Studies on the Anemia of Disseminated Malignant Neoplastic Disease. II. Study of the Life Span of the Erythrocyte

By George A. Hyman, Alfred Gellhorn and Jane L. Harvey

The importance of an increased rate of hemolysis in the pathogenesis of anemia of malignant disease has been demonstrated previously by showing that the life span of the erythrocyte from normal donors is shortened when transfused into patients with leukemia, lymphoblastoma, and a variety of disseminated carcinomas.\(^{1-14}\) In addition, direct Cr\(^{31}\) labeling of the red cells of patients with these conditions discloses an intrinsic shortening of the life span of the patient's own red cells.\(^{14, 15}\)

This evidence suggests the presence of a humoral factor in cancer patients which destroys erythrocytes. The experiments reported below were designed to determine whether in addition to a hemolytic factor there is also a defect in the erythrocytes of cancer patients which increases their susceptibility to destruction.

Twenty-three patients with biopsy proven advanced neoplastic disease were selected from the wards of the Francis Delafield Hospital in New York City (see table 1). Since there is no evidence that cancer can be transmitted by transfusion, 53 healthy volunteers were chosen from among the inmates at Sing Sing Prison in New York to receive blood from these patients.

**Methods**

The Ashby technic for the study of red cell life span by differential agglutination\(^{15-20}\) was employed 16 times, while in 52 instances Cr\(^{31}\) was used for red cell labeling\(^{21-27}\). The two technics were combined in 8 instances. Patients with hemoglobin values of 11 Gm. or higher were selected for study. The blood was kept at room temperature and given within four hours of its removal by phlebotomy. In the Ashby studies blood was completely typed and Coombs crossmatched with suitable volunteers. In cases where group O blood was given, 2 cc. anti-A and anti-B substances were added. When Cr\(^{31}\) was employed 75 microcuries of Cr\(^{31}\) (sodium chromate) with specific activity averaging 1.7 mc/mg. in 1 cc. solution were added to the whole blood or to the red cell suspension. The blood was then mixed thoroughly, left at room temperature, and used for transfusion approximately two to four hours later at which time 85 to 95 per cent of the chromium binding had occurred (see table 2).

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TABLE 1.—Type of Neoplasm Studied (23 Patients)

<table>
<thead>
<tr>
<th>Type of Neoplasm</th>
<th>Number of Patients</th>
<th>Average Duration of Life (mos. from onset of study)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alive</td>
<td>Dead</td>
</tr>
<tr>
<td>Carcinoma of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Breast</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Nasal</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Ovary</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Larynx</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Prostate</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Unknown site</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Hodgkins Disease</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Multiple Myeloma</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Chronic Lymphatic Leukemia</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Reticulum cell sarcoma</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Malignant Melanoma</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Lymphoeytic Lymphosarcoma</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>11</td>
</tr>
</tbody>
</table>

* One half of patients died less than 2 months after phlebotomy.

TABLE 2.—Red Cell Absorption of Chromium 51 (Normal and Neoplastic Red Cells)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Pts.</th>
<th>Type of Study</th>
<th>% Cr⁵¹ Attached to RBC</th>
<th>% Cr⁵¹ Remaining in Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>9</td>
<td>Pt. whole blood to volunteer</td>
<td>93.7 90.7-97.1</td>
<td>6.3 2.9-9.3</td>
</tr>
<tr>
<td>II (a)</td>
<td>9</td>
<td>Pt's. RBC's to volunteer</td>
<td>88.5 82.6-94.8</td>
<td>11.5 5.2-17.4</td>
</tr>
<tr>
<td>(b)</td>
<td>6</td>
<td>Pt's. RBC's to patient</td>
<td>89.9 81.7-94.1</td>
<td>10.1 5.9-18.3</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>Volunteer whole blood to volunteer</td>
<td>93.0 90.6-96.5</td>
<td>7.0 3.5-9.1</td>
</tr>
<tr>
<td>IV</td>
<td>17</td>
<td>Volunteer whole blood to patient</td>
<td>91.2 82.6-96.0</td>
<td>8.8 4.0-17.4</td>
</tr>
</tbody>
</table>

RESULTS

FOUR MAIN TYPES OF EXPERIMENTS WERE PERFORMED:

1. Transfusion of whole blood from patients into volunteers.

Transfusion of whole blood from 15 patients into normal volunteers was studied. In six instances chromium labeling and Ashby labeling were done simultaneously (see Charts 1A and 1B). It can be seen (Chart 1B) that a normal life span of 120 days was achieved either by the chromium or Ashby labeling technic in three instances in which life span studies were carried out as completely as possible. Similar results were suggested by the slope of the curves obtained in nine studies when chromium technic alone was used (Charts 1C and 1F) or Ashby technic alone was used (Charts 1D and 1E).

2. Transfusion of red cells from patients into volunteers.

Experiments on the transfusion of red cells from 15 patients with advanced neoplastic disease were carried out. The red cells were allowed to settle for 4
hours prior to the transfusion studies and the plasma separated off. In 9 instances the red cells were labeled with Cr$^{51}$ and transfused into volunteers (see Chart 2A). There was moderate to considerable shortening of the life span in some instances, whereas other curves approached normal.
A second study was performed utilizing the separated red cells from patients after Cr$^{51}$ labeling. In six instances one half of the cells were transfused into a volunteer and one half reinfused into the donor patient (see Charts 2B-1 and 2B-2). In two instances the red cell survival in the volunteer was longer than in
the patient, in two the reverse was true, and in two no significant difference was noted.

Considerable variability can be noted. Thus there is a general tendency to a mild to moderate shortening of the life span of red cells from patients handled in this way (Chart 2C).
3. Transfusion of whole blood from volunteers into volunteers.

The third type of experiment consisted of control studies. Whole blood from 12 volunteers was labeled by the Cr\textsuperscript{51} technic and transfused into other volunteers (see Charts 3A and 3B). In these Cr\textsuperscript{51} studies it can be seen that a 120 day red
cell life span was attained in some instances and that this life span was suggested by the slopes of curves in additional studies. In four experiments employing the Ashby technic, an entirely normal life span of approximately 120 days was obtained when blood from volunteers was given to normal recipients (see Chart 3C).
4. Transfusion of whole blood from volunteers into patients.

The fourth group of experiments involved the transfusion of whole blood from healthy volunteers to patients, employing Cr\(^{51}\) labeling of normal cells in 17 studies (see Charts 4A and 4B). Considerable shortening of the life span of these
red cells was noted in seven of these studies when compared to the other curves, although this is not apparent if only the 50 per cent cell survival time were used. Although these studies were not carried out to completion, it could be seen that the slope of six of the curves suggested a normal life span.

**DISCUSSION**

In our experience the use of Cr\textsuperscript{51} labeling in the study of the red cell life span in patients with neoplastic disease is quite feasible, simple and useful.\textsuperscript{5, 7, 9, 10, 13, 28} The value obtained with Cr\textsuperscript{51} labeling by us is exceedingly close to that obtained by Ashby studies performed simultaneously (see Charts 1A, 1B and 3B).

However it should be emphasized here that this view is a minority opinion. Necheles\textsuperscript{22}, Ebaugh\textsuperscript{23}, and Weinstein\textsuperscript{25} have indicated a normal Cr\textsuperscript{51} 50 per cent red cell survival time of 25 to 40 days, unlike the 50 to 60 day Ashby value. Elution of Cr\textsuperscript{51} has been considered a factor in this variance of the 50 per cent values. The authors and others such as Osgood\textsuperscript{34} believe the 50 per cent point often may be quite misleading, and curves should be interpreted only if they have been carried out to near completion. In addition Mollison\textsuperscript{28} using Cr\textsuperscript{51}, feels that there may be more than one type of cell population, and that a small percentage (6%) may disappear in the first 24 hours.

Others such as Sutherland\textsuperscript{24} feel as we do, that Cr\textsuperscript{51} and Ashby survival studies parallel each other, and that elution of Cr\textsuperscript{51} is not a significant factor in vivo: and this has been our impression as well. In future studies it would seem that Cr\textsuperscript{51} labeling would give adequate information while avoiding the well recognized hazards of transfusion which may occur in Ashby studies.\textsuperscript{29, 30}

There apparently is no advantage of using infusions of red cell rather than transfusions of whole blood in analyzing life span problems in such patients, since there is an apparent shortening of the red cell life span of such blood, pos-
sibly secondary to even the slight handling involved in the removal of the plasma (see Charts 2A, 2B1, 2B2 and 2C, as compared to Charts 1A–F and 3A–C). In any event, it seems likely that the small amount of plasma transfused with the whole blood in the patient to volunteer studies is destroyed in a few days in the recipient, in addition to being extremely well diluted.

The most important finding in this study would appear to be in the fact that the slopes of the curves of cell destruction strongly suggest that whole blood of patients injected into volunteers tends to have a normal life span as compared with the shortened life span seen after the injection of volunteer whole blood into patients, even though the studies in most instances could not be extended to a full 120 days. (See Charts 1A through F.) This finding of a normal life span of Cr\(^{51}\) labeled “neoplastic” red cells is actually underlined further by the suggestion of some authors such as Mollison\(^{28}\) that the elution rate from abnormal erythrocytes may be increased. This is in contrast to studies of the patient’s own cells, labeled in vitro with radio-chromium, which frequently have a considerable shortening of their life span when they are reintroduced into the patient’s own circulation.\(^{14,15}\) The shortening of the life span in many instances when normal blood is transfused into patients with neoplastic disease (see Charts 4A1 and 4A2) is confirmed by our previous experience.\(^6\)

The absence of any indication of an intrinsic corpuscular abnormality re-emphasizes the importance of a hemolytic factor which does not reside in the erythrocytes, in the genesis of the anemia of cancer. It is not intended to imply that this hemolytic factor is specifically related to the anemia of cancer inasmuch as increased hemolysis has also been demonstrated in a number of chronic diseases involving the liver, kidney, lungs, joints, etc.\(^{29,31-33}\) The studies here presented offer evidence which may help to characterize the hemolytic factor further. It
can be seen in the graphs depicting the disappearance rate of patients' erythrocytes when transfused to a healthy recipient (viz., Group 1F), that the early part of the curve is relatively steep which is followed by a change in the slope with a more gradual decline. Thus, if the survival time of the erythrocytes were calculated by extrapolating the curves from the observed early points, an erroneously short life span would be obtained. In order to find out the true life span of the cancer patient's red blood cells in a normal circulation, it is necessary to continue the observations for 120 days. This biphasic type of curve suggests that the cancer patient's erythrocytes may be coated with a hemolytic factor which leads to their destruction in a normal environment just as it did when the cells were in the patient's own circulation. With time, however, either due to dilution in the normal recipient's plasma or to failure of renewal, the hemolytic factor fails to be effective and the patient's erythrocytes then are destroyed at a normal rate. This speculative interpretation of the experimental observations leads to the conclusion that the hemolytic factor is humoral and that its actions on red blood cell survival are reversible. Experiments are now in progress which will attempt to elucidate as well as demonstrate a hemolytic plasma factor present in patients with advanced neoplastic disease.

**SUMMARY**

1. A study of the life span of the erythrocyte in patients with advanced neoplastic disease was performed by transfusing blood from 23 hospitalized patients into 53 healthy volunteers.

2. The Ashby technic for red cell labeling was used alone in 16 instances, Cr$^{51}$ technic in 52 instances, and a combination of the 2 technics in 8 instances.
3. Normal or nearly normal life spans were suggested by the slopes of curves in 15 instances where whole blood from patients was transfused into volunteers.

4. Some shortening of the life span of the patient’s red cells was noted when these cells, separated from the plasma, were transfused into volunteers or back into patients, possibly related to the handling during plasma removal.

5. Control studies using either Cr⁵¹ or Ashby labeling technic indicated a normal life span of approximately 120 days in the volunteers employed could be achieved by each method.

6. Values obtained with Cr⁵¹ were so similar to those using Ashby labeling that the easier Cr⁵¹ technic is recommended for future studies.

7. The normal erythrocyte transfused into patients with neoplastic disease may have a moderate to considerably shortened life span.

8. These studies seem to demonstrate an absence of an intrinsic defect in the erythrocytes of patients with neoplastic disease, and further favor the presence of a hemolytic plasma factor of considerable importance in the pathogenesis of the anemia of cancer.

SUMMARIO IN INTERLINGUA

1. Un studio del duration de vita del erythrocytos de patientes con avantiata morbo neoplastic esseva executate per transfundere sanguine ab 23 patientes hospitalisatea in 53 voluntarios normal.

2. Le technica de Ashby pro le etiquettage de erythrocytos esseva usate in 16 casos. Le technica a Cr⁵¹ esseva usate in 52 casos. Le duo technicas esseva combine in 8 casos.

3. Normal o quasi normal durationes de vita esseva indicate per le curvas in 15 casos in que sanguine integre habeva essite transfundite ab le patientes a in le voluntarios.

4. Un certe reduction del duration de vita del erythrocytos del patientes esseva notate quando iste cellulas, separate ab le plasma, esseva transfundite a in voluntarios o retrofundite a in le patientes. Isto esseva possibilemente influentiate per le manipulation durante le ablation del plasma.

5. Studios de controlo per medio del technica de etiquettage a Cr⁵¹ o per medio del technica de Ashby indicava que un normal duration de vita de circa 120 dies esseva attingibile in le voluntarios per le un e le altere del duo technicas.

6. Le valores obtenite per medio del technica a Cr⁵¹ esseva si simile al valores obtenite per le etiquettage de Ashby que le plus simple technica a Cr⁵¹ es recomendate pro studies futur.

7. Le erythrocyto normal transfundite a in patientes con morbo neoplastic ha un leve- o considerablemente reducide duration de vita.

8. Iste studios pare demonstrar le absentia de un defecto intrinsec in le erythrocytos de patientes con morbo neoplastic. Illos reinfortia le these del presentia de un factor plasmatic hemolytic que possede un considereable importantia in le pathogenese del anemia de cancer.

REFERENCES

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Studies on the Anemia of Disseminated Malignant Neoplastic Disease. II. Study of the Life Span of the Erythrocyte

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