The Hemolytic Anemia of Human Bartonellosis

By CÉSAR REYNAFARJE AND JOSÉ RAMOS

HUMAN bartonellosis, or Carrion's disease, is characterized by rapid development of severe hemolytic anemia in the acute stage of the disease caused by infestation of the erythrocytes by Bartonella bacilliformis. The bacterium described by Barton in 1905 is visible in the red cells at the acute stage, so that their examination makes the diagnosis very easy (fig. 1). Fever of sudden onset and irregular course, generalized bone pain, and general malaise are the most frequent symptoms which accompany the anemic phase. There follows a quiescent period in which the parasite disappears from the red blood cells, the anemia improves and, after a variable time, the second stage begins. This is characterized by the appearance in the skin and in some organs of nodules composed by histoid granulomata with capillary proliferation, to which the disease owes its name, verruga peruana.

Although studies had been made of this anemia since 1898, its hemolytic character was first described by Monge in 1915. Hemolysis was demonstrated by the elevated indirect bilirubin, the increased phagocytosis of erythrocytes seen in autopsies, the indications of red cell regeneration, i.e., reticulocytosis and bone marrow hyperplasia, and by the increase in fecal urobilinogen. Other characteristics have been described by Hurtado, Merino and Pons.

However, some aspects of the hemolytic process in this disease could not be adequately studied until recent developments in hematologic technics became available. In the past three years we have carried out studies related to red cell survival, the mechanical fragility or parasitized red cells, metabolism of iron, immunohematologic observations, the site of sequestration of the red cells, and other investigations of the hemolytic process in this disease. Some of the findings have been presented in preliminary form. In the present paper we are evaluating all the studies, as well as reviewing the anemia of human bartonellosis in general.

MATERIALS AND METHODS

Twenty-eight patients with bartonellosis were studied, 23 of them in the anemic stage and 5 in the eruptive stage.

At the beginning of the study, most of the patients in the anemic stage were at the height of their illness with a high percentage of parasitized red cells (as much as 80 to 100 per cent in many of them). Red cells were marked with radioactive chromium for red cell life-span studies. In some cases approximately 300 μc of the isotope in the form of sodium chromate were injected intravenously, whereas in others the erythrocytes whose survival was to be studied were previously tagged by incubating 20 cc. of blood with 100 μc of Cr-51. The study on
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the mechanical fragility of the erythrocytes was carried out according to the method of Shen, Castle, and Fleming. In all anemic patients the production of red cells and the metabolism of iron were studied following the method of Humff,17 by injecting intravenously 10 μc. of Fe-59 with serum globulin fraction IV-7. The technic of Moore,18 was used to determine inert plasma iron. Investigation of the sites of sequestration of red cells was carried out by the method described by Jandl et al.,19 and consisted chiefly in injecting red cells tagged with Cr-51 and counting the radioactivity over the heart, spleen and liver with a scintillation counter of directional type at intervals after the transfusion of 15 minutes, 24 hours, and then every 3 to 7 days. Both the patients in the anemic stage and those in the eruptive period were examined for the possible existence of agglutinins against red cells by the Coombs’ autoglobulin method.20 Patients were also tested for cold agglutinins and hemolysins. The sternal bone marrow was studied; the blood volume, by means of Fe-59; free protoporphyrin in the red cells, by the method of Grinstein and Watson;21 the total and fractionated bilirubin, using the technic of Malloy and Evelyn;22 fecal urobilinogen, by Watson’s method;23 reticulocytosis, by Brecher’s method;24 the diameter of nonreticulated red cells, and the hemoglobin and hematocrit, by the usual methods.

RESULTS

Life Span of Erythrocytes

First the longevity of the red cells was studied in six patients. The results are shown in figures 2 and 3 in the form of curves relating the proportion of surviving red cells to the time in days. In three of the patients (fig. 2), the erythrocytes were labeled by injecting sodium chromate intravenously. In three other cases (fig. 3), blood was drawn from the patient, incubated with radioactive chromium, and then injected.

As may be seen in both figures, there was a rapid fall in the curves in all the cases, indicating that great destruction of red cells occurred in the first few days. Later, the curve tended to follow the line of the zone of normal variation (shaded zone, fig. 3) obtained from measurements in ten healthy subjects, signifying that part of the red cells had escaped the increased destruction. It is of particular interest that this occurred in patients whose red cells were 100 per cent parasitized (Note the upper right corner in fig. 2 and 3). Note also, that there was no major difference in the results obtained by either method of labeling the cells.

In order to clarify the controversy over the use of blood transfusions in treating human bartonellosis,25,26 we studied the red cell survival of transfused blood from healthy subjects given to patients with high degrees of parasitization. The results are presented in figure 4; although the curve of red cell survival fell rapidly the first few days in the five patients studied, it later became modified to follow that of the healthy subjects. This indicates that the donor cells were promptly destroyed in a certain proportion, probably because they were parasitized, but that a great portion of them had normal survival times. This varied with the degree of parasitization of the recipient and the duration of parasitization in the red cells. Although it is difficult to estimate the quantity of red cells that escaped destruction, one can infer that it was not less than 50 per cent in the majority of the cases.

In figure 5 we show on the left side the results that were obtained on injecting red cells from a patient in the eruptive stage into one with 100 per
cent of his red cells parasitized. Under these conditions, the survival curve fell within the zone of normal variation; that is, there was no increase in the destruction of these cells, presumably because they were not parasitized, in spite of the fact that the patient had been infected for almost a month, with evidence of major red cell destruction indicated by an appreciable increase in the indirect bilirubin. Former studies of this phenomenon indicated that it was due to a resistance to destruction developed by the red cells in patients in the eruptive stage.

On the right side of figure 5, we show the results obtained when red cells from a patient who had just progressed from the parasitized phase were given to a patient with 100 per cent parasitization of his erythrocytes. In this instance the survival curve fell rapidly, indicating that the red cells were probably parasitized and destroyed. If one assumes that the red cells can ac-
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Fig. 2.—Autogenous red cell survival in 3 patients with human bartonellosis. Red cells were labeled by injecting sodium chromate intravenously.

Fig. 3.—Autogenous red cell survival in 3 patients with human bartonellosis. Red cells had previously been incubated with sodium chromate.
Fig. 4.—Survival of red blood cells from normal individuals transfused into patients with human bartonellosis.

Fig. 5.—(Left): Survival of red cells transfused from a patient in the eruptive phase of Carrion's disease into one with 100 per cent red cell parasitization. (Right): Survival of red cells transfused from a patient who had just recovered from parasitized phase into one with 100 per cent of his red cells parasitized.

quire resistance in the eruptive stage of the disease, this last observation would indicate that they are not yet able to acquire resistance in the anemic stage of the disease.

Mechanical Fragility of Erythrocytes

The possibility that one of the factors explaining major destruction of red cells in human bartonellosis might be that of an abnormality in osmotic fragility has been raised by Hurtado, Merino and Pons. They noted fairly often a slight increase in fragility but attached no great importance to it in explain-
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Table 1.—Mechanical Fragility of Red Cells in 37 Normal Subjects and in 21 Patients with Human Bartonellosis

<table>
<thead>
<tr>
<th></th>
<th>Normal (Hemolysis %)</th>
<th>Bartonellosis (Hemolysis %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Blood Mean</td>
<td>4.99 ± 0.25</td>
<td>10.56 ± 0.96</td>
</tr>
<tr>
<td>Incubated Mean</td>
<td>15.75 ± 0.95</td>
<td>18.9 ± 1.22</td>
</tr>
</tbody>
</table>

Table 2.—Mechanical Fragility of Red Cells During and After Parasitization with Bartonella Bacilliformis

<table>
<thead>
<tr>
<th>No</th>
<th>Parasitized</th>
<th>Fresh Blood</th>
<th>Non-Parasitized</th>
<th>After Incubation</th>
<th>Non-Parasitized</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BC</td>
<td>13.8</td>
<td>8.5</td>
<td>25.0</td>
<td>16.1</td>
</tr>
<tr>
<td>2</td>
<td>DN</td>
<td>10.0</td>
<td>7.0</td>
<td>18.1</td>
<td>19.2</td>
</tr>
<tr>
<td>3</td>
<td>JM</td>
<td>17.3</td>
<td>7.0</td>
<td>21.3</td>
<td>21.5</td>
</tr>
<tr>
<td>4</td>
<td>JR</td>
<td>15.8</td>
<td>5.5</td>
<td>22.8</td>
<td>13.7</td>
</tr>
<tr>
<td>5</td>
<td>DN</td>
<td>15.0</td>
<td>6.6</td>
<td>20.5</td>
<td>8.9</td>
</tr>
<tr>
<td>6</td>
<td>HL</td>
<td>3.1</td>
<td>6.5</td>
<td>20.3</td>
<td>17.9</td>
</tr>
<tr>
<td>7</td>
<td>TT</td>
<td>9.4</td>
<td>5.0</td>
<td>13.2</td>
<td>15.3</td>
</tr>
<tr>
<td>8</td>
<td>RM</td>
<td>9.1</td>
<td>2.2</td>
<td>24.7</td>
<td>14.8</td>
</tr>
<tr>
<td>9</td>
<td>JA</td>
<td>8.9</td>
<td>4.6</td>
<td>14.9</td>
<td>17.7</td>
</tr>
<tr>
<td>Mean</td>
<td>11.37</td>
<td>5.88</td>
<td>20.08</td>
<td>16.12</td>
<td></td>
</tr>
<tr>
<td>±S.E.</td>
<td>±1.50</td>
<td>±0.69</td>
<td>±3.26</td>
<td>±1.21</td>
<td></td>
</tr>
</tbody>
</table>

The mechanical fragility of red cells remained to be studied. In table 1 are the results of such a study in 21 patients. As can be seen, the average percentage of hemolysis of fresh blood reached levels of 10.56 per cent ± 0.96 in contrast to 4.99 per cent ± 0.25 seen in 37 normal subjects. However, in 4 of the 20 patients the mechanical fragility was unaltered. In the blood incubated for 24 hours, the index of hemolysis was less changed, as is evident on comparing the average obtained in the 20 patients, 18.9 per cent ± 1.22 with that obtained in 37 healthy subjects, 15.7 per cent ± 0.95. It should be pointed out that there was no relation between the rate of increase of mechanical fragility and the parasitization of red cells.

It is interesting to note that the mechanical fragility became normal when the parasitization of red cells had disappeared completely and the anemia had improved. As can be seen in table 2, in those patients that were studied at the time of discharge from the hospital, the mechanical fragility in fresh blood was 5.88 per cent ± 0.69, and in the incubated blood 16.12 per cent ± 1.21, which is very nearly normal.

Products of Hemoglobin Catabolism

The results obtained in the present study show a great variability in the levels of bilirubin, not only in the total amount, but also in the relation between the direct and indirect fractions (see table 3). Thus we have found cases in which there was an increase of both types of bilirubin, with predominance of the indirect fraction; others in which there was increase of both types of bilirubin without predominance of either fraction, and, finally, only a
Table 3.—Bilirubin Determinations in 10 Patients with Human Bartonellosis

<table>
<thead>
<tr>
<th>Case</th>
<th>B. Direct (mg. per 100 cc.)</th>
<th>B. Indirect (mg. per 100 cc.)</th>
<th>B. Total (mg. per 100 cc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.70</td>
<td>5.40</td>
<td>14.10</td>
</tr>
<tr>
<td>2</td>
<td>0.26</td>
<td>0.64</td>
<td>0.90</td>
</tr>
<tr>
<td>3</td>
<td>0.21</td>
<td>1.08</td>
<td>1.29</td>
</tr>
<tr>
<td>4</td>
<td>0.68</td>
<td>0.60</td>
<td>1.28</td>
</tr>
<tr>
<td>5</td>
<td>0.80</td>
<td>1.70</td>
<td>2.50</td>
</tr>
<tr>
<td>6</td>
<td>1.19</td>
<td>1.47</td>
<td>2.66</td>
</tr>
<tr>
<td>7</td>
<td>0.77</td>
<td>1.92</td>
<td>2.69</td>
</tr>
<tr>
<td>8</td>
<td>0.42</td>
<td>0.42</td>
<td>0.84</td>
</tr>
<tr>
<td>9</td>
<td>3.41</td>
<td>2.11</td>
<td>5.56</td>
</tr>
<tr>
<td>10</td>
<td>0.60</td>
<td>1.81</td>
<td>2.41</td>
</tr>
</tbody>
</table>

part of the patients had an increase exclusively of the indirect bilirubin. Since in this disease hepatic complications frequently occur, the bilirubinemia is apparently caused not only by great destruction of red cells, but also by the hepatic lesions which alter the parenchyma as well as the bile canaliculi.

The fecal urobilinogen determination could not be made because wide-spectrum antibiotics had been given to the patients in the majority of the cases. Only in a few cases were we able to collect feces for a period of 24 hours before the administration of antibiotics, finding in them levels over 700 mg./day. This increase is well confirmed by previous investigations.10,11

Coombs’ Test

In order to investigate the existence of auto-agglutinins as a possible cause of the massive destruction of red cells in human bartonellosis, the Coombs’ test was made both by the direct and indirect method in 10 patients in the anemic stage and in 5 in the eruptive stage. All the tests were negative. All tests for cold agglutinins and hemolysins were negative.

These observations removed the possibility that one of the mechanisms of red cell destruction in Carrion’s disease might be agglutination or hemolysis of immunologic type. Repeated studies in the past, using different methods, also failed to demonstrate agglutinins in this disease.27-29

Sites of Sequestration and Destruction of Red Cells

Three of our patients in the anemic phase were explored for sites of sequestration of red cells, by labeling with Cr-51 cells withdrawn from the patients, as described under MATERIALS AND METHODS above, and reinjecting the blood to note where the labeled cells had gone. Values were obtained with scintillation counter held over the areas of the spleen, liver, and heart. In figure 6, the results are compared with those obtained in normal subjects. The index of increase of sequestration of red cells in the spleen and liver is a function of time in the figure. The index was calculated by dividing the radioactivity found over the spleen and liver by that found in precordial area. In 2 patients, who at the time of the test had parasitization and anemia of mild degree, the increase of sequestration in the liver and spleen was not marked, but in the third, who had 90 per cent parasitization and severe anemia, this
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Fig. 6.—Sites of sequestration of red cells in 3 patients with human bartonellosis.

index was very marked. It is interesting to note that for some unknown reason the organ in which sequestration appeared to be most intense varied from patient to patient.

The data here presented give support for the concept proposed by Aldana, of destruction of red cells in the reticuloendothelial system.
Table 4.—Iron Turnover Rate in Eleven Bartonellosis Patients

<table>
<thead>
<tr>
<th>Case</th>
<th>Plasma Fe Turnover Rate (mg. Fe/day/Kg.)</th>
<th>Red Cell Fe Turnover Rate (mg. Fe/day/Kg.)</th>
<th>Plasma Iron Turnover Per 100 cc.</th>
<th>Total Plasma Iron (mg.)</th>
<th>Reticulocytes (Gm./R.B.C. %)</th>
<th>Hemoglobin (Gm./100 cc.)</th>
<th>Parasitized R.R.B.C. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.50</td>
<td>1.26</td>
<td>117.6</td>
<td>3.16</td>
<td>9.8</td>
<td>4.5</td>
<td>38</td>
</tr>
<tr>
<td>2</td>
<td>1.71</td>
<td>1.38</td>
<td>187.1</td>
<td>6.88</td>
<td>30.0</td>
<td>5.1</td>
<td>40</td>
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<tr>
<td>3</td>
<td>0.22</td>
<td>0.19</td>
<td>36.4</td>
<td>0.68</td>
<td>0.2</td>
<td>13.0</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>0.76</td>
<td>0.63</td>
<td>47.9</td>
<td>1.30</td>
<td>3.2</td>
<td>5.6</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>0.78</td>
<td>0.68</td>
<td>104.5</td>
<td>3.10</td>
<td>10.0</td>
<td>4.5</td>
<td>28</td>
</tr>
<tr>
<td>6</td>
<td>0.74</td>
<td>0.57</td>
<td>141.5</td>
<td>3.58</td>
<td>3.0</td>
<td>10.1</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>0.50</td>
<td>0.49</td>
<td>80.0</td>
<td>2.51</td>
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<td>6.7</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>0.47</td>
<td>0.44</td>
<td>59.7</td>
<td>1.52</td>
<td>2.7</td>
<td>9.2</td>
<td>90</td>
</tr>
<tr>
<td>9</td>
<td>1.02</td>
<td>0.94</td>
<td>142.6</td>
<td>4.36</td>
<td>3.9</td>
<td>4.0</td>
<td>96</td>
</tr>
<tr>
<td>10</td>
<td>2.26</td>
<td>1.90</td>
<td>122.2</td>
<td>3.07</td>
<td>19.0</td>
<td>6.6</td>
<td>27</td>
</tr>
<tr>
<td>11</td>
<td>2.37</td>
<td>2.01</td>
<td>100.0</td>
<td>3.55</td>
<td>28.0</td>
<td>4.3</td>
<td>36</td>
</tr>
<tr>
<td>Mean</td>
<td>1.12</td>
<td>0.95</td>
<td>103.4</td>
<td>3.06</td>
<td>10.3</td>
<td>6.6</td>
<td>54.4</td>
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<tr>
<td>±S.E.</td>
<td>±0.22</td>
<td>±0.18</td>
<td>±13.7</td>
<td>±0.50</td>
<td>±1.0</td>
<td>±0.83</td>
<td>±11.7</td>
</tr>
</tbody>
</table>

Mean Values in 12 Normal Subjects

| Mean | 0.43 | 0.37 | 93.8 | 2.14 | 0.5 | 15.1 |
| ±S.E. | ±0.02 | ±0.02 | ±3.9 | ±0.15 | ±0.02 | ±0.34 |

Test for Red Cell Production and Iron Metabolism

In order to make a quantitative study of red cell formation as a response to the great blood destruction in bartonellosis, 11 patients in the anemic stage of the disease were tested. In table 4, we present the results of both the plasma iron turnover rate and the RBC iron turnover, the data being expressed in milligrams of iron per day per kilogram of weight. In every case, except for case 3, the figure for RBC iron turnover was higher than the average normal (0.35 mg./Kg.). Consequently, the total daily plasma turnover rate of iron by these patients was also increased. However, it may be seen that the increase in iron uptake varied from patient to patient. It depended on several factors: the degree of anemia at the time the test was done, the intensity of parasitization, and the presence or absence of secondary infection. In fact, in the cases of intense parasitization such as cases 7 and 8, the bone marrow response expressed by iron used for red cell formation was less than could be expected, probably due to the action of the infection on the erythropoietic organs. On the other hand, when the acute infectious stage was subsiding and there were no secondary infections, the production of red cells reached enormous proportions, five times greater than normal, as in cases 10 and 11. In case 3, there was a depression of red cell formation with iron utilization half of normal, but in this case the parasitization of red cells was only 2 per cent; there was a secondary Salmonella infection; and the anemia was not marked.

The curves of iron uptake by the bone marrow in 7 patients, presented in figure 7, shows a deviation to the left of the zone of normal variation (shaded area) obtained in 5 healthy subjects. This deviation indicates that the red cells prematurely entered the circulation from the bone marrow, due to the great peripheral demand. The other fact that can be inferred from these curves...
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is that after they reach their maximum they fall but later tend to recover. This indicates that a good part of the recently formed cells were rapidly destroyed. Paralleling the course of iron metabolism, the reticulocyte count in the peripheral blood reflected the bone marrow response to the peripheral stimulus of anemia disturbed at the start by the intensity of the toxic process. In fact, in the cases studied in which the parasitization was of great intensity, the reticulocyte response was poor in spite of the degree of anemia (cases 7 and 8). However, the reticulocytosis was of great intensity when the infectious process was subsiding, as can be seen in the cases in which the parasitism had diminished (cases 2, 5, 10 and 11).

Histology of the Bone Marrow

The study of the anatomic substrate of the erythropoietic tissues by bone marrow biopsy was carried out in 6 patients in the anemic phase. In figure 8 are shown the individual results. As can be seen, the production of nucleated red cells was increased in all of them, but also there was great individual variation. Thus in case 1, the total number of nucleated red cells was 59.5 per cent, while in case 2 it was 29.5 per cent. In the erythrocytes series the more mature forms predominated, although the increase in young forms, including proerythroblasts, was notable. There was also an appreciable increase of red cells in mitosis. In the granulocytic series, as well, an increase of the elements of intermediate maturation could be seen in response, undoubtedly, to the intense infectious process. The megakaryocyte series showed no significant changes.

These data on the bone marrow confirms the earlier findings of Carvallo and Urteaga. Other Determinations

Besides measuring hemoglobin and hematocrit to determine the degree of
Fig. 8.—Bone marrow in 6 patients with human bartonellosis.

Fig. 9.—Blood volume in human bartonellosis. Graphic representation of mean values in 11 patients.
anemia, the blood volume in 11 patients was studied (fig. 9). In all cases, as expected, there was an appreciable diminution in the mass of red cells, but at the same time there was a compensatory increase in plasma, approximately in the same proportion, so that the total volume was not greatly affected. This can be seen in the corresponding graph along with the average of observations made in 12 healthy subjects.

Changes in the diameter of red cells were also studied. Hurtado, Merino and Pons in a detailed study found a great variation in the diameter of the red cells, with a predominance of those of large size. The average red cell volume was also increased and the authors concluded that the anemia of human bartonellosis was macrocytic. It was thought, however, that the macrocytosis was due simply to the presence of numerous reticulocytes. To clarify this problem the diameter of nonreticulated red cells was measured. The resulting Price-Jones curves revealed that the increase in the diameter of the erythrocytes in this disease was independent of the reticulocytosis. In figure 10 are shown the results of this study, compared with the normal curve obtained in 5 healthy subjects.

It seemed interesting to study the free protoporphyrins in the red cells as well. In table 5 is presented the average obtained in 7 patients with bartonellosis, compared to that obtained in 12 normal subjects. The increase of this hemoglobin precursor was marked, reaching 190.4 μg per hundred cc. of red cells in the Bartonella patients, while the level in the healthy subjects was 30.6 μg.

Fig. 10.—Price-Jones curves of red cells without reticulum in 4 patients with human bartonellosis.
Table 5.—**Free Erythro-Protothorin in Human Bartonellosis**

<table>
<thead>
<tr>
<th>No.</th>
<th>Erythro-Protoporphyrins (gammas/100 cc.)</th>
<th>Hemoglobin (Gm./100 cc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>104.0</td>
<td>5.1</td>
</tr>
<tr>
<td>2</td>
<td>45.0</td>
<td>13.0</td>
</tr>
<tr>
<td>3</td>
<td>254.0</td>
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<td>3.5</td>
</tr>
<tr>
<td>5</td>
<td>441.0</td>
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</tr>
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<td>6</td>
<td>221.0</td>
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</tr>
<tr>
<td>7</td>
<td>132.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>190.0 ± 49.5</td>
<td>7.0 ± 1.2</td>
</tr>
</tbody>
</table>

Mean Values in 12 Normal Subjects

| Mean ± S.E. | 30.6 ± 4.5 | 15.1 ± 0.34 |

**DISCUSSION**

The presence of Bartonella bacilli in the red cells of patients suffering with Carrion's disease necessitates their removal by the reticuloendothelial system, so that their stay in the circulation is greatly shortened and is the basis for the anemia produced. The life span of the red cells in the typical patient with bartonellosis is thus greatly diminished. In some cases, only 50 per cent of the tagged red cells can be found by the sixth day. However, not all the parasitized red cells are removed from the circulation. This conclusion is based on the observation that in those cases in which all the red cells were parasitized at the time of labeling, a good part of them had a normal behavior from the point of view of survival, indicating that they rid themselves of the organism. It is not clear how the red cells accomplish this, but it is known that the spleen has the capacity of removing foreign particles from red cells without altering their structure. This would be facilitated in this disease by the fact that Bartonella, as has been demonstrated by Aldana, is not inside the red cell, but adherent to it.

As to the normal and compatible red cells that were injected into the bartonellosis cases in the midst of the stage of parasitized red cells, it should be emphasized that more than 50 per cent of them escaped destruction. These findings support the use of blood transfusions in the patients with very low hemoglobin levels. It is also interesting to observe the results of injection of cells from a patient in the second stage of the disease into a patient in the acute stage with 100 per cent parasitization of the red cells. The red cells of the patient in the eruptive stage, in which there is no anemia, presumably were not parasitized, for their survival time was normal. If this observation is confirmed by later studies, one may conclude that in the second stage the red cells can acquire resistance to the organism.

The study of the sites of sequestration of the red cells, as well as the increase in the products of catabolism of hemoglobin, indicate, however, that the reticuloendothelial system is responsible for removal of the red cells from the circulation in this disease. It is not clear whether the phagocytes, in destroy-
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The Bartonella bacillus, also destroy the red cell, or whether the bacillus has already produced alterations in the surface of the red cells, changing their enzyme content and thus making them more fragile and more easily phagocytosed. Nor is it known why in some cases the major destruction is accomplished preferentially in the spleen and in others in the liver, as may be inferred from the present study of the index of sequestration of the red cells.

Another factor that contributes to the massive destruction of red cells in bartonellosis is the fragility of the erythrocytes to mechanical action. This alteration does not occur in all patients, however, and it is not related to the degree of anemia or to the intensity of parasitization. Apparently it is more closely related to the changes in red cell morphology, which were marked in most cases or, perhaps, to the biochemical changes in the red cell that Bartonella may produce.

Since the Coombs' test and other tests for agglutinins or hemolysins were all negative, the possibility that an immunologic mechanism causes the anemia appears to be excluded.

When one considers the mechanism of greater production as a response to increased destruction of red cells, such as occurs in this anemia, one sees by the Fe-59 test that at the onset of the disease, when marked parasitization exists, the bone marrow is prevented by "toxicity" from responding adequately to the peripheral demand. This defective erythropoietic response in addition to an exaggerated destruction explains the rapid development of anemia in this disease. The iron metabolism studies show that in the patients in whom the infection is subsiding, erythropoiesis is three to five times greater. The reticulocytosis in the peripheral blood parallels the blood regeneration. Similarly, histologic examination of the bone marrow shows that erythropoiesis reaches its peak only when the infectious process is subsiding.

With respect to the synthesis of hemoglobin inside the erythroblasts of the bone marrow, it has been seen that it is deficient during the anemic stage and that it is reflected in the appreciable increase of protoporphyrins not saturated with iron in the red cells of these patients. This appears to be the explanation of the hypochromia that was described by Hurtado et al. It is necessary to make clear the fact that the above alteration does not depend in any way on an iron deficiency, for this element is increased in absolute figures in the plasma of these patients, and according to previous investigations it is also increased in the depot organs.

SUMMARY

A study of the processes of formation and destruction of blood has been carried out, in addition to other investigations of the physiopathology of the anemia of human bartonellosis. From the results obtained the following conclusions may be drawn:

1. The life-span of the red cells parasitized by Bartonella bacilliformis is greatly shortened. However, not all the parasitized red cells are prematurely destroyed.

2. Red cells from normal subjects are partially destroyed when they are injected into infected patients. More than 50 per cent of them survive normally.
3. The mechanical fragility of the red cells is increased in the majority of the cases.

4. The index of sequestration of red cells by the liver and spleen was increased in the three patients studied. Also, the products of catabolism of hemoglobin were increased in all the patients studied.

5. The increased production of red cells as a response to the great destruction was prevented at first, but later it reached its peak, being in some cases five times greater than normal.

6. The search for agglutinins and hemolysins was negative.

7. The amount of free protoporphyrins in the red cells was increased, indicating that there was some interference to the synthesis of hemoglobin that would also explain the hypochromia of the red cells.

8. The increase in the diameter of the red cells was independent of the actual amount of reticulocytes.

**Summario in Interlingua**

Esseva interprendite un studio del processos de formation e de destruction del sanguine, insimul con altere investigationes del physiopathologia del anemia de bartonellosis human. Ab le resultatos le conclusiones sequente esseva derivate.

1. Le superviventia del erythrocytos parasitivate per Bartonella bacilliformis es multo reducite. Nonobstante, non omne le parasitivate erythrocytos es destruite prematurmente.

2. Erythrocytos ab subjectos normal es Partialmente destruite quando illos es injucite in patientes infectate. Plus que 50 pro cento de illos supervive normalmente.

3. Le fragilitate mechanic del erythrocytos es augmentate in le majoritate del casos.

4. Le indice de sequestration de erythrocytos per le hepate e le splen esseva augmentate in le tres patientes studiate. De plus, le productos del catabolismo de hemoglobin es esseva augmentate in omne le patientes studiate.

5. Le augmentate production de erythrocytos in responsa al grande destruction esseva prevenite al initio, sed plus tarde illo attingeva su culmine—in certe casos a un nivello de cinque vices illo del norma.

6. Le cerca pro agglutininas e hemolysinas remaneva negative.

7. Le quantitate de protoporphyrinas libere in le erythrocytos esseva augmentate, indicante que il habeva un certe interferentia in le synthese de hemoglobin que etiam explicarea le hypochromia del erythrocytos.

8. Le augmento in le diametro del erythrocytos esseva independente del numeration de reticulocytos.

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