Hemolysis and Erythropoiesis

I. Influence of Intraperitoneal Administration of Whole Hemolysates on the Recovery of Bled Dogs, as Measured by Changes in the Total Erythrocytic Volume.

By LUIS SÁNCHEZ-MEDAL, JUAN LABARDINI AND ALVAR LÓRÍA

SUGGESTIVE EVIDENCE that the oral or parenteral administration of hemolysates increases the rate of erythropoiesis in bled animals was reported early in this century when it was even postulated that erythrocytic destruction products are the main regulating factors of the normal bone marrow activity. Since 1940, however, the study of the relationship between hemolysis and erythropoiesis has received the attention of very few investigators.

Our clinical observations are in accord with the concept that erythrocytic production in hemolytic anemia is greater than in the therapeutic response of anemia caused by iron or vitamin B₁₂ deficiencies. These observations and the inconclusive evidence regarding the stimulatory action of hemolysates prompted the study of the problem, using more accurate procedures.

In this paper it is reported that intraperitoneal administration of hemolysates to bled dogs increases by one and five tenths times the erythrocytic production as compared with the use of intraperitoneal saline in the same dogs.

METHODS

Four normal adult dogs, weighing 22 to 24 Kg., were kept in individual cages under constant hygiene, temperature and diet conditions. Preliminary to the initiation of the study, the animals were submitted to repeated intraperitoneal punctures for their adaptation to the subsequent experimental procedures.

Each animal was used as its own control and therefore underwent two periods of study. In each period, the dogs were rendered anemic by three 22 to 25 ml. per Kg. bleedings, on alternate days. On the day following the third bleeding, their erythrocytic volume was measured by the Cr⁵¹ method. Immediately afterwards, they received daily injections of: (a) 150 mg. of intramuscular iron-dextran, as Imferon, until the amount of iron removed through the bleedings was replaced; (b) 1.5 ml. per Kg. of isotonic saline, intraperitoneally, throughout the control period, or an equal volume of an autohemolysate during the test period. Fourteen to 18 days later, the erythrocytic volume was determined again with the Cr⁵¹ method. A 2- to 3-month interval was allowed between both periods. Two animals received the saline first and the hemolysate in the second period (dogs 2 and 3); a reversed sequence was followed in the other two (dogs 1 and 4).

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*We are indebted to Lakeside Laboratories of Mexico for the Imferon kindly supplied for this investigation.
Duplicate hematocrit and hemoglobin determinations in venous blood were performed two or three times a week.

The autohemolysates were prepared with blood obtained in the first bleeding—that is, when the dog was non-anemic. The erythrocytes were washed thrice with saline and resuspended in a volume of distilled water equal to the amount of the original plasma, and freezed and thawed as many times as necessary to obtain a hemolysis of 97 per cent or more. The degree of hemolysis was controlled by microscopic cell counting. The hemolysates contained 14.6 to 16.0 Gm. of hemoglobin per 100 ml.; the daily dose of hemolysate per Kg. given to the animals was equivalent to approximately 0.75 ml. of packed red cells.

Hemoglobin determinations were made by the cyanmethemoglobin method. The packed cell volume was determined in Wintrobe tubes centrifuged at 2318 g. during 30 minutes. The erythrocytic volume (EV) was determined as described elsewhere.\(^1\)

The erythrocytic volume gained during each period (final EV minus initial EV) divided by the weight of the animal and the days elapsed between both Cr\(^51\) estimations gave the erythrocytic increase in terms of ml. of red cells per day and per Kg. For the computation of the hemoglobin mass (HbM), the following formula was used:

\[
HbM = \frac{EV \times \text{Gm. of Hb in 100 ml. of blood}}{\text{Hematocrit in per cent} \times 0.96}
\]

Factor 0.96 was used to correct the hematocrit for trapped plasma.\(^5\)

EV and HbM increases observed in a given animal during the hemolysate period were compared with his own saline period values.

**RESULTS**

Before the start of the investigations, the animals had a mean hemoglobin of 18.2 Gm. per 100 ml., a mean hematocrit of 54.6, and a mean value of 33.3 for the MCHC. The weight of the animals did not vary more than one Kg. throughout the entire experiment. After the bleedings, the degree of anemia was similar in both experimental periods (table 1); at the end of the periods, lasting 15.0 and 15.1 days as an average, saline administration gave lower venous hemoglobin values than those following hemolysate. Hence, the average daily gain in venous hemoglobin was 0.258 Gm. per 100 ml. in the control experiments and 0.366 when the hemolysate was used.

Table 2 gives the initial and final EV, the per Kg. per day gain in EV and the ratio between the latter in the hemolysate period over the saline period. In every case this ratio was above 1, indicating that erythrocytic production was higher when the animals were injected with the hemolysate. This ratio ranged from 1.126 to 2.411 with a mean of 1.568.

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### Table 1.—Peripheral Blood Values at Start and End of Each Period of Study*  

<table>
<thead>
<tr>
<th>Dog</th>
<th>Initial</th>
<th>Final</th>
<th></th>
<th>Initial</th>
<th>Final</th>
<th></th>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb (Gm. x 100 ml.)</td>
<td>Hct. (%)</td>
<td>MCHC (%)</td>
<td>Hb (Gm. x 100 ml.)</td>
<td>Hct. (%)</td>
<td>MCHC (%)</td>
<td>Saline</td>
<td>Hemolysate</td>
<td>I</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S H</td>
<td>S H</td>
<td>S H</td>
<td>S H</td>
<td>S H</td>
<td>S H</td>
<td>I</td>
<td>F</td>
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</tr>
<tr>
<td>1</td>
<td>8.2 8.7</td>
<td>12.8 14.6</td>
<td>25.7 27.6</td>
<td>35.7 43.9</td>
<td>31.9 33.0</td>
<td>31.5 33.2</td>
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<td></td>
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<td>2</td>
<td>9.9 7.9</td>
<td>14.9 15.0</td>
<td>33.2 28.0</td>
<td>40.4 45.8</td>
<td>29.8 30.1</td>
<td>29.2 30.1</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>3</td>
<td>8.5 8.2</td>
<td>12.8 14.7</td>
<td>25.8 25.7</td>
<td>37.3 43.9</td>
<td>32.3 32.9</td>
<td>31.9 33.4</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>4</td>
<td>8.2 9.6</td>
<td>10.4 12.1</td>
<td>25.6 29.3</td>
<td>32.2 36.4</td>
<td>32.0 32.2</td>
<td>32.7 33.2</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>8.7 8.6</td>
<td>12.6 14.1</td>
<td>27.7 27.4</td>
<td>39.4 43.5</td>
<td>31.5 32.0</td>
<td>31.3 32.4</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* S = saline period; H = hemolysate period; I = initial value; F = final value.
Table 2.—Erythrocytic Volumes at Start and End of Each Period of Study

<table>
<thead>
<tr>
<th>Dog</th>
<th>Initial EV (ml.)</th>
<th>Final EV (ml.)</th>
<th>EV gain (ml./Kg./day)</th>
<th>Initial EV (ml.)</th>
<th>Final EV (ml.)</th>
<th>EV gain (ml./Kg./day)</th>
<th>Hemolysate Gain</th>
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<tbody>
<tr>
<td>1</td>
<td>267</td>
<td>497</td>
<td>0.655</td>
<td>287</td>
<td>562</td>
<td>0.738</td>
<td>1.126</td>
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<tr>
<td>2</td>
<td>516</td>
<td>870</td>
<td>1.228</td>
<td>396</td>
<td>889</td>
<td>1.580</td>
<td>1.286</td>
</tr>
<tr>
<td>3</td>
<td>343</td>
<td>588</td>
<td>0.762</td>
<td>354</td>
<td>710</td>
<td>1.106</td>
<td>1.451</td>
</tr>
<tr>
<td>4</td>
<td>378</td>
<td>493</td>
<td>0.340</td>
<td>463</td>
<td>739</td>
<td>0.820</td>
<td>2.411</td>
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<tr>
<td>Mean</td>
<td>376.0</td>
<td>612.0</td>
<td>0.746</td>
<td>375.0</td>
<td>725.0</td>
<td>1.061</td>
<td>1.568</td>
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</table>

Table 3.—Hemoglobin Mass Values at Start and End of Each Period of Study

<table>
<thead>
<tr>
<th>Dog</th>
<th>Initial HbM (Gm.)</th>
<th>Final HbM (Gm.)</th>
<th>HbM gain (Gm./Kg./day)</th>
<th>Initial HbM (Gm.)</th>
<th>Final HbM (Gm.)</th>
<th>HbM gain (Gm./Kg./day)</th>
<th>Hemolysate Gain</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>86.9</td>
<td>167.7</td>
<td>0.237</td>
<td>92.3</td>
<td>190.7</td>
<td>0.258</td>
<td>1.088</td>
</tr>
<tr>
<td>2</td>
<td>157.0</td>
<td>267.7</td>
<td>0.377</td>
<td>118.2</td>
<td>273.2</td>
<td>0.500</td>
<td>1.326</td>
</tr>
<tr>
<td>3</td>
<td>113.1</td>
<td>197.8</td>
<td>0.258</td>
<td>115.2</td>
<td>242.5</td>
<td>0.389</td>
<td>1.507</td>
</tr>
<tr>
<td>4</td>
<td>123.5</td>
<td>162.4</td>
<td>0.112</td>
<td>154.7</td>
<td>250.6</td>
<td>0.281</td>
<td>2.508</td>
</tr>
<tr>
<td>Mean</td>
<td>120.12</td>
<td>198.90</td>
<td>0.246</td>
<td>120.10</td>
<td>239.25</td>
<td>0.357</td>
<td>1.607</td>
</tr>
</tbody>
</table>

A similar comparison of the HbM values is presented in table 3. The hemolysate/saline ratio of hemoglobin gain ranged from 1.088 to 2.508 with a mean of 1.607.

Figure 1 shows, comparatively, the speed with which the venous hematocrit rose in the control and in the test experiments, in each dog.

In all instances, increments in HbM and EV were higher than increments in venous hemoglobin and hematocrit. This can be seen in table 4: the mean venous hematocrit at the end of the eight experimental periods was 50.63 per cent higher than the initial hematocrit, whereas the final EV was 79.6 per cent higher than the initial one. A similar phenomena was observed in respect to venous blood hemoglobin concentration and hemoglobin mass.

DISCUSSION

Previous investigations on the erythropoietic action of hemolysates are objectionable on several points:

1. Responses were judged by differences observed in reticulocyte per cent values and/or by the speed with which blood concentrations in erythrocytes and hemoglobin increased. At present the limited value that reticulocyte counts have for quantitating erythropoiesis is well recognized. Erythrocyte and hemoglobin concentrations are influenced by changes in blood volume and these changes are known to occur in anemia and during the recovery phase.

2. The iron removed during the induced anemia was not restored. Its lack could have limited the recovery of the control animals whereas this element was provided to the test animals through the hemolysate. In some experiments, 5 ml. of hemolyzed blood per Kg. and per day were in-
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Fig. 1.—Change in venous hematocrit during the control (●−−●) and test (●—●−●) experimental periods. Time in days, on abscissae, and units of venous hematocrit above the initial value, on ordinates.

jected; this was equivalent to parenterally administering about 2.5 mg. of iron daily per Kg. of body weight.

(3) Different animals were used in the control and in the test experiments Whipple and Robscheit-Robbins26, 31 observed that some dogs of their colony constantly regenerated more hemoglobin than others. The present study confirms their observations: dog 2 produced two to nearly four times as much red cells as dog 4, with dogs 1 and 3 giving intermediate values (table 2). There is no reason to believe that rabbits and rats, which were used in previous investigations, do not share the same individual differences demonstrated by Whipple et al. in dogs.

In the present work, the response was judged by determining the increases of erythrocytic volume measured with an accurate method (Cr51); in this manner the plasma volume variations can be disregarded. In accordance with previous observations,16 apparently the plasma volume did not decrease as much as the red cell volume increased—that is, the increments of EV were not reflected by changes of equal magnitude in the venous hematocrit (table 4).

Iron was early provided in our experiments in an equal amount to that lost through the bleedings. The replaced iron can be considered as sufficient since the experiments were concluded before the animals reached complete normalcy. Another precaution taken2, 31 was to adapt the dogs to the environmental conditions and experimental procedures, which on the other hand were the same on both control and test periods. Finally, the fact that the production of each animal when receiving the hemolysate was compared with its own control production greatly minimized errors.
Table 4.—Comparison of the Increments in Total and Peripheral Blood Values in the Recuperation Phases of the Experimental Dogs

<table>
<thead>
<tr>
<th>Dog</th>
<th>Period</th>
<th>Hct Gain† (%)</th>
<th>EV Gain† (%)</th>
<th>Hb Gain† (%)</th>
<th>HbM Gain† (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S</td>
<td>50.5</td>
<td>86.1</td>
<td>56.0</td>
<td>92.9</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>59.0</td>
<td>95.8</td>
<td>67.8</td>
<td>106.6</td>
</tr>
<tr>
<td>2</td>
<td>S</td>
<td>48.7</td>
<td>68.6</td>
<td>50.5</td>
<td>70.5</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>84.4</td>
<td>124.4</td>
<td>89.8</td>
<td>131.1</td>
</tr>
<tr>
<td>3</td>
<td>S</td>
<td>41.8</td>
<td>71.4</td>
<td>44.7</td>
<td>74.8</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>70.8</td>
<td>100.5</td>
<td>79.2</td>
<td>110.5</td>
</tr>
<tr>
<td>4</td>
<td>S</td>
<td>25.7</td>
<td>30.4</td>
<td>28.8</td>
<td>31.4</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>24.2</td>
<td>59.6</td>
<td>26.0</td>
<td>61.9</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>50.63</td>
<td>79.60</td>
<td>55.10</td>
<td>84.96</td>
</tr>
</tbody>
</table>

*S = saline; H = hemolysate.
†Hct = venous hematocrit; EV = erythrocytic volume; Hb = venous hemoglobin; HbM = hemoglobin mass. Gains were computed by formula: (final value - initial value)/100/initial value.

The present study furnishes strong evidence that hemolysates enhance the recovery from the anemia produced by blood removal in dogs, an effect apparently not due to its iron content. This is contrary to the findings of Finch et al.8 who observed no differences between the bone marrow activity of bled and phenylhydrazine-treated rabbits. The incongruity could be explained by the relatively short time of observation (3 and 6 days) used by these authors, since it could possibly be that the effect of hemolysates takes more than 6 days to become evident, as suggested by the comparison of the venous hematocrit increments observed during both periods (fig. 1). In three of our four dogs the hematocrit curves differ clearly only on the final days of study. Furthermore, Finch and co-workers made comparisons between different lots of rabbits and their methods seem more susceptible to error.

The mechanism of hemolysate action on erythropoiesis is unknown. If hemopoietine production is dependent only on the degree of anoxia4,14,22 (i.e., blood hemoglobin levels in the case of anemia), the results of the present study cannot be ascribed to differences in the amount of circulating hemopoietine: blood hemoglobin concentrations were similar at the start of both test and control experiments. However, the possibility that factors other than anoxia may stimulate production of hemopoietine can not be disregarded; it is conceivable that red cell constituents may stimulate production of this hormone. Many other alternate possibilities also exist, although one, reutilization of red cell constituents, seems unlikely in view of the findings of Bale et al.1 So far, we are inclined to believe that hemolysates indirectly stimulate red cell production through a humoral mechanism different from anoxic hemopoietine.

**SUMMARY**

The effect of RBC hemolysates on the recovery from anemia due to blood removal was studied in four dogs. For that purpose each dog was bled on two occasions to obtain similar degrees of anemia, and the erythrocytic pro-
duction was measured during the 14- to 18-day lapse following each bleeding. A 2- to 3-month interval was allowed between bleedings. The erythrocytic production was evaluated by Cr⁵¹ determinations of the circulating erythrocyte volume (EV) after each bleeding and at the end of the 14- to 18-day lapse. During the control study, the dogs received iron intramuscularly and saline intraperitoneally; the latter was substituted by hemolyzed RBC during the test study. The erythrocytic production, in terms of ml of EV gained per Kg. and per day, in every animal was higher during the test study: 1.126 to 2.411 (mean, 1.568) times the production of the same dog during the control study.

The results furnish strong evidence that the products of erythrocytic destruction have an enhancing effect on the recovery of anemia induced by bleedings in dogs.

**SUMMARIO IN INTERLINGUA**

Le effecto de hemolysatos de erythrocytos super le restablimento ab anemia causate per le extraction de sanguine esseva studiate in quatro canes. Pro ille objectivo, omne le canes esseva sanguinate in duo occasiones usque a simile grados de anemia, e le production de erythrocytos esseva mesurate durante le intervallo de 14 a 18 dies post le un e le altere sanguination. Un periodo de 2 a 3 menses esseva intercalate inter le duo sanguinationes. Le production de erythrocytos esseva evalutate per medio del uso de Cr⁵¹ in determinationes del circulante volumine de erythrocytospost omne sanguination e al fin del intervallo de 14 a 18 dies. In le studio de controlo, le canes recipeva ferro per via intramuscular e solution salin per via intraperitonee. Iste ultime esseva reimplaciate per hemolysate erythrocytos durante le test. Le production erythrocytic, exprimite in ml de volumine erythrocytic ganiate per kg per die, esseva plus alte in omne le animales durante le test, i.e. 1,126 a 2,411 vices o, al media, 1,568 vices le valor trovate in le mesme can durante le studio de controlo.

Le resultatos forni forte indicationes que le productos del destruction erythrocytic exerce un effecto promotori super le restablimento ab anemia inducite in canes per sanguination.

**REFERENCES**


HEMOLYSIS AND ERYTHROPOIESIS I

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