Siderocytes and the Spleen

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A SIDEROCYTE is a red blood cell with one or more granular inclusion bodies which contain enough ferric iron to give a positive Prussian-blue reaction. When siderocytes were first observed by Bizzozero[1] he interpreted the iron-staining granules to be a product of hemoglobin degradation. It is now known that about 50 per cent of the erythroblasts in normal bone marrow contain these granules[8] and it is generally accepted that siderocytes in the peripheral blood are descendants of the "sideroblasts" in the bone marrow. There are few if any siderocytes in the blood of normal persons; even in diseases characterized post-splenectomy by many siderocytes, they are rare when the spleen is intact.[6] The appearance after splenectomy of red cells with iron inclusion bodies has stimulated much speculation regarding the effect of the spleen on this phenomenon. Pappenheimer[10] suggested that the granules might be related to "certain intra-erythrocytic parasites." McFadzean and Davis[8] commented

> Since normoblasts containing the inclusions were equally abundant in the marrow both before and after splenectomy, it seems probable that the spleen is concerned with the removal of the affected erythrocytes from the peripheral blood rather than with the suppression of their formation. An obvious corollary of this theory is that a red cell containing inclusion bodies is a defective cell fated to rapid elimination from the circulation, a processin which the spleen plays a major part. According to this view, abnormal erythropoiesis resulting in the production of inclusion-containing cells may constitute a major aetiological factor in the cases of haemolytic anaemia under consideration.

This concept was accepted by others.[7, 9]

In 1948, a clinical experience indicated that the spleen might not destroy the red cells with iron inclusion bodies.[2] A patient with hereditary nonspherocytic hemolytic anemia was studied. Before splenectomy 0.1 per cent of the red cells in his peripheral blood were siderocytes. Pigment studies and red cell survival experiments indicated that he was turning over almost 50 Gm. of hemoglobin per day. After splenectomy the degree of anemia and the rate of hemolysis had not substantially changed but the siderocyte count increased to 45 per cent. If, before splenectomy, the spleen had been destroying the siderocytes, removal of the spleen and sparing of the siderocytes should have resulted in some abatement of the hemolytic disease. It did not. This suggested that the spleen was capable of removing the inclusion body without destroying the red cell that contained it. An experiment was designed to test this proposition.[3]

MATERIALS AND METHODS

The donors of the six transfusion experiments were patients who, for one reason or another, had their spleens removed and following splenectomy developed siderocytosis.

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TABLE 1.—*The Loss of Siderin Inclusion Bodies After Transfusion of Siderocytic Blood*

	1	2	3	4	5	6
1. Donor (all splenectomized)	Hereditary spherocytosis	Thalassemia minor	Hereditary spherocytosis	Polycythemia Vera		Hodgkin's disease
2. Donor's siderocyte count (per cent RBC)	14	57	62	20	18	38
3. Size of transfusion (ml. RBC)	480	20	250	275	280	105
4. Recipient	Hypoplastic anemia without splenomegaly	Uremic infant with anemia	Normal	Hodgkin's disease in remission	Acute leukemia 3 mo. postsplenectomy	Hypoplastic anemia 2 weeks postsplenectomy
5. Recipient's siderocyte count (per cent RBC)	0	0	0	0	3.8	4.2
6. Time from start of transfusion to first post-transfusion sample (minutes)	90	50	30	16	20	15
7. Anticipated increase in siderocytes post-transfusion (See Methods) (per cent recipient's RBC)	5(100%)*	17(100%)	5.3(100%)	2.8(100%)	6(100%)	4.5(100%)
8. Actual increase in siderocytes (per cent recipient's RBC)	3(60%)	14.3(87%)	4.6(84%)	2.8(100%)	6(100%)	4.2(93%)
9. Transfused siderocytes remaining (per cent recipient's RBC)						
at 1 hour	1.5(30%)	—	—	1.3(54%)	5.8(97%)	4.6(100%)
at 2 hours	1.0(20%)	13.6(80%)	3.0(57%)	—	4.5(75%)	3.7(82%)
at 4 hours	0.3(6%)	7.2(42%)	1.6(30%)	0	5.5(92%)	3.8(84%)
at 6 hours	—	5.7(30%)	—	—	4.0(67%)	3.3(73%)
at 24 hours	—	0.8(5%)	1.0(19%)	0	4.6(77%)	3.6(80%)
10. Transfused RBC remaining (percentage of those found immediately after transfusion)						
at 4 hours	100 (Ashby)	—	100 (Ashby) 92(Cr^{51})	—	96(Cr^{51})	100 (Ashby)
at 6 hours	—	84(Cr^{51})	—	100(Cr^{51})	—	—
at 24 hours	—	—	77 (Ashby) 73 (Cr^{51})	100(Cr^{51})	89(Cr^{51})	— 92 (Ashby)
11. If reduction of siderocyte counts were due to *destruction* of the erythrocytes that contain iron granules the values in line 10 above at 4 or 6 hours would have been (See Methods)	90	70	65	80	No significant loss of siderocytes	No significant loss of siderocytes

* Percentages in parentheses indicate the proportion remaining of the total quantity of siderocytes transfused into the recipient.

Donor 1 was a 21 year old soldier with hereditary spherocytosis. Five weeks after splenectomy his siderocyte count was 14 per cent, reticulocyte count 0.7 per cent and hematocrit 48 per cent. A one-liter phlebotomy was performed into ACD solution and the blood was transfused into a 71 year old man with anemia and a hypoplastic bone marrow; his spleen was not enlarged. The first 500 ml. were transfused rapidly, the second relatively slowly so that the entire transfusion required 90 minutes.

Donor 2 was a 56 year old woman whose spleen had been removed 10 years before because of undiagnosed anemia. At the time of this experiment, study of the woman and her family revealed a familial abnormality of the red cells indistinguishable from thalassemia. The donor's blood had 57 per cent siderocytes, 3.2 per cent reticulocytes, hematocrit 26 per cent. The blood was transfused into an 18 month old child with anemia associated with chronic renal insufficiency.

Donor 3 was a 42 year old retired soldier with hereditary spherocytosis. Four months after splenectomy, his siderocyte count was 68 per cent, reticulocyte count 1 per cent, hematocrit 44 per cent. The blood was transfused into a 20 year old soldier, a healthy volunteer.

Experiments 4 and 5 were done on the same day with blood from the same donor. The donor was a 77 year old man with polycythemia vera whose spleen had been accidentally ruptured and was removed 4 years before. At the time of phlebotomy his hemoglobin concentration was 18 Gm. with approximately 20 per cent siderocytes. The recipient in experiment 4 was a 20 year old man with Hodgkin's disease which was apparently in complete remission at the time. The recipient in experiment 5 was a 26 year old man with acute leukemia whose spleen had been removed three months before because of severe thrombocytopenia in the presence of a bone marrow with many megakaryocytes. At the time of this transfusion he was again thrombocytopenic and the transfusion raised his platelet count from 5,000 to 75,000. He also experienced a nonhemolytic transfusion reaction about 30 minutes after completion of the transfusion.

Donor 6 was a 36 year old retired soldier with Hodgkin's disease whose spleen was removed two years before because of hemolytic anemia. At the time of phlebotomy, his blood contained 38 per cent siderocytes; hemoglobin was 6.2 Gm. per 100 ml. The recipient was a 20 year old soldier with anemia associated with hypoplasia of the bone marrow whose spleen had been removed three weeks earlier. It was hoped that the splenectomy would improve his hematologic condition, but it did not. It was found that his spleen was normally shaped, but it weighed only 16 Gm. Before splenectomy the patient's peripheral blood had no siderocytes. Three weeks later, at the time of the siderocyte transfusion, 4 per cent of his erythrocytes had siderin granules. Howell-Jolly bodies and nucleated red cells were enumerated during differential leukocyte counts. Before splenectomy there were 5 nucleated red cells and 98 Howell-Jolly bodies per 200 leukocytes (WBC 7300). Four months after splenectomy there were 6 nucleated red cells and 46 Howell-Jolly bodies per 200 leukocytes (WBC 2100). The siderocytes were 2 per cent of 1000 red cells counted. At the time of all of these counts the patient's hemoglobin concentration was 8 Gm. per 100 ml. Survival studies of red cells tagged with Cr^{51} indicated that hemolytic disease did not contribute to his anemia.

Blood volume of the recipients was computed from the dilution of the transfused blood after the transfusion had been completed. The transfused cells were identified post-transfusion either by an isotopic tag (Cr^{51}) or by differences in blood groups (Ashby). These and other determinations were performed by standard methods used in this laboratory.[4] When Cr^{51} was used, the radioactive sodium chromate was added to the entire amount of blood to be transfused and the mixture incubated at room temperature for about 45 minutes. Ascorbic acid (200 mg.) was added to stop the binding of the chromium and the blood was transfused without washing the red cells. The transfusions were begun within one hour after the blood was taken from the donor.

Siderocytes were stained by immersing thin, methanol-fixed blood smears in a concentrated, acidified solution of potassium ferrocyanide by a method previously described.[2] The siderocytes were counted by oil-immersion microscopy (×970) in a field made small by partially obstructing the eye piece with a paper diaphragm pierced by a small hole.

Only 3 to 5 complete red cells could be seen at one time and only cells completely within the field were counted. Each cell was carefully and individually brought into focus to determine whether or not inclusion bodies were present. A thousand or more red cells were counted. Analysis of repeated counts indicated that counting this number of cells achieved an accuracy of approximately ±10 per cent at siderocyte levels of 5 to 10 per cent and ±20 per cent at levels of about one per cent.

The anticipated increase of the recipient's siderocyte count after transfusion was calculated thus:

$$Ac = \frac{Dv \times Dc}{Dv + Rv}$$

Where

Ac = Anticipated increase of recipient's siderocyte count (per cent recipient's RBC).
Dv = Volume of donor's RBC transfused (ml).
Dc = Siderocyte count of donor's blood (per cent donor's RBC).
Rv = Recipient's red cell volume before the transfusion (ml).

Case 3 is an exception. Blood volume was not measured and the anticipated increase is an estimate based on extrapolation of a curve formed by plotting the siderocyte counts against time.

The figures in line 11 of table 1 were derived as follows:

$$A = 100 - (DOS)$$

Where A (the figure in line 11) = Anticipated survival of transfused red cells, 4 or 6 hours posttransfusion, if the decreasing siderocyte count were due to destruction of red cells that contained siderin granules (percentage of cells present immediately after transfusion).

100 = Ashby or Cr^{51} value at end of transfusion.
D = Siderocyte count of donor's blood (per cent donor's RBC).
O = Observed increase of recipient's siderocyte count, stated as percentage of the anticipated increase (Ac in the first equation above).
S = Siderocyte loss: recipient's siderocyte count at 4 or 6 hours divided by his posttransfusion siderocyte count (line 8, table 1), and the result subtracted from 100.

RESULTS are tabulated in table one. In the four recipients with spleens the siderocytes disappeared promptly. At four hours, less than half of the transfused siderocytes remained. At the same time few, if any, of the transfused red cells had been lost. This indicates that the iron-containing granules were lost from the red cells but the cells were not destroyed. Transfusions were given to two subjects who had no spleen. At 24 hours, 80 per cent of the transfused siderocytes were still present in their peripheral blood and 90 per cent of the transfused red cells were still present. In these two subjects, the siderin granules were not removed from the transfused red cells.

DISCUSSION

The experiments demonstrated that when blood that contained considerable numbers of siderocytes was transfused into a recipient with a spleen, few of the erythrocytes disappeared from the recipient's circulation during the time that almost all of the iron-staining inclusion bodies were removed. Neither red cells nor inclusion bodies were lost when siderocytic blood was transfused into a recipient without a spleen. The least fanciful interpretation of these results is that the spleen is able to cause the removal of the inclusion bodies without destroying the red cells that contain them.

The erythroblast needs iron to make hemoglobin and it probably gets it by

taking up dissolved iron from ambient tissue fluids. The presence of siderin granules in an erythroblast indicates that more iron is present in the cell than was required for the hemoglobin it has synthesized. This iron may provide a store for further hemoglobin synthesis. As the cell nears maturity and the formation of hemoglobin diminishes, the supply of component parts is probably allowed to decrease: less iron taken in, less porphyrin produced. In normal blood there is a small amount of erythrocyte protoporphyrin, probably in the youngest cells, and there are a few siderocytes, indicating that supply and demand of component parts do not come out even every time. It seems probable that the siderin granules in normal erythroblasts are usually dissolved or metabolized to utilize the iron.

Following splenectomy for diseases not involving the red cells there is little increase in the siderocyte count.[6] In diseases of the erythron the persistence after splenectomy of iron granules in many of the circulating erythrocytes indicates that the cells, as erythroblasts, had taken up more iron than they were able to use.

The experiments described above demonstrate that the spleen is somehow able to remove the inclusion bodies, but the manner in which this is done has not been demonstrated. It may be extruded as a particle, or, as Dacie[5] has suggested, it may be rapidly metabolized under the influence of some splenic function. The presence of iron-staining granules in splenic cells from patients with siderocytosis[10] suggests that the granules may be removed rather than metabolized.

After splenectomy in certain conditions other intra-erythrocytic inclusion bodies may become more numerous: red-cell nuclei, Howell-Jolly bodies, malarial plasmodia, organisms of bartonellosis, Heinz bodies. Is it possible that a normal function of the spleen is to assist the circulating red cells to rid themselves of any inclusion bodies as it does the siderocyte? If true this might be called the "pitting function" of the spleen.

The amount of splenic tissue that is needed may be different for various functions of the spleen. For example, the recipient of transfusion number four had a spleen removed which weighed only 16 Gm. Before splenectomy he had Howell-Jolly bodies in his red cells and a few nuclei but no siderocytes. After splenectomy there were siderocytes in addition to the other red cell inclusions. Evidently an 16 Gm. spleen was sufficient to rid the red cells of their iron granules, but it was not enough to dispose of Howell-Jolly bodies and red cell nuclei.

Summary

1. Blood containing high concentrations of siderocytes was transfused into four recipients with spleens and into two who lacked a spleen. In the circulation of recipients with spleens the siderin granules rapidly disappeared while the transfused red cells remained. In the recipients who had no spleen both the granules and the red cells remained.

2. It is concluded that the spleen is somehow able to bring about the removal of siderin inclusion bodies without destroying the red cells that contain them.

Summario in Interlingua

1. Sanguine continente alte concentrationes de siderocytos esseva transfundite in quatro recipientes con splenes e duo qui non habeva un tal. In le circulation

del recipientes con splenes le granulos de siderina dispareva rapidemente durante que le transfundite erythrocytos remaneva. In le recipientes sin splen, tanto le granulos e le erythrocytos remaneva.

2. Es formulate le conclusion que le splen, in un maniera o un altere, es capace a effectuar le elimination del inclusiones de siderina sin destruer le erythrocytos que contineva los.

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