Clearance Kinetics of Haptoglobin-Hemoglobin Complex in the Human

By DYREL A. FAULSTICK, JEROME LOWENSTEIN AND MARVIN J. YIENGST

ONE OF THE interesting properties of haptoglobin is the ability to bind hemoglobin, forming what has been referred to as the haptoglobin-hemoglobin (Hp-Hb) complex. This complex has been isolated from serum and found to have a molecular weight of approximately 310,000, depending on the degree of polymerization of the haptoglobin molecule, and is composed of one haptoglobin and two hemoglobin molecules. The complex is not excreted by the kidney and the clearance kinetics and site of destruction have been of interest to several investigators.

Results from a number of studies related to plasma clearance kinetics of the Hp-Hb complex in humans are not in agreement. Laurell and Nyman\(^2\) studied the disappearance of Hp-Hb complex in two human subjects following plasma levels of hemoglobin from 107–13 mg./100 ml. and concluded that the clearance was rectilinear at a rate of 13 mg./100 ml./hr. Gabrieli\(^1\) reported a rectilinear clearance rate for the bound complex when plasma Hb levels ranged from 10 to 260 mg./100 ml. and concluded that the clearance was unchanged throughout this range. Jandl\(^4\) followed plasma hemoglobin values in three humans after the injection of 2.8 Gm. of hemoglobin and observed an exponential fall. Lathem\(^5\) administered Hb in excess of the binding capacity in 13 human subjects and observed both rectilinear and exponential clearances of the complex. The plasma levels of hemoglobin were in a similar range to those noted above with values from 180–40 mg./100 ml.

Plasma disappearance rate studies of the Hp-Hb complex have been extended to animals with general agreement as to the type of clearance mechanism involved. Franklin\(^6\) followed clearances of the complex in rabbits and concluded that the rates were more or less exponential. Since the rabbit has a relatively low plasma haptoglobin concentration, he elevated the level using human serum or \(\alpha-2\)-globulins and obtained peak plasma haptoglobin levels of 25–50 mg./100 ml. Murray\(^7\) produced haptoglobin-rich rabbit plasma by subcutaneous injection of turpentine, harvested it and combined the haptoglobin with Fe\(^59\) hemoglobin. He followed the disappearance of injected complex in rabbits and observed an exponential clearance. His plasma haptoglobin levels in the examples shown probably did not exceed 30–40 mg./100 ml. He also noted that about 56 per cent of the counts cleared from the plasma was found in the liver; less than 5 per cent was in the spleen, lung, kidney, and marrow, and about 40 per cent was in the remaining parts of the carcass. It is not known what role recirculation of the iron tag played in distributing the counts in the organs mentioned. Garby and Obara\(^8\) in a study on rats, followed

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the plasma disappearance rate and the organ uptake of Fe\textsuperscript{59} tagged hemoglobin. Initial plasma levels were about 12 mg./100 ml. and all of the hemoglobin was present as the complex. The plasma clearances showed an exponential decrease and the organ uptake results were similar to those of Murray.

The present studies were undertaken in an attempt to examine further the kinetics of removal of the complex from circulating plasma in man.

**MATERIALS AND METHODS**

These studies were carried out on nine control male subjects and six male subjects with bronchogenic carcinoma. Serum haptoglobin determinations were made by combining an aliquot of the serum sample with a known amount of hemoglobin. Haptoglobin-hemoglobin complex was separated from free hemoglobin by paper electrophoresis using a phosphate buffer (pH = 7.0, ionic strength 0.10) at 9 milliamperes in a Durrum-type cell for 16 hours. The amounts of free and bound hemoglobin were determined photometrically by a modification of the method described by Lathem.\textsuperscript{5}

Autogenous hemoglobin solutions were prepared under sterile conditions by lysis of packed red cells collected in acid-citrate-dextrose solution. Hemolysis was accomplished by addition of distilled water and followed by freezing and thawing. The latter was found to increase the yield of hemoglobin considerably above that obtained from distilled water hemolysis alone. Hypertonic saline was added to restore isotonicity and the mixture centrifuged at 2000 rpm for two hours at 0 C. to remove the stromal elements. Selected samples of the hemoglobin solutions were examined spectrophotometrically for the presence of methemoglobin. None of the samples showed any indication of an absorption peak or inflection in the recordings at 635 or 500 Å. Quantities of hemoglobin varying from 1.5 to 6.4 Gm. were given intravenously over a 15-minute period. The volume injected never exceeded 130 ml. Five-ml. blood samples were collected from an indwelling venous needle into siliconized graduated centrifuge tubes containing 0.4 ml. of acid-citrate-dextrose solution. In most cases blood was withdrawn without venous stasis or the use of a syringe. Between samples the needle was flushed with a dilute solution of heparin (2 mg. per ml.). Three to five ml. of blood was allowed to pass through the needle prior to obtaining the sample for analysis, to free the sample of any diluent. Plasma hemoglobin determinations were carried out by the method of Crosby and Furth.\textsuperscript{9} All samples were analyzed in duplicate, checked by paper electrophoresis and showed no free hemoglobin. Hemoglobin standards were measured over the entire range of concentrations encountered experimentally. The control or pre-injection plasma hemoglobin blank was subtracted from post-infusion values. The urine was collected from each patient during the experiment and all samples were free of hemoglobin.

Nine male subjects (aged 42–77 years) who were free of any infectious, systemic, cardiac or hepatic disease were chosen for control subjects. The study was repeated on each of these individuals; in the first procedure the total circulating haptoglobin was 90–95 per cent saturated with hemoglobin while during the repeat study approximately half this amount was used. A period of two weeks was allowed to lapse between individual repeat studies. Six male subjects (aged 48–72 years) who had bronchogenic carcinoma were also examined. The serum haptoglobin level in these individuals was always over 200 mg./100 ml. as contrasted to the normal values in the controls, which averaged 140 mg./100 ml. Of these six subjects, five met the above control criteria (the sixth being jaundiced from hepatic metastases at the time of study).

**RESULTS**

The maximum plasma-bound hemoglobin values in the nine control subjects ranged from 100–175 mg./100 ml. In all cases the values were followed to 30 mg./100 ml. and in most to about 20 mg./100 ml. One cause of error in the
### Table 1.—Clearance Rates of Haptoglobin-Hemoglobin Complex from Plasma

<table>
<thead>
<tr>
<th>Subject</th>
<th>Test</th>
<th>Age</th>
<th>Dose (free) (mg./Kg. B.W.)</th>
<th>Max. plasma level (bound) mg./100 ml.</th>
<th>Deviation from linear fit</th>
<th>Karith.</th>
<th>$\sqrt{1-r^2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>II</td>
<td>82.0</td>
<td>143</td>
<td>.210</td>
<td>.084</td>
<td>.200</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>I</td>
<td>72.0</td>
<td>150</td>
<td>.300</td>
<td>.078</td>
<td>.243</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>II</td>
<td>35.8</td>
<td>85</td>
<td>.261</td>
<td>.179</td>
<td>.247</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>I</td>
<td>71.7</td>
<td>175</td>
<td>.340</td>
<td>.063</td>
<td>.226</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>I</td>
<td>44.5</td>
<td>112</td>
<td>.273</td>
<td>.089</td>
<td>.267</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>II</td>
<td>73.0</td>
<td>121</td>
<td>.212</td>
<td>.078</td>
<td>.259</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>I</td>
<td>35.5</td>
<td>72</td>
<td>.215</td>
<td>.110</td>
<td>.141</td>
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<tr>
<td>8</td>
<td>I</td>
<td>65.0</td>
<td>166</td>
<td>.308</td>
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<td>.195</td>
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</tr>
<tr>
<td>9</td>
<td>I</td>
<td>32.4</td>
<td>102</td>
<td>.275</td>
<td>.167</td>
<td>.212</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
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<td></td>
<td></td>
<td></td>
<td>.256</td>
<td>.092</td>
<td>.232</td>
</tr>
<tr>
<td>Mean</td>
<td>I</td>
<td>71.1</td>
<td>138.8</td>
<td>.018</td>
<td>.012</td>
<td>.018</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>II</td>
<td>36.3</td>
<td>80.1</td>
<td>.016</td>
<td>.023</td>
<td>.024</td>
<td></td>
</tr>
</tbody>
</table>

| **Patients—Bronchogenic Carcinoma** | | | | | | | |
| 1 | 48 | 92.5 | 150 | .357 | .063 | .217 | | |
| 2 | 65 | 62.0 | 88 | .328 | .045 | .141 | | |
| 3 | 66 | 58.5 | 85 | .304 | .045 | .200 | | |
| 4 | 67 | 89.5 | 141 | .281 | .063 | .161 | | |
| 5 | 67 | 61.0 | 113 | .328 | .045 | .259 | | |
| 6 | 72 | 62.2 | 121 | .275 | .134 | .226 | | |
| Mean | | | | | .299 | .066 | .201 | |
| Mean | I | 71.0 | 116.3 | .017 | .016 | .020 | | |

*Plot of concentration (mg./100 ml.) vs. time.

†Plot of log concentration vs. time.

‡Test I—approximately 90 per cent of haptoglobin combined with hemoglobin.

§Test II—approximately 45 per cent of haptoglobin combined with hemoglobin.

The experimental procedure was from hemolysis which occurred during blood sampling. Although it was not possible to monitor the amount of hemolysis in each sample, the mean value in 24 samples drawn before hemoglobin infusion was 3.1 mg./100 ml. with an S.D. of 2.1 mg./100 ml. In view of this error, hemoglobin determinations below 20 mg./100 ml. cannot be considered reliable for clearance kinetics.

Results were plotted as both arithmetic and semilog graphs and the best straight line fit calculated by the method of least squares for each. The slope of the regression line and coefficient of alienation ($\sqrt{1-r^2}$), where r represents the correlation between concentration and time, were calculated for each.
Fig. 1.—Arithmetic plot of plasma haptoglobin-hemoglobin complex concentrations following an intravenous infusion of hemoglobin in five subjects.

graph (table 1) as an index of the fit of the observations to a linear regression. In all experiments, the straight line on the arithmetic plot was a better fit than on the semilog plot. Experimental results from five subjects are shown in figures 1 and 2 for comparison of arithmetic and semilog plots. It is clear that the semilog plots are nonrectilinear.

The average disappearance rate of the bound hemoglobin for the controls during the first study was 15.4 mg./100 ml./hr. and on the repeat study was 13.0 mg./100 ml./hr. The average rate for the patients with bronchogenic carcinoma was 17.9 mg./100 ml./hr.

**DISCUSSION**

It would appear that the plasma clearance kinetics of Hp-Hb complex in the range followed is definitely zero order in the human. This was noted in both the control subjects and those with bronchogenic carcinoma. The disappearance rate was not significantly different between the two groups.

Laurell and Nyman suggested that the reticuloendothelial system may be
Fig. 2.—Semilog plot of plasma haptoglobin-hemoglobin complex concentrations following an intravenous infusion of hemoglobin in the same five subjects as shown in figure 1.

the major organ involved in the clearance of the complex. Jayle and Boussier\(^1\) noted that the haptoglobin molecule binds two molecules of hemoglobin in forming the complex. The dose of complex may be approximated by the following equation: \[ \text{Complex (mg./Kg. B. W.)} = \frac{\text{Hb (mg./Kg. B. W.) injected}}{310,000 \div 2 \times 68,000} \]

where 310,000 is the molecular weight of the complex and 68,000 that of hemoglobin. Substances such as colloidal chromic phosphate and iron and carbon suspensions that are known to be cleared by the reticuloendothelial system of animals have exhibited an exponential disappearance from the plasma with the rate being inversely proportional to the dose given. This relationship results in a constant \( K \times D \) value, where \( K \) is the rate of disappearance and \( D \) the dose of complex given, except for small doses where the rate (\( K \)) becomes constant. In our nine control subjects a doubling of the amount of hemoglobin injected (\( D \)) resulted in an increase of only 15 per cent in \( K \) which is regarded as insignificant. However, it is interesting that in no case did the rate decrease when the dose was increased. Goodness of fit to a rectilinear regression was not significantly influenced by the amount of hemoglobin administered.

The zero order disappearance rate observed in our work and its probable independence of dose might incline one to believe that the complex is not cleared by the reticuloendothelial system. Some substances cleared by this system have exponential disappearance rates in animals which are inversely
related to the dose given provided the dose is in the “critical dose” range. Plasma levels attained (61–175 mg./100 ml. of plasma) may have been above the critical dose. Halpern\textsuperscript{11} has reported exponential decreases of heat-coagulated human serum albumin in humans using doses of 10 mg./Kg. B. W. and Lelieves that this is cleared by the reticuloendothelial system. It is difficult to compare different substances that may be cleared by this system on a dosage basis using mg./Kg. B. W. as the measure. It seems more reasonable to assume that phagocytic clearance rates are influenced by the size and number of particles presented rather than by the weight of the particles. If this is true, then it is hard to compare clearance kinetics of carbon suspensions with heat-coagulated serum albumin or Hp-Hb complex on a weight basis.

The results of this study are in general agreement with those of Laurell and Nyman\textsuperscript{2} and of Gabrieli.\textsuperscript{3} Clearance of the complex was linear above 10–20 mg./100 ml. of plasma with an essentially constant rate of elimination in these three studies. The experiment of Lathem and Worley\textsuperscript{5} shows similar disappearance rates for the complex but they concluded that half of their subjects showed a linear and half an exponential decrease. These authors state that “the characteristics of the disappearance curves were not consistent” and give an error of ±7.0 per cent for plasma hemoglobin analyses in concentrations in excess of 20 mg. per cent. Examination of their plasma disappearance curves for bound hemoglobin suggests that the removal may be rectilinear rather than exponential.

Garby and Noyes,\textsuperscript{12} using hemoglobin tagged with Fe\textsuperscript{59}, concluded that over a 60-minute period, when small amounts of hemoglobin (1.3–3.5 mg./Kg. B. W.) were administered, the rate of removal followed a first order reaction in humans. Plasma hemoglobin levels ranged from about 1 to 10 mg./100 ml. In view of these results it is possible that at low plasma levels the clearance of the haptoglobin-hemoglobin complex proceeds at a first order rate, whereas at high plasma levels the clearance is a zero order reaction.

**SUMMARY**

In 15 human subjects the rate of clearance of haptoglobin-hemoglobin complex was rectilinear with respect to time between plasma levels of 175–20 mg. of bound hemoglobin.

**SUMMARIO IN INTERLINGUA**

In 15 subjectos human le clearance del complexo haptoglobina-hemoglobina esseva un function rectilinee del tempore intra le nivellos de 175 e 20 mg de ligate hemoglobina per 100 ml de plasma.

**ACKNOWLEDGMENTS**

The authors are indebted to Dr. N. W. Shock for suggestions and advice in the design of these experiments. The technical assistance of Miss Margaret Sellmayer and Mrs. Ramona Dorcas is gratefully acknowledged.

**REFERENCES**

1. Jayle, M. F., and Boussier, G.: Les séromucoïdes du sang leur relations avec les mucoprotéïnes de la substance fondamentale du tissu con-
2. Laurell, C. B., and Nyman, M.: Studies on the serum haptoglobin level in
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