Haptoglobin Metabolism in Polycythemia Vera

By Stephen Krauss

PLASMA HAPTOGLOBIN (Hp) is a well-characterized α2-glycoprotein which possesses the ability to bind hemoglobin (Hb), forming the haptoglobin-hemoglobin (Hp-Hb) complex. Under physiologic conditions, it is estimated that 20–40 per cent of the Hp catabolized daily is degraded via complex formation with hemoglobin released during normal intravascular hemolysis. The hemoglobin thus made available for Hp binding constitutes about 10 per cent of the total Hb catabolized daily as a result of normal red cell senescence. Once formed, the Hp-Hb complex is cleared rapidly from the circulation with a half-life of 9–30 minutes so that little Hp-Hb complex can be detected in the plasma at any time (~0.3 mg./100 ml.). That portion of plasma Hp not removed as the Hp-Hb complex is catabolized by another, slower route, similar, perhaps, to that by which other plasma proteins are catabolized. Thus, the normal disappearance curve of radioiodinated Hp (T½ of 2.8–4 days) is the resultant of both catabolic processes. When the degree of intravascular hemolysis increases above normal levels, as in hemolytic states, plasma Hp is low or absent, since the increased formation and clearance of Hp-Hb is unaccompanied by any acceleration of Hp production. In a variety of diverse pathologic states (infection, neoplasia, trauma) characterized by connective tissue injury and repair, Hb levels increase as a result of increased hepatic synthesis of the glycoprotein in response to an unknown, possibly humoral, mediator, while the degradation rate remains essentially unchanged. Where inflammation and hemolysis coexist, the Hb levels may be low, normal, or elevated, depending upon which of the opposing influences predominates at the time of observation.

In polycythemia vera, the occurrence of diminished Hp levels is not uncommon, in the absence of overt signs of hemolysis at a time when the red cell mass may be either elevated or normal as a result of prior treatment. In an effort to define the mechanism of this hypohaptoglobinemia, and clarify the role of Hp in Hb catabolism in this condition, the turnover of 125I-labeled human Hp was studied in patients with polycythemia vera.
MATERIALS AND METHODS

Patients

The subjects for this study were drawn from a large group of patients being followed in the Polycythemia Vera Clinic of the Department of Hematology, as part of a long-term study of myeloproliferative disorders. The polycythemia patients were selected to include a wide range of Hp values; all had received 32P and/or chemotherapy in the past. Five were being treated by phlebotomy alone at the time of the study, and were considered to have active disease; two had recently completed courses of chemotherapy (chlorambucil and cyclophosphamide, respectively) and were in remission. The 4 subjects without polycythemia vera were also drawn from the hospital population and included 1 man with diabetic retinopathy, 1 man with “spurious” polycythemia (i.e., hematocrit and hemoglobin at the upper limit of the normal range with a normal red cell mass as determined by the 51Cr method), and 2 patients with erythrocytosis of unknown etiology. These latter cases, a male and a female, showed an elevation of the RBC mass but no other evidence for either a myeloproliferative disorder or a causative factor for secondary erythrocytosis. The woman was receiving an oral contraceptive (Ortho-Novum, Ortho). In most instances, the patients were studied at the Clinical Research Center of the Mount Sinai Hospital, where complete 24-hour urine collections were assured. In all cases informed consent was obtained from the patients prior to inclusion in the study.

Preparation of 125I-Labeled Hp

Hp of types 2–1 and 2–2 was prepared from normal donor plasma (ACD), by a method previously described.6 The purity of the final product was established by vertical starch gel electrophoresis.9 Radioiodination of human Hp was carried out with 125I, using an iodine monochloride method as set forth in a prior communication.6 All procedures were carried out at 4 C. and all buffers contained Merthiolate (Thimerosal, Lilly) to retard bacterial growth. Merthiolate was removed during the final dialyses after iodination. Sterilization was then achieved by a passage of the iodinated Hp solution through a sterile, disposable millipore filter (pore size 0.22 μ), and confirmed by culture of the filtrate in several media. Confirmation of the purity of the labeled Hp was obtained by starch gel electrophoresis, with and without added hemoglobin, followed by radioautography (Fig. 1).

Procedure

All patients received Lugol’s solution, 10 drops three times daily, beginning two days prior to injection of isotope and continuing throughout the study. From 30–100 μc. of

Fig. 1.—Radioautograph of starch-gel electrophoresis of 125I-haptoglobin. Migration is upward. Free Hp is on the left, and the Hp-Hb complex is on the right. Note the multiple bands characteristic of Hp type 2–2, the absence of contaminating proteins, and the characteristic retardation in mobility of the Hp-Hb complex.
125I-labeled Hp (S. A. 100 μc./mg.) were injected intravenously from a calibrated syringe, and citrated plasma obtained 10, 20, and 30 minutes, and six hours after injection, and, subsequently, daily for 10 days. Later in the study, blood was obtained at 2-hour intervals for the first 12 hours, and thereafter, at least twice daily when possible. Twenty-four hour urine collections were obtained throughout the study period. In 3 subjects “clearance” studies were performed10: blood was sampled at the beginning and end of a 2-hour period, during the third or fourth day of the study, and all urine voided during this interval was collected. Adequate urine flow was insured by an intravenous infusion of 5 per cent dextrose in water, or by ingestion of at least 1000 ml. of water before and during the collection period. One ml. aliquots of all plasma and urine samples were counted for radioactivity in a Picker well-type scintillation counter. In order to establish that the radioactivity measured in plasma represented protein-bound radioactivity, plasma proteins were precipitated with an equal volume of 10 per cent trichloracetic acid (TCA), and, after centrifugation, the precipitate and supernatant were counted separately. One ml. aliquots of urine from these subjects were also treated with equal volumes of 10 per cent TCA after addition of unlabeled carrier protein, and then counted. Portions of urine were dialyzed, and the dialyzed urine and dialysates counted for radioactivity. Over 95 per cent of the urine radioactivity was found to be dialyzable, and not precipitable with TCA.

Calculations

The analysis of the data is based on the model of an open 3-compartment mammillary system, first described in the plasma protein turnover studies of Berson and Yalow,10,11 which involves the following assumptions11:

a) The turnover of the labeled protein is similar to that of the native one.
b) A steady state exists during the observation period.
c) All newly synthesized protein enters the intravascular compartment.
d) The labeled iodine released in the process of protein catabolism is excreted almost immediately, and reutilization does not occur.

The analysis also makes use of the observation, noted initially in turnover studies with 131I-labeled albumin,10 later extended in studies with fibrinogen,12 IgG13 and IgM,14 that the urinary excretion of radioactive iodine per day expressed as a per cent of the radioactivity remaining in the plasma is a constant (k1) throughout the period of observation (except possibly during the first few days when any denatured protein would be expected to be degraded, giving higher k1 values).

From measurements of plasma and urine activity, and the plasma Hp concentration, the following were derived: 1) Plasma Hp pool (Gm.); 2) Fractional catabolic rate (per cent per day, k1); 3) Plasma Hp turnover rate (mg./kg./day); and 4) the half-life (T1/2) of plasma Hp which was estimated from the slope of the second component of the curve. The first, a steep component, may be due to two factors; equilibration of the labeled protein with an extravascular pool and/or accelerated catabolism of any denatured protein, while the second component is taken to represent catabolism of the native protein, equal to its rate of synthesis if steady-state conditions prevail. The fractional catabolic rate was also calculated from the plasma radioactivity curve.

Since this study deals with a protein with a rapid turnover rate, clearance studies were carried out, as described by Berson,10 to determine the degradation rate over a short time interval. In this determination, the mean plasma radioactivity over a 2-hour period in cpm./ml. is divided into the total urine radioactivity (cpm.) excreted during that time, giving the volume of plasma cleared of radioactivity in 2 hours. Having determined the plasma Hp concentration, the amount of Hp degraded in 2 hours is calculated: Hp concentration (mg./100 ml.) × volume of plasma cleared (ml.) = total plasma Hp degraded in 2 hours.

Results

The results are summarized in Table 1, where the patients are listed in order of increasing half-time (T%) of plasma radioactivity.
Table 1.—$^{125}$I-Labeled Haptoglobin Turnover Studies in Polycythemia Vera

<table>
<thead>
<tr>
<th>Patient</th>
<th>Plasma Hp (mg./100 ml.)</th>
<th>Hp Type</th>
<th>Plasma volume (ml.)</th>
<th>T% Plasma Radioactivity (days)</th>
<th>Fractional Catalytic Rate (%day)*</th>
<th>Hp turnover (mg./kg./day)</th>
<th>Clinical Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.M.</td>
<td>29</td>
<td>2-2</td>
<td>4800 (2773)</td>
<td>0.80</td>
<td>126 (99)</td>
<td>16</td>
<td>P.V. Recent chlorambucil Rx Hct. = 50%</td>
</tr>
<tr>
<td>D.S.</td>
<td>126</td>
<td>2-2</td>
<td>4060 (2758)</td>
<td>3.47</td>
<td>131 (77)</td>
<td>75</td>
<td>P.V. — active Phlebotomies Hct. = 47%</td>
</tr>
<tr>
<td>F.A.</td>
<td>16</td>
<td>2-2</td>
<td>5000 (3625)</td>
<td>0.56</td>
<td>151 (50)</td>
<td>18</td>
<td>P.V. — active Phlebotomies RBC mass = 32 ml./kg.</td>
</tr>
<tr>
<td>E.F.</td>
<td>63F¹</td>
<td>144</td>
<td>4500 (2800)</td>
<td>4.03</td>
<td>68 (50)</td>
<td>35</td>
<td>P.V. — active Phlebotomies RBC mass = 28.2 ml./kg.</td>
</tr>
<tr>
<td>R.T.</td>
<td>26F²</td>
<td>57</td>
<td>2079</td>
<td>2.91</td>
<td>60 (41)</td>
<td>21</td>
<td>Erythrocytosis of unknown etiology Hct. = 53%</td>
</tr>
<tr>
<td>M.M.</td>
<td>65F²</td>
<td>200</td>
<td>2500</td>
<td>5.00</td>
<td>53 (39)</td>
<td>37</td>
<td>P.V. in remission after cyclophosphamide Rx Diabetes mellitus Hct. = 43%</td>
</tr>
</tbody>
</table>

*Denotes catalytic rate calculated using 0.025 mg. of $^{125}$I-Hp.
<table>
<thead>
<tr>
<th>O.I.</th>
<th>66M</th>
<th>35</th>
<th>2-2</th>
<th>4200</th>
<th>1.12</th>
<th>2.2</th>
<th>43 (32)</th>
<th>7</th>
<th>P.V. — active Phlebotomies</th>
<th>Hct. = 39%</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.E.</td>
<td>53M</td>
<td>82</td>
<td>2-2</td>
<td>3879</td>
<td>3.17</td>
<td>2.5</td>
<td>39 (28)</td>
<td>15</td>
<td>Erythrocytosis of unknown etiology Phlebotomies</td>
<td>Hct. = 49%</td>
</tr>
<tr>
<td>A.W.</td>
<td>69M</td>
<td>180</td>
<td>2-2</td>
<td>2380</td>
<td>6.10</td>
<td>2.6</td>
<td>24 (27)</td>
<td>32</td>
<td>P.V. — active Phlebotomies</td>
<td>Hct. = 48%</td>
</tr>
<tr>
<td>N.L.</td>
<td>27M</td>
<td>170</td>
<td>2-1</td>
<td>2586</td>
<td>4.40</td>
<td>2.8</td>
<td>31 (35)</td>
<td>19</td>
<td>Control. Diabetes mellitus Hct. = 42%</td>
<td></td>
</tr>
<tr>
<td>M.B.</td>
<td>65M</td>
<td>120</td>
<td>2-2</td>
<td>3217</td>
<td>3.86</td>
<td>4.1</td>
<td>10 (17)</td>
<td>26</td>
<td>Control. Relative Polycythemia Hct. = 50%</td>
<td></td>
</tr>
</tbody>
</table>

* Derived from urinary excretion of radioactivity. Figures in parentheses derived from plasma curve only (see text).
† Estimated on the basis of body weight.
1 Additional control values for the Tt of radioiodinated Hp include 3.0 days and 4.0 days.7
1 125I-Hp 2-2 Batch 9/27/67
2 125I-Hp 2-2 Batch 1/20/68
3 125I-Hp 2-1 Batch 4/18/68
4 125I-Hp 2-2 Batch 7/9/68
In 6 of 7 patients with polycythemia vera, the T3% was shortened, when compared to values in 2 control subjects. Of the 6 patients with accelerated disappearance of plasma radioactivity, 3 had low plasma Hp levels as well. One (D.S.), had a chronic stasis dermatitis and thrombophlebitis which may have provided a stimulus for increased Hp synthesis. Of 2 subjects with erythrocytosis of unknown etiology, one showed a shortened T3%; this patient had Hp levels which fluctuated around the lower limit of normal, but was taking an oral contraceptive (Ortho-Novum, Ortho) which contains mestranol, one of a group of estrogenic compounds known to lower Hp levels\(^1\) by a mechanism not well defined. The types of plasma radioactivity curves obtained are illustrated in Figure 2. The biphasic curve in patient A.W. with a normal Hp level was similar to that obtained in the control subjects, while the rapid decrease in plasma radioactivity in patients B.M. and F.A. was characteristic of patients with hypohaptoglobinemia.

The rate of appearance of radioactivity in the urine (Figs. 3 and 4) formed the basis of one method of calculating the fractional catabolic rate (Table 1). This rate exceeded 40 per cent in all subjects with a shortened T3% including 6 subjects with polycythemia vera, while in 2 control subjects and patient J.E.

Fig. 2.—Plasma radioactivity curves of 3 patients with polycythemia vera. The upper curve, in a patient with a normal Hp level, resembles that of control subjects, and demonstrates an initial steep slope, and a second, slower component, from which the T3% was derived. The 2 lower curves, in the patients with depressed Hp values, demonstrates marked shortening in the T3% as observed in patients with hemolysis. The same batch of \(^{125}\)I-Hp was employed in these subjects.

with idiopathic erythrocytosis and normal Hp levels, the fractional catabolic rate ranged from 10–39 per cent.

Despite wide variations in half times of plasma radioactivity and fractional catabolic rates, Hp turnover, i.e., the weight of Hp synthesized (and degraded) daily, varied within relatively narrow limits with the exception of patient D.S. Thus, patients B.M., F.A., and O.L., with low plasma Hp levels, had values for Hp turnover of 16, 18 and 7 mg./kg./day, respectively, compared to values of 19 and 26 in two control subjects. In patient D.S., the Hp turnover is high (75 mg./kg./day), probably because of the increased
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Fig. 3.—Urinary excretion of radioactivity and plasma levels in patient A. W. with normal Hp concentration. This pattern is like that seen in control subjects.

Fig. 4.—Urinary excretion of radioactivity and plasma levels in patient F. A. with very low Hp level. Note rapid excretion of radioactivity, with 50 per cent being excreted in the first 2 days.
fractional catabolic rate is accompanied by an increase in the rate of Hp synthesis as a result of chronic inflammation (thrombophlebitis and stasis dermatitis). Because of the short half-life and rapid turnover of $^{125}$I-labeled Hp, the degradation rate was also determined over a 2-hour period using a clearance technic. In the control subject M.B. ($T_\frac{1}{2} = 4.1$ days) clearance studies were performed on days 3 and 5, during the second, slower phase of the plasma disappearance curve. Values obtained were 188 mg. Hp degraded in 2 hours on day 3, and 115 mg. Hp degraded in 2 hours on day 5. Since this patient was in a steady state, these values extrapolate to a turnover rate of 34/mg./kg./day on day 3 and 21 mg./kg./day on day 5, thus showing fair agreement with the figure for Hp turnover (26 mg./kg./day) derived from the average daily fractional catabolic rate (Table 1). In contrast, a similar study on patient O.L. ($T_\frac{1}{2} = 2.2$ days) showed 116 mg. Hp degraded in 2 hours, giving a turnover rate of 14 mg./kg./day, a value considerably higher than that derived from the average daily fractional catabolic rate (7 mg./kg./day, Table 1). The discrepancy results because the $k_1$ in this patient, as in other subjects with shortened plasma half-times, was not constant, but reached a peak on day 3 when the clearance study was performed. The inconstancy in the $k_1$ values calculated from the daily urinary excretion of radioactivity also offers the most likely explanation for the observed discrepancy between the fractional catabolic rate derived from the plasma disappearance curve and that based upon urinary excretion.

As indicated in Table 1, plasma volumes calculated from the dilution of $^{125}$I-Hp were unusually high in those patients with accelerated disappearance.
of $^{125}$I-Hp. Thus, in patients E.F. and F.A., blood volume determination ($^{51}$Cr) revealed that the $^{125}$I-Hp "plasma" volume exceeded the calculated plasma volume by 38 and 67 per cent, respectively. Where the T½ of the labeled Hp was less rapid, e.g., in patients A.W., N.L., and M.B., values for plasma volume derived from $^{125}$I-Hp dilution were more reasonable.

The distribution of labeled Hp in body compartments was estimated in a control subject from data for plasma and urine radioactivity (Fig. 5). At the equilibrium point, indicated by the highest value on the plot of extravascular activity, from 25–40 per cent of the labeled Hp was distributed extravascularly, and the extravascular labeled Hp mass was from $\frac{1}{8}$ to $\frac{1}{5}$ of the plasma $^{125}$I-Hp mass at equilibrium. This distribution might be expected in the case of a large molecule like Hp 2–2 (M.W. > 220,000). With Hp 1–1 (M.W. 90,000), the extravascular mass has been found to be about equal to the intravascular mass at equilibrium time.\(^2\) Note that the fractional catabolic rate, plotted at the bottom of the graph, remains relatively constant at about 30 per cent per day throughout the study.

A similar graph in patient F.A. (Fig. 6) with a shortened T½ and low Hp level, reveals that by the end of day 1, 50 per cent of the radioactivity is extravascular, while only 13 per cent is intravascular. Of note is the fact that the fractional catabolic rate derived from the urinary excretion of radioactivity is not constant, but reaches a peak on day 3, probably reflecting, in part, delayed degradation products from days 1 and 2.

It may be noted (Table 1) that 10 of the 11 subjects had the phenotype

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**Fig. 6.**—Graph of intravascular, extravascular and total body radioactivity in patient F. A. with low level (15.5 mg./100 ml.) Note that by the end of day 1, 50 per cent of the radioactivity is extravascular, while only 13 per cent is intravascular. The very large fractional catabolic rate on the third day probably reflects excretion of $^{125}$I-Hp degradation products from the previous 2 days.
Hp 2-2, a distribution deviating markedly from the usual distribution of Hp phenotypes. There was no intentional selection of patients with regard to Hp phenotype; the small size of the sample population offers a partial explanation for the observed distribution.

**DISCUSSION**

The data presented here suggest that patients with active polycythemia vera catabolize Hp more rapidly than normal, resulting in many instances in low plasma Hp levels. It is hypothesized that this accelerated Hp catabolism reflects increased Hp-Hb formation, but the evidence for this is largely indirect, since overt hemolysis was not present. Thus, the patient with the shortest T% and lowest Hp levels (F.A.) had a normal RBC survival by the 51Cr method (T% = 28 days). It has been said that low Hp levels occur during hemolysis when RBC destruction increases by two- to fourfold (51Cr T% < 20 days).

The existence of a relatively small population of circulating erythrocytes which are more susceptible to intravascular lysis might result in increased during hemolysis when RBC destruction increases by two- to fourfold (51Cr technic, would be unaffected. Normally about 10 per cent of the 6-7 Gm. of hemoglobin catabolized daily as a result of red cell senescence and death is released intravascularly. This figure is obtained from a consideration of the plasma hemoglobin level (about 0.3 mg./100 ml.) and the turnover of tracer amounts of Hp-Hb complex (T% = 9-30 minutes). The mass of the Hb thus catabolized would be expected to increase in untreated polycythemic patients in whom the RBC mass was elevated, even if fractional Hb breakdown remained normal. None of our patients had frank erythrocytosis at the time of the study; in 2 with evidence of accelerated Hp catabolism, however, the RBC mass, determined with 51Cr labeled erythrocytes, was normal.

Increased intramedullary destruction of erythrocytes might also provide a source of hemoglobin for Hp binding, but could only be detected by sensitive methods for determining total heme catabolism. Thus, estimation of CO production as a measure of total heme catabolism, or studies with 14C glycine on the early appearance of 14C-labeled heme pigments in stool might contribute information on the source of hemoglobin available for Hp binding.

The fact that values for Hp turnover remain within relatively narrow limits, despite wide variation in the T% and fractional catabolic rate, suggests that increased Hp catabolism per se is not accompanied by increased Hp synthesis. Similar conclusions have been drawn by others in studies on 125I-Hp metabolism in hemolytic states. The normal rate of Hp synthesis would thus appear to be the limiting factor in the amount of Hp catabolized, in the absence of factors such as inflammation which increase Hp synthesis.

The elevated fractional catabolic rate of Hp which accompanies the rapid disappearance of plasma radioactivity in our polycythemic subjects has also been observed in hemolytic anemia, where the catabolic rate is inversely proportional to the Hp level. This inverse relationship between the plasma level of the protein and its catabolic rate is also seen in the case of transferrin,
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where the catabolic rate rises above normal as the transferrin level falls below normal. In inflammatory states, however, where Hp levels are increased, the fractional catabolic rate remains relatively unchanged, as does the plasma disappearance time, so that increased plasma levels must be the result of increased Hp synthesis. This latter pattern, where the mass, but not the proportion, of protein catabolized daily increases with increased plasma levels, is also seen in the catabolism of fibrinogen, the behavior of which closely parallels that of Hp in the “acute phase” response. In contrast, the fractional catabolic rate of gamma globulin (IgG) increases as the concentration increases, while with albumin the catabolic rate decreases with decreasing plasma concentration.

The data suggest that the T3 of plasma radioactivity, the rate of urinary excretion of radioactivity, and the fractional catabolic rate derived from these determinations are the most useful parameters of overall Hp catabolism, particularly in those circumstances where the plasma Hp level is influenced by a coexistent inflammatory process. The present study also indicates the need for sensitive technics to determine small increases in the intravascular or intramedullary release of hemoglobin, so that such data may be correlated with information obtained from 125I-Hp studies.

SUMMARY

1. Turnover studies with 125I-labeled haptoglobin (Hp) were performed in 7 patients with polycythemia vera, 2 patients with erythrocytosis of unknown etiology, and 2 control subjects.

2. The T3 of plasma radioactivity was shortened in 6 of the 7 patients with polycythemia vera; 3 of these had diminished plasma Hp levels but lacked other evidence of hemolysis.

3. The fractional catabolic rate exceeded 40 per cent/day in all subjects with a shortened half-time of plasma radioactivity.

4. Increases in the fractional catabolic rate were not accompanied by increases in Hp turnover (mg/kg/day), suggesting that accelerated Hp catabolism per se does not provide a stimulus to Hp production.

5. It is concluded that patients with polycythemia vera catabolize Hp more rapidly than nonpolycythemic subjects, possibly because of increased formation and removal of the haptoglobin-hemoglobin complex.

SUMMARIO IN INTERLINGUA

1. Studios del rotation de haptoglobina (Hp) esseva effectuate con haptoglobina marcate a 125I in 7 patientes con polycythemia ver, 2 patientes con erythrocytosis de mcognite etiologia, e 2 subjectos de controlo.

2. Le tempore de medie valor del radioactivitate in le plasma esseva reducite in 6 del 7 patientes con polycythemia ver. In 3 del 6 le nivellos plasmatic de Hp esseva reducite in le absentia de altere evidentia de hemolyse.

3. Le fractional prorata catabolic excedeva 40 pro cento per die in omne le subjectos con un reducite tempore de medie valor del radioactivitate in le plasma.

4. Augmentos in le fractional prorata metabolic non esseva accompaniate de augmentos in le rotation de Hp (mg/kg/die), suggestionante que un accelerate catabolismo de Hp per se non provide un stimulation del production de Hp.
5. Es conclusionate que pacientes con polycythemia ver catabolisa Hp plus rapidamente que subjectos nonpolycythemic, possibilemente a causa de un augmentate formation e elimination del complexo de haptoglobina e hemoglobina.

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REFERENCES

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