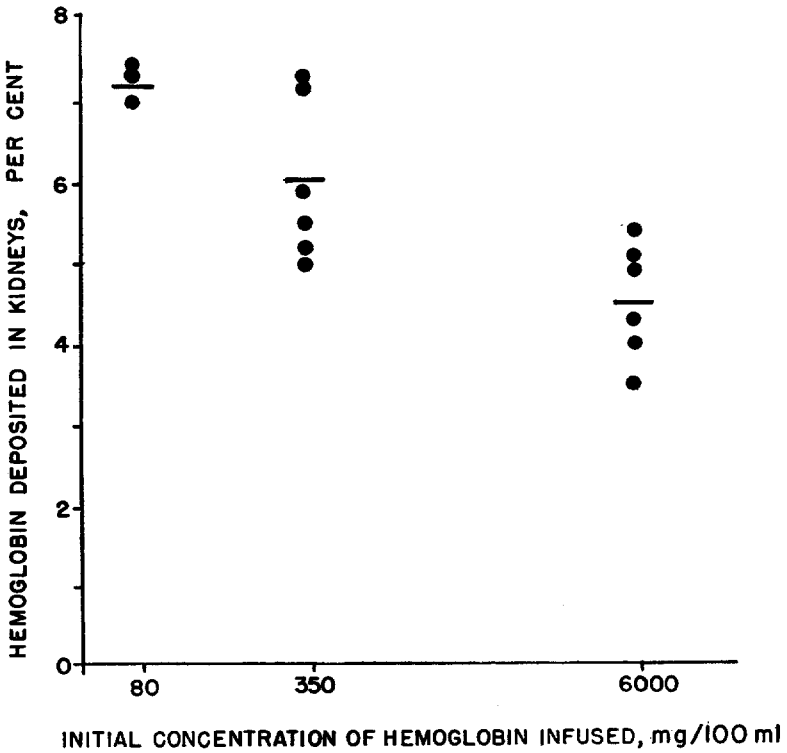

FIG. 4. Renal uptake in rabbits following the intra-arterial injection of differing concentrations of ^{59}Fe rabbit hemoglobin.

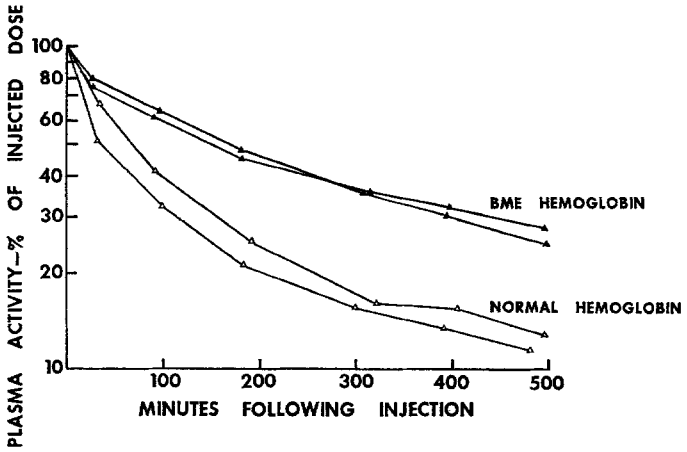

FIG. 5. Plasma disappearance of labeled canine hemoglobin. Dogs were injected with 200 mg/kg of either untreated ^{51}Cr hemoglobin (△) or BME ^{51}Cr hemoglobin (▲).

gen and serum glutamic oxalacetic transaminase determinations were normal 24 and 48 hr after the infusion.

In Vitro Studies.—Unlabeled rat carboxyhemoglobin treated with BME had a considerably faster mobility on Sephadex G-100 than untreated ^{59}Fe carboxyhemoglobin (Fig. 6). Similar results were obtained when the oxy derivatives of both rat and human hemoglobins were tested. Thus, under physiologic con-

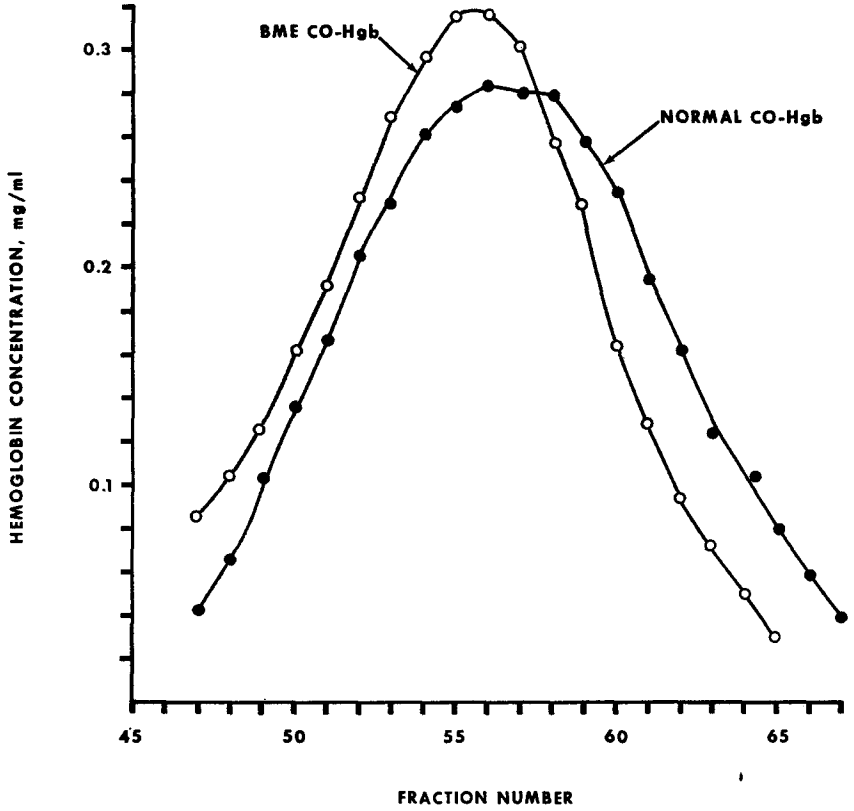

FIG. 6. Elution pattern following chromatography of a mixture of unlabeled rat BME carboxyhemoglobin and untreated ^{59}Fe carboxyhemoglobin on Sephadex G-100.

ditions of pH and ionic strength, BME hemoglobin had a higher mean mol wt, due to a reduced degree of dissociation into $\alpha\beta$-dimers (6). Earlier sedimentation velocity measurements, done under nonphysiologic conditions, also showed that human BME hemoglobin dissociated less readily into subunits (7).

In the following experiments human blood and hemoglobin were employed. A gasometric method was used to measure oxygen dissociation curves in order that hemoglobin solutions in plasma could be compared directly with whole blood. A representative experiment is shown in Fig. 7. Under physiologic con-

ditions (pH 7.40, 37°C), fresh whole blood had a P_{50}[5] of 26.4 mm Hg, the partial pressure of oxygen at which hemoglobin was half saturated. A mixture of freshly prepared hemoglobin solution and plasma (5 g/100 ml) from the same blood specimen had an oxygen dissociation curve considerably to the left, showing increased oxygen affinity as indicated by the lower P_{50} value of 17.5 mm Hg. No ferrihemoglobin was detectable in this mixture. The oxygen dissociation

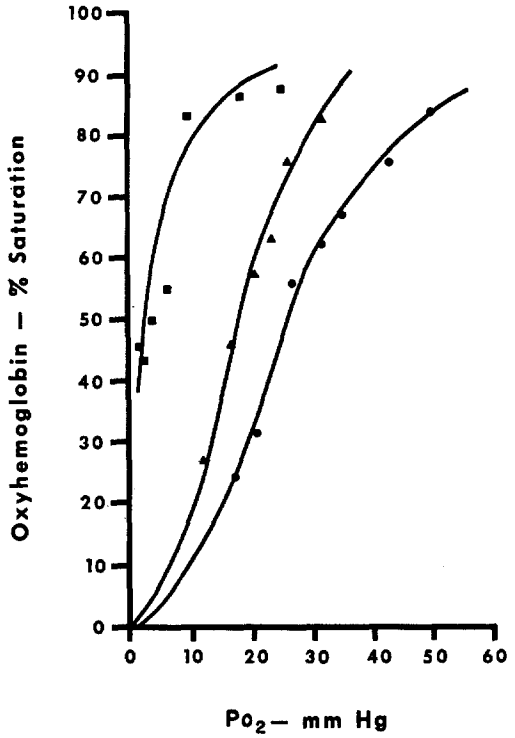

FIG. 7. Oxygen dissociation curves on whole blood (●), untreated human hemoglobin in plasma (▲), and human BME hemoglobin in plasma (■), 37°C, pH 7.4.

curve of dialyzed hemoglobin in plasma was slightly further to the left with a P_{50} of 17.0 mm Hg. Mixtures of hemoglobin and plasma from five other donors also had considerably lower P_{50} values than the corresponding whole blood specimens. These data have been presented in more detail in another report (20). Others have also observed a higher oxygen affinity of saponin and freeze-thaw hemolysates compared to whole blood (21, 22). The oxygen dissociation curve of BME hemoglobin in plasma was hyperbolic with a very high oxygen

[5] Oxygen affinity.

affinity: P_{50}, 3 mm Hg; n,[6] 0.9. Since the very low oxygen pressures required for this curve were difficult to measure accurately, there was necessarily more scatter than in the other two curves represented in Fig. 7. Using a spectrophotometric method which is better suited to measuring oxygen equilibria on hemoglobins of such high affinity, Simon and Konigsberg obtained a P_{50} of about 2.2 mm Hg (corrected to pH 7.40, 37°C) and an n of 1.00 (7).

The rate of auto-oxidation of human BME hemoglobin under physiologic

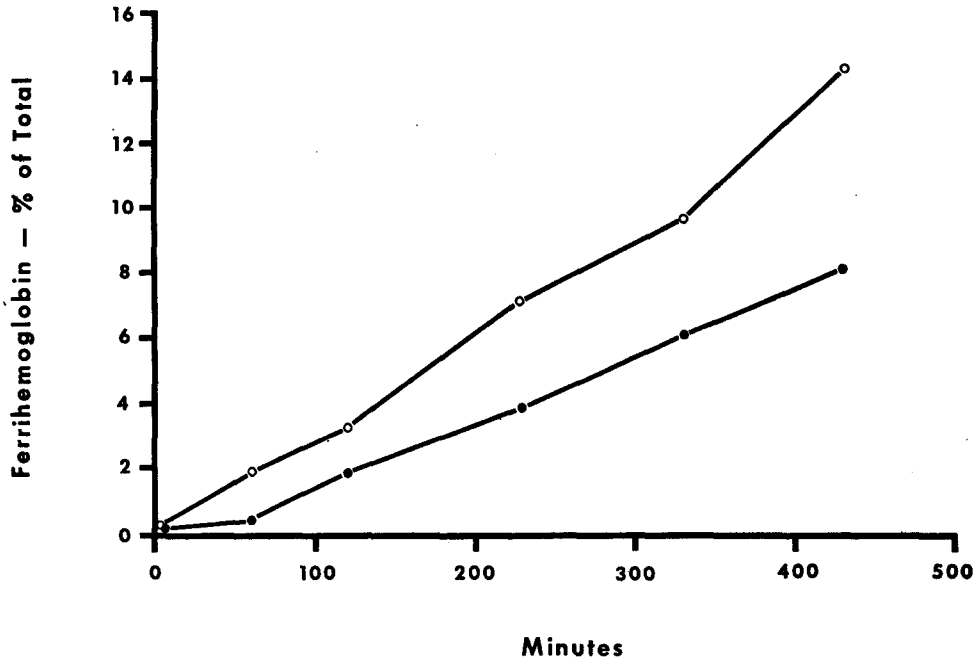

FIG. 8. Rate of auto-oxidation of untreated human hemoglobin (○) and human BME hemoglobin (●), 37°C, pH 7.4.

conditions was compared with that of untreated hemoglobin. In each of three experiments, one of which is shown in Fig. 8, ferrihemoglobin accumulated less rapidly when hemoglobin was reacted with BME. BME hemoglobin and untreated hemoglobin had equal rates of alkali denaturation.

DISCUSSION

These studies were designed to test the hypothesis that hemoglobin ($\alpha_2\beta_2$) is filtered by the glomerulus primarily as a half molecule ($\alpha\beta$). The equilibrium

[6] Coefficient in Hill equation: $\log Y/(1 - Y) = n(\log PO_2 - \log P_{50})$ where Y = oxyhemoglobin/oxyhemoglobin + deoxyhemoglobin.

between the tetramer and the dimer is reached so rapidly that physical separation of the two forms is not possible. Thus it was necessary to study the glomerular filtration of hemoglobin under conditions in which the dissociation equilibrium was shifted in one direction or the other.

As in any self-associating system, the relative formation of hemoglobin subunits is enhanced by decreasing concentration. One could predict that, if the above hypothesis were correct, the renal clearance of low plasma concentrations of hemoglobin would be greater than that of high concentrations. However, it would be difficult if not impossible to show these clearance differences in the intact animal, since at low levels of plasma hemoglobin the molecule is largely absorbed in the proximal tubule of the nephron. Monke and Yuile found that renal hemoglobin clearance in dogs was extremely variable below a plasma level of 400 mg/100 ml (23). To circumvent this, we injected a small (1 ml) volume of varying concentrations of ^{59}Fe hemoglobin directly into the renal arteries of rabbits. Since no activity was detected in the urine, even after the injection of high concentration (60 mg/ml), it is assumed that the filtered hemoglobin remained trapped in the kidneys. Since this is a highly indirect way of estimating glomerular filtration, the results cannot be considered conclusive. Nevertheless, the observed inverse relationship between renal uptake and concentration of the injected hemoglobin suggests that dilute hemoglobin was more readily filtered.

There is some variability in the extent to which various animal hemoglobins dissociate into subunits (5, 6). Cat and dog hemoglobins were selected because they differ widely in this respect, cat hemoglobin forming dimers much more readily than dog hemoglobin. (6). The greater excretion of cat hemoglobin by the rat kidney can be attributed to increased glomerular filtration. Any difference in the tubular absorption of cat and dog hemoglobins seems unlikely since the ^{51}Cr kidney activity was the same in the two groups. Hemoglobin which was reacted with the sulfhydryl reagent BME showed both a decreased tendency to dissociate into dimers and a reduced renal clearance in the rat. The rapid plasma disappearance of untreated rat hemoglobin was due primarily to its extensive renal excretion. The disappearance of plasma hemoglobin was prolonged in nephrectomized rats or intact rats (and dogs) receiving BME hemoglobin.

Although hemoglobin has several properties which would make it a suitable plasma expander (24, 25, 26), it disappears from the plasma much more rapidly than other plasma substitutes such as albumin or clinical dextran. Whether or not hemoglobin per se is nephrotoxic, it would be advantageous if its renal excretion could be curtailed. The experiments cited above suggest that this can be accomplished by reacting hemoglobin with BME.

One unique advantage offered by hemoglobin as a plasma expander would be its ability to bind and unload oxygen reversibly. The reaction of hemoglobin

with ligands such as oxygen appears to be dependent upon subunit dissociation. Models recently proposed by Benesch and associates (27) and Guidotti (28) predict that if liganded hemoglobin has an increased extent of dissociation into dimers it will have a reduced oxygen affinity and vice versa. Cat hemoglobin, and the human variant, hemoglobin Kansas (29, 6), both of which have an increased degree of dimer formation, have unusually low oxygen affinities. Conversely, as predicted from the above models, BME hemoglobin has a very high affinity. The oxygen dissociation data shown in Fig. 1 were obtained from hemoglobin solutions in plasma, thus simulating conditions of hemoglobin serving as a plasma expander. The oxygen affinity of BME hemoglobin is so high that in the physiologic range of PO_2, virtually no oxygen would be unloaded to tissues. However, solutions of untreated hemoglobin in plasma also had a relatively high oxygen affinity, compared to whole blood, as shown by a shift to the left of the oxygen dissociation curve. Because of the difference in oxygen affinity between hemoglobin circulating in plasma and that in red cells, most of the oxygen transport would be carried on by the red cells, even under conditions where a large proportion of the blood volume were replaced by the hemoglobin solution. After dogs were infused with large amounts of hemoglobin solution, Rabiner and his associates found that the mean oxygen content of arterial plasma samples was 3.60 vol/100 ml, representing 97% saturation (25). However, the mean oxygen content of mixed venous plasma was only 13% less. Since the red cell hemoglobin was presumably in excess of the plasma hemoglobin, the total contribution of the plasma to oxygen exchange would be correspondingly less.

If a hemoglobin solution is to serve as a plasma expander, it is critical that the formation of ferrihemoglobin (methemoglobin) be minimized, whether during preparation, storage, or after infusion. Ferrihemoglobin increases the oxygen affinity of the remaining active hemoglobin. Furthermore, upon oxidation of hemoglobin, its heme groups become less firmly bound to globin. When mixtures of ferrihemoglobin and plasma are incubated at 37°C, hemes readily transfer to albumin and hemopexin, leaving a denuded unstable globin (30). Finally, some reports from the earlier literature have indicated that ferrihemoglobin had greater renal toxicity than oxyhemoglobin (31, 32). As shown in Fig. 8, BME hemoglobin had a reduced rate of auto-oxidation, under physiologic conditions of temperature, pH, and ionic strength. For this reason, BME hemoglobin would probably have greater stability in plasma than untreated hemoglobin.

SUMMARY

The glomerular filtration of hemoglobin ($\alpha_2\beta_2$) was studied under conditions in which its dissociation into $\alpha\beta$ dimers was experimentally altered. Rats receiving hemoglobin treated with the sulfhydryl reagent bis(*N*-maleimido-

methyl) ether (BME) showed a much lower renal excretion and prolonged plasma survival as compared with animals injected with untreated hemoglobin. Plasma disappearance was also prolonged in dogs receiving BME hemoglobin. Gel filtration data indicated that under physiological conditions, BME hemoglobin had impaired subunit dissociation. In addition, BME hemoglobin showed a very high oxygen affinity and a decreased rate of auto-oxidation.

Glomerular filtration was enhanced under conditions which favor the dissociation of hemoglobin into dimers. Cat hemoglobin, which forms subunits much more extensively than canine hemoglobin, was excreted more readily by the rat kidney. The renal uptake of ^{59}Fe hemoglobin injected intra-arterially into rabbits varied inversely with the concentration of the injected dose.

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