The Hemolytic Effect of Ionizing Radiations and Its Relationship to the Hemorrhagic Phase of Radiation Injury

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The anemia produced by whole body exposure to lethal doses of ionizing radiations develops more rapidly than would be expected from cessation of erythropoiesis together with the normal attrition of cells. In large measure the excess loss of red cells is accounted for by hemorrhage, incident to the thrombocytopenia which occurs as the result of bone marrow aplasia. It also has been frequently suggested that hemolysis contributes to the rapid onset of anemia. Some authors have described a precipitous decline in the peripheral red cell values prior to the development of thrombocytopenia. Schwarz and Davis reported an increased bilirubin excretion in dogs during the first week after irradiation which could signify either an increased destruction of circulating red cells or the development of alternate pathways of pigment metabolism consequent to marrow aplasia. Wright, however, reported that an increased destruction of peripheral red cells in rabbits could not be detected prior to the 7th–10th day after whole body irradiation. A delayed onset of hemolysis was also observed by Sheets and his colleagues in patients with malignancy following partial body exposure to x-irradiation.

The delay in onset of hemolysis reported by these authors might suggest that the red cells are not directly damaged by ionizing radiations. In support of this are studies in which erythrocytes exposed to 2000 r in vitro have been shown to survive normally upon subsequent transfusion into normal recipients. Changes in osmotic properties of the red cell did not occur following in vitro irradiation of cells unless more than 20,000 r were used.

During the period of thrombocytopenia followed irradiation, the lymph nodes are markedly hemorrhagic. Wood and his associates have noted that the thoracic duct lymph becomes sanguinous and contains as many as $1 \times 10^6$ RBC per cu.mm. Presumably a large part of the cells extravasated into the tissues enter the lymphatics. Many are destroyed in the lymph nodes but significant numbers are returned to the general circulation via the thoracic duct. A similar phenomenon has been observed in thrombocytopenia due to other causes. It has been

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suggested that this extravascular cycle affects red cell survival. However, was unable to demonstrate an alteration in the survival characteristics of normal rabbit cells injected into serous cavities upon the return of the red cells to the general circulation. Whether this is also true for cells injected or extravasated subcutaneously or intramuscularly and for cells from irradiated animals has not been established.

The present study was undertaken to determine whether red cell damage could be demonstrated consistently prior to thrombocytopenia. In addition, the possible role of the extravascular cycle, incident to thrombocytopenia, in producing red cell injury was explored by determining the behavior of both extravasated normal cells and cells from irradiated animals upon their return to the general circulation.

MATERIAL AND METHODS

Adult mongrel dogs were used throughout the study. The irradiator was a Co source with geometry. The dose rate was measured with a rate meter calibrated by the National Bureau of Standards for Co gamma radiation. Doses are reported as roentgens measured free in air along the central axis of the exposure chamber. The tissue dose at the midline of the dog would be approximately 83 percent of the air dose. With this source 475 r has been found to be a uniformly lethal dose. The methods employed in determining hematocrit, reticulocytes and platelet levels have been described previously. The degree of suppression of erythropoiesis was estimated by Fe incorporation as well as by changes in peripheral reticulocyte levels and hematocrit.

Red cell survival was measured with Cr labeled cells. The dogs were typed for the canine red cell agglutinogens A, B, C, D, E, and F described by Young and his associates and cross matched prior to transfusion. The red cells were tagged by introducing 0.1-0.5 cc. of an aqueous solution of radioactive sodium chromate, NaCrO, into fresh whole blood collected in acid citrate dextrose (ACD). 5 to 15 y of chromium metal were added per ml. of red cells. In those experiments in which blood was transfused intravenously, 50-150 μc. of Cr with a specific activity of 1 to 2.5 mc. per mg. were used. The method of tagging red cells and the determination of Cr activity in this laboratory have been described. The survival characteristics of red cells extravasated into tissues and then returned to the general circulation were determined by injecting Cr labeled red cells extravascularly. Cells from both normal and irradiated dogs were injected into normal and irradiated recipients. For these studies Cr with a specific activity of 20-25 mc. per milligram was required to insure sufficient activity upon return of a small sample to the general circulation; 6-8 ml. of whole blood collected in ACD was tagged so that the final concentration of chromium metal was approximately 5 y/ml. of packed red cells. Cells so labeled were then injected either intraperitoneally, intramuscularly, or subcutaneously. When given intramuscularly, half of the blood (3-4 ml.) was given in the femoral region of each leg. When given subcutaneously, 1 ml. was injected in each of six sites along the vertebral column. A more direct approach to the effect of the extravascular cycle on the survival of red cells in the irradiated animal was afforded by transfusing red cells collected from the thoracic lymph of lethally irradiated dogs into normal recipients. The lymph was collected by inserting a polyethylene catheter into the thoracic duct at a point just prior to its entry into the jugular vein. The procedure was carried out under nembutal anesthesia. The lymph was allowed to flow by gravity into a bottle containing ACD. The flow rate of lymph averaged 1 ml. per minute. Over a 3 hr. period, 200 ml. were collected, cen-

Air dose rates reported in all earlier publications had been determined with a rate meter calibrated by the National Bureau of Standards for x-rays and must be multiplied by a factor of 1.19 to obtain values corresponding to the present calibration against the National Bureau of Standards Co standard.

We are indebted to Dr. Esther Hardenbergh for the performance of the operative procedures.
trifuged at 1500 g, and all but 15 ml of the supernatant decanted. The red cells were re-
suspended in the remaining supernatant and tagged with Cr\textsuperscript{51} in the usual fashion. Midway
during the collection period 30 ml of whole blood was obtained from the femoral vein,
tagged with Cr\textsuperscript{51} and transfused into a second normal recipient.

Determination of the rate of loss of Cr\textsuperscript{51}. A precise determination of the elution rate re-
quires simultaneous measurement of cell survival by differential agglutination and Cr\textsuperscript{51}. With these measurements the elution rate (k) can be estimated\textsuperscript{17} (by least squares assuming
that all points are equally well determined) from:

\[ k_i = \frac{\sum \left( \log \frac{A}{C} \right) t_i}{\sum t_i^2} \]

where \( t_i \) is the \( i \)th day following transfusion, \( k_i \) the estimate of \( k \) for the first \( t \) days follow-
ing transfusion, \( A \) is the per cent of the starting value for differential agglutination and \( C \) is
the similar value for Cr\textsuperscript{51}.

This permits determination of the elution rate in situations where this remains constant
throughout the period of study. In the irradiated animal, however, it was observed that the
rate of loss of Cr\textsuperscript{51} in excess of that due to senescence was inconstant. In these circumstances
estimates of \( k \) were computed for an initial five day period and then for ten day periods for
the duration of each study. Where there were not enough observations in a ten day period
to allow the fitting of a firm least squares line, the time period covered by a single line was
extended so that at least five to seven observations were included. The formula used for
fitting these lines was the usual least squares equation for a straight line which is not forced
through an 0,0 point:

\[ k_{t_2-t_1} = \frac{\sum \left( t_i - \bar{t} \right) \left( \log \frac{A}{C} \right)_i - \bar{t} \log \frac{A}{C} \bar{t}}{\sum (t_i - \bar{t})^2} \]

This gives the elution constant for the time interval \( t_1 \) to \( t_2 \), where \( \bar{t} \) is the arithmetic mean
of times of observation for that time interval and \( \log \frac{A}{C} \) is the arithmetic mean of the \( \log \frac{A}{C} \)
observations for the same interval.

When values for differential agglutination are not available such as in the case of auto-
transfusion of Cr\textsuperscript{51} tagged cells, in vivo tagging with Cr\textsuperscript{51}, or following small homotrans-
fusions, a direct measurement of Cr\textsuperscript{51} elution cannot be made. An estimate, however, may
be obtained using a theoretical curve of senescence. This is constructed by drawing a
straight line on arithmetic paper from the Cr\textsuperscript{51} value at 0 time to the point of extinction.
This line theoretically represents the loss of cells from senescence. Differences in the esti-

dated point of extinction of ±10% did not alter the \( k \) values importantly. In the absence
of hemolysis in either donor or recipient, values obtained from this curve should be identical
with the true values for differential agglutination and formula (1) will give the correct
elution rate. In the presence of hemolysis the \( k \) value obtained in this fashion includes
loss of Cr\textsuperscript{51} both from elution and hemolysis.

The values for Cr\textsuperscript{51} activity are usually expressed as counts per minute/ml. of packed
cells, since the red cell mass is more constant than the blood volume.\textsuperscript{18} This activity repre-
sents a ratio of tagged to non-tagged cells. To obtain a representative survival curve, the
red cell mass must remain constant and each chromated cell which is destroyed must be
replaced by a newly formed non-tagged cell. A changing red cell mass introduces errors.
When the production rate exceeds the rate of destruction, there is a dilution of the remaining
chromated cells with untagged cells, thus lowering the activity per unit of packed

As a consequence one overestimates the rate of destruction. Conversely, if the
rate of production is less than the rate of destruction, one underestimates the rate of de-
struction. When there is virtual absence of production such as is the case in the lethally
irradiated animal, the ratio of tagged to non-tagged cells will not be altered except from
elution or selective destruction of red cells. Consequently, following a dose of radiation
which produces complete red cell aplasia any loss of Cr activity per unit of packed cells must result either from an increased elution rate or selective destruction of tagged cells. In either event a change in the rate of Cr loss per unit cells signifies damage to the tagged population.

**Determination of uptake and survival of Cr labeled cell introduced extravascularly.**

Following the line of reasoning advanced by Scow and Cornfield:

The number of tagged cells, $C_2$, present (in the blood) at times $t_2$

$$= C_1 \times (1 - \text{departure rate}) + \text{net new cells introduced during the time interval } t_1 \text{ to } t_2.$$

This "difference equation" then provided the basis for beginning the computation over again at time $t_2$, defining this as a starting level and going on to time $t_3$ and so on.

If the proportion of tagged red cells present at any time $t$ is given by the formula

$$Y_t = e^{-kt}\left(\frac{T-t}{T}\right)$$

where $T$ is the potential life span and $k$ is the "loss" rate per day (including both chromium elution and random destruction) and if, in addition, there is a uniform transport rate R per day moving tagged cells from tissues to the circulation during the interval $t_1$ to $t_2$, then the net number of new cells introduced into the circulation during the time interval $t_1$ to $t_2$ is given by

$$\int_{t_1}^{t_2} Re^{-k(t-t_1)} \left[\frac{T-(t-t_1)}{T}\right] dt$$

Integration and some algebraic manipulation yield the final form for the difference equation:

$$C_2 = C_1 e^{-k(t_2-t_1)} \left[\frac{T-(t_2-t_1)}{T}\right] + \frac{R}{Tk} \left[\frac{T-1}{k} - \left(\frac{T-1}{k} - \frac{T_2-t_1}{T}\right) e^{-k(t_2-t_1)}\right]$$

Or, in general for interval $t_1$ to $t_{i+1}$ writing $t_{i+1} - t_1 = t_i$,

$$C_{i+1} = C_i e^{-k_1(t_1)} \left[\frac{1}{k_1} - \frac{\Delta t_i}{T}\right] + \frac{R_i}{Tk_i} \left[\frac{T-1}{k_i} - \left(\frac{T-1}{k_i} - \frac{T_1}{k_1}\right) e^{-k_1(t_1)}\right].$$

This last form allows computation of the number of cells remaining in circulation, $R$, the rate per day of transport of tagged cells into the circulation, and $k$, the "constant" of random destruction and chromium elution.

From the formula above, a worksheet* can be set up which permits calculation of the successive $C_i$, the numbers of tagged cells. To do this, initial estimates have to be made of $T$, $k$, and $R$. It becomes clear very quickly whether these are adequate to give a good fit to the observed data, and what changes appear necessary. Although mathematically it appears that more than one combination of $R$'s and $k$'s could yield a solution, operationally unique solutions were obtained. This undoubtedly results from the fact that uptake $R$ occurs for only 4-8 days. The $R$'s can be functionally related if some biologic mechanism can be postulated which seems to describe the transport phenomenon adequately, as was done by Scow and Cornfield, or can be left free of such a relation, if no simple mechanism seems to be adequate, as was done here.

**Results**

1. **The Anemia of Whole Body Irradiation**

Changes in peripheral red cell values were determined after whole body exposure to ionizing radiations in doses ranging from sublethal (180 r) to supralethal

*A simple worksheet and a chart of the observed and fitted points can be obtained from the authors.
Fig. 1.—Peripheral hematocrit and platelet values following whole body exposure to doses of ionizing radiations varying from sublethal (180 r) to midlethal (385 r). It is to be noted that a major drop in hematocrit occurs only after the onset of severe thrombocytopenia associated with higher doses of irradiation.
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Fig. 2.—Fe$^{59}$ incorporation in dogs after irradiation compared with the normal. Note the suppression of Fe$^{59}$ incorporation at all levels and the virtual absence of iron incorporation after 385 r.

(600 r). A precipitous decline in hematocrit occurred only after the onset of thrombocytopenia (fig. 1). Exposure to 180 r, which did not result in hemorrhagic levels of thrombocytopenia, was followed by a gradual decline in hematocrit compatible with suppression of erythropoiesis together with the normal attrition of cells. A mild hemolytic component, however, could not be excluded. After higher doses of radiation there was also a gradual decline in hematocrit during the first week. With the onset of severe thrombocytopenia between the 7th and 10th post-radiation day, however, there was a more rapid decline in hematocrit. Due to the generalized hemorrhagic tendency during this period, the presence or absence of hemolysis could not be established from hematocrit changes alone.

Suppression of erythropoiesis was demonstrated after doses of 180 r. Only an occasional reticulocyte was seen and iron incorporation was decreased (fig. 2). Three hundred r produced a pronounced depression of red cell production as evidenced by the disappearance of reticulocytes from the peripheral blood and an even lower iron incorporation. In those dogs which survived this dose, there was a return of red blood production between the 18th and the 25th day, at which time reticulocytes reappeared in the peripheral blood. Following 385 r there was virtually complete cessation of erythropoiesis. In these animals not only were reticulocytes absent from the peripheral blood but in some animals there was no utilization of intravenously injected radio-iron. In those animals surviving this amount of radiation (usually with the aid of antibiotics) there was renewed red cell production between the 20th and 30th days after irradiation.
It was apparent from the foregoing studies that injury to the circulating red cells could be detected only by a study of the survival of the red cells of irradiated animals. Accordingly, the survival of red cells collected from irradiated dogs was determined following transfusion into normal recipients (fig. 3). Normally canine cells survive 90–120 days. The initial apparent half time* of Cr⁴¹ labeled cells in normal dogs varies from 21–30 days but in most instances is in the range of 23–27 days.² It has been observed that 1–2% of the Cr⁴¹ remaining in the cells is eluted daily. Normally the elution appears to be constant. However, in dogs where there is red cell damage it has been observed that the elution rate is initially greater than normal and progressively declines throughout the period of study.² An inconstant elution rate also has been occasionally observed in an apparently normal dog although the initial rates have not been elevated.

The survival of cells collected from dogs on the 3rd, 5th, 10th and 12th days

* The initial apparent half time refers to that time in which the Cr⁴¹ activity decreases from 100% to 50%. Since the Cr⁴¹ curve is the product of a linear loss due to senescence and random loss (due to elution and, if present, hemolysis) the decrease in Cr⁴¹ activity is not an exponential function. The time required for the initial decrease in activity from 100–50% is not the same as that required for a decrease from 50–25% of activity. The measure of the initial decrease from 100–50%, nevertheless, has some practical usefulness for purposes of comparison, and is designated the apparent initial half time. It is not a half life in the usual sense which implies a constant exponential rate.
**Table 1.**—Calculated Chromium "Elution" Following Transfusion of Red Cells from Irradiated Donors into Normal Recipients

<table>
<thead>
<tr>
<th>Days After Transfusion</th>
<th>Rate* ± Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dog 837 3rd day†</td>
</tr>
<tr>
<td>1-10</td>
<td>.012 ± .001</td>
</tr>
<tr>
<td>11-20</td>
<td>.016 ± .003</td>
</tr>
<tr>
<td>21-40</td>
<td>.010 ± .0004</td>
</tr>
<tr>
<td>41-60</td>
<td>.010 ± .0004</td>
</tr>
</tbody>
</table>

These rates for Cr\(^{51}\) elution following transfusion of cells from irradiated donors into normal recipients were computed from the data shown in figure 1.

* The rate of loss of Cr\(^{51}\) in excess of that anticipated from senescence. This includes loss from both hemolysis and elution.

† Refers to the post-irradiation day on which the cells were collected.

![Graph](https://via.placeholder.com/150)

**Fig. 4.**—The rate of disappearance of Cr\(^{51}\) following auto-transfusion is shown. Note the decrease in activity (per unit ill) following irradiation.
TABLE 2.—The Calculated Cr\textsuperscript{51} "Elution" Rate in Dogs Receiving Whole Body Radiation

<table>
<thead>
<tr>
<th>Days After Transfusion</th>
<th>Rate\textsuperscript{a} ± Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dog 678 Normal (5.0y)</td>
</tr>
<tr>
<td>0–10</td>
<td>.019 ± .014</td>
</tr>
<tr>
<td>11–20</td>
<td>.011 ± .005</td>
</tr>
<tr>
<td>21–30</td>
<td>.013 ± .000</td>
</tr>
<tr>
<td>31–45</td>
<td>.019 ± .001</td>
</tr>
<tr>
<td>46–64</td>
<td>.012 ± .001</td>
</tr>
<tr>
<td>Days After Irradiation</td>
<td></td>
</tr>
<tr>
<td>0–1</td>
<td>.026 ± .004</td>
</tr>
<tr>
<td>5–10</td>
<td>.021 ± .002</td>
</tr>
<tr>
<td>11–15</td>
<td>.061 ± .012</td>
</tr>
<tr>
<td>16–20</td>
<td>.059 ± .003</td>
</tr>
<tr>
<td>21–25</td>
<td>.06 ± .003</td>
</tr>
<tr>
<td>26–35</td>
<td>.06 ± .003</td>
</tr>
</tbody>
</table>

The experimental curves from which these rates were computed (except for Dog 730 and 678) are shown in fig. 4–8. The animals were transfused 22–54 days prior to irradiation, except in the case of Dog 730 where normal tagged cells were given immediately after irradiation. The time designated "days after irradiation" follows immediately after the last recorded day under "days after transfusion" so that for Dog 997 which was irradiated on the 54th day after transfusion, the 0–4th day after irradiation corresponds to the 54th–58th day after transfusion.

\textsuperscript{a} The concentration of chromium per cc of cells used in tagging. In dog ST4 the cells were tagged in vivo and the concentration given refers to the fraction entering the red cell.

after exposure to 475–600 r was determined (fig. 3). Survival of cells collected on the third day was normal and the elution was constant (table 1). Although the initial apparent Cr\textsuperscript{51} half times of 21 days and the over-all "elution" rate of cells collected on 5th and 10th day were normal, the rate of elution was inconstant. These values were initially increased and subsequently declined suggesting red cell damage. The loss of Cr\textsuperscript{51} activity following transfusion of cells collected after the 10th day was clearly increased. Since no new cell production occurred between the time of irradiation and the collection of blood for transfusion, it was anticipated that the age distribution of the transfused cells would be distorted accordingly. The observed differences, however, were greater than could be accounted for by the abnormal age distribution of the transfused cells.

Even though auto-transfusion is feasible with the Cr\textsuperscript{51} technic, it is impossible to directly evaluate hemolysis in the irradiated animal since the loss of Cr\textsuperscript{51} as the result of hemolysis cannot be separated from the much greater loss due to hemorrhage secondary to thrombocytopenia. It appeared likely, however, that the presence of red cell damage following irradiation could be determined indirectly if irradiation damage altered the chromium elution or produced selective destruction as had been observed in other hemolytic states.\textsuperscript{2} The doses of radiation used in these studies produced virtual cessation of erythropoiesis as evidenced by the absence of reticulocytes and the low Fe\textsuperscript{59} incorporation in the face of a falling hematocrit. Under these circumstances where there is no replacement of tagged cells with newly formed non-tagged cells, the ratio of Cr\textsuperscript{51} tagged to non-tagged cells should remain constant, since there would be a proportional loss
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Fig. 5.—Cr⁵¹ “elution” plotted on a semilogarithmic scale. The cells were tagged with 15γ of Cr per ml. of cells. The sharp increase in the rate of Cr⁵¹ loss per unit cell following irradiation (385 r) indicates the presence of red cell damage. The second apparent increase in the rate of Cr⁵¹ loss is due to dilution from the outpouring of newly formed non-tagged cells following bone marrow regeneration.

of tagged and non-tagged cells from hemorrhage or hemolysis. Any loss of Cr⁵¹ activity above the normal elution per unit of cells would reflect an alteration in the rate of elution of Cr⁵¹ or selective destruction of Cr⁵¹ tagged cells.

With the view in mind of determining the presence of red cell damage in this fashion, the rate of Cr⁵¹ loss following irradiation was determined in a group of 14 dogs in which the normal elution rate had been established during a 20-55 day period prior to irradiation. Seven of these dogs received autotransfusions. In the others the cells were tagged with the in vivo technic. The concentration of chromium used in tagging ranged from 0.1–15 γ per ml. of cells. The doses of radiation varied from 300–450 r. In all animals there was an abrupt decrease in the Cr⁵¹ activity per unit cells on the 1st–3rd day after irradiation (fig. 4). This was reflected in the elution curve by a sharp increase in the rate of elution (fig. 5-7; table 2). The change in the rate of elution when cells were tagged with 15 γ/ml. of cells (fig. 5) was greater than when 0.1 γ was used (fig. 7). The difference between 0.1 and 5 γ was less marked (fig. 6, 7). The magnitude of this
change was not influenced by the dose of radiation within the range studied. Following the initial increase there was a progressive decline in the rate of Cr²⁵¹ loss. In those dogs that survived a mid-lethal dose of radiation, there was a second decrease in the Cr²⁵¹ concentration per unit cells occurring between the 16th and 30th post-irradiation day. Presumably this resulted from dilution of the remaining Cr²⁵¹ activity by the outpouring of newly formed non-tagged cells, since this event coincided with the reappearance of reticulocytes.

Normal cells were transfused into irradiated recipients on six occasions, to determine whether the effects of irradiation were indirect. Cells were collected from the dog to be irradiated immediately prior to exposure to the Co⁶⁰ source, tagged with Cr²⁵¹ and autotransfused 1–3 hours following irradiation. In these dogs there was a significant increase over normal in the initial rate of Cr²⁵¹ loss which then declined progressively (fig. 8, table 2). With the resumption of red cell production there was again an apparent increase in Cr²⁵¹ loss.
3. Survival of Extravasated Red Cells upon Return to the General Circulation

A. Survival of normal cells. Approximately 60 per cent of Cr$^{51}$ tagged normal autogenous red cells were returned to the general circulation following intraperitoneal injection. The peak circulating radioactivity was attained on the 4th post-injection day. The survival of those cells reaching the general circulation appeared to be normal (fig. 9). The apparent initial Cr$^{51}$ half time was 25 days. The Cr$^{51}$ elution rate was constant amounting to 1.5 per cent.

In 2 normal dogs 20 per cent of autogenous red cells injected intramuscularly into the femoral region were returned to the general circulation over a five-day period. The survival of these cells fell within the normal range with apparent initial half times of 24 and 26 days (fig. 9). The Cr$^{51}$ elution rates were constant being 1.5 and 1.4 per cent respectively.
Fig. 8.—The rate of loss of Cr\textsuperscript{51} from normal cells transfused into irradiated recipients is plotted on a semilogarithmic scale. The marked increase in Cr\textsuperscript{51} loss is contrasted with the normal loss of Cr\textsuperscript{51} following transfusion of normal cells into normal recipients. The apparent change in the rate of loss of Cr between the 18th-25th post-transfusion day is the result of the outpouring of newly formed non-tagged cells following bone marrow regeneration.

As might be expected when autogenous cells were injected subcutaneously, fewer cells were returned to the circulation than when such cells were injected intramuscularly or intraperitoneally. Only about 1 per cent of the injected cells were returned to the general circulation and the absorption was slower extending over a 7-8 day period (fig. 9). When an attempt was made to fit these data using a constant $k$, the deviation of the fitted from the observed experimental points made it obvious that elution and/or hemolysis were not constant throughout the survival study. Moreover, the rate of Cr\textsuperscript{51} loss other than from senescence during the initial phase of the curve was markedly increased above normal. The rate of
loss of \( {\text{Cr}}^{45} \) from hemolysis and elution in one instance was initially 5 per cent and decreased by the 50th day to 2 per cent. In a second experiment the initial loss of 4 per cent decreased to 1.5 per cent by the 50th day. The values for the apparent initial half times of the recirculating cells were 14 and 15 days.

B. Survival of cells from irradiated dogs when injected intramuscularly into normal dogs. Cells collected from each of 2 dogs on the first and fourth day following whole body exposure to 385 r were tagged with high specific activity \( {\text{Cr}}^{45} \) and injected intramuscularly into normal compatible recipients. The peak intravascular radioactivity was attained on the 5th day. The survival of the cells from the irradiated donors was significantly shortened (fig. 10). The average rate of loss of \( {\text{Cr}}^{45} \) in excess of that due to senescence was 6 per cent. Cells collected from donors on the 4th post-irradiation day were even more rapidly destroyed on return to the general circulation. In these studies the rapidity of loss of cells prevented the use of a mathematical model to determine the percentage absorption. An approximation by extrapolation, however, indicated that from 5–12 per cent of the injected cells were absorbed. It was considered unlikely that isoinmunization accounted for the rapid rate of destruction of red cells in these experiments since hemolysis was apparent immediately after transfusion.

When normal cells were injected intramuscularly into irradiated recipients it was not possible to quantify the rate of hemolysis of the injected cells due to the concomitant loss of cells from hemorrhage. The presence or absence of selective damage to the tagged cells, however, could be determined by changes in the ratio of tagged to untagged cells as had been done previously. Using this model it was evident that there was selective damage to the tagged normal cells (fig. 11). Again the possibility of isoinmunization arose but this was considered unlikely in the irradiated animal.
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INTRAVASCULAR SURVIVAL OF CANINE RED CELLS FROM NORMAL AND IRRADIATED DOGS FOLLOWING INTRAMUSCULAR INFUSION INTO NORMAL DOGS

Fig. 10.—The uptake and survival of cells from irradiated donors following intramuscular infusion into normal recipients is contrasted with that of normal cells injected intramuscularly.

Fig. 11.—The survival of Cr\textsuperscript{31} tagged normal cells after infusion into dogs which had received 475 r 2-3 days prior to the infusion. The Cr\textsuperscript{31} activity is determined as cpm/ml of packed cells so that any loss of Cr\textsuperscript{31} activity represents a selective effect on the tagged cells.
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Fig. 12.—The survival of cells collected from the thoracic duct lymph of an irradiated dog and transfused into a normal recipient is compared with the survival of cells collected from a peripheral vein of the same dog on the 3rd and 12th post-irradiation days.

C. Survival of cells collected from the thoracic duct. Erythrocytes were collected from the thoracic duct of 2 dogs 8 and 12 days following whole body exposure to 475 r whole body irradiation. During the middle of the period of collection of thoracic lymph, cells were obtained from the femoral vein for a comparative study of the survival of thoracic duct and peripheral vein erythrocytes. Cells collected from the thoracic duct on the 12th post-irradiation day had a shorter survival on transfusion into a normal recipient than those collected from the femoral vein (fig. 12). The rate of Cr\(^{51}\) loss due to elution and hemolysis from cells collected from the thoracic duct was not constant but progressively decreased from 7 to 3 per cent over a 60-day period. The mean value for k was 4.8 per cent; the apparent initial half time 7 days. In the peripheral blood the elution rate was also inconstant, declining from 2.8 to 1.4 per cent with a mean value of 1.8 per cent. The apparent initial half time was 16 days. The recipients of both thoracic duct and venous blood from a second irradiated dog developed iso-antibodies which became apparent on the 20th post-transfusion day. The rates of destruction, determined for the period prior to the development of the iso-antibodies, again indicated that thoracic duct erythrocytes were destroyed at a more rapid rate than peripheral vein red cells.

DISCUSSION

Post-radiation anemia from cessation of red cell production and from blood loss during the thrombocytopenic phase of radiation injury has been well studied and can be ascribed to bone marrow injury. The aim of the present investigation was to establish whether ionizing radiations also damage circulating red cells, and to clarify, if possible, the mechanism of this injury. Measurement of red cell survival appeared to be the most suitable method for such an investigation, both because of its sensitivity and because of the physiologic significance of
shortened red cell survival. Cells tagged with Cr\(^{51}\) were used for these studies because of the ease with which isotope measurements of high precision can be made and because the survival of Cr\(^{51}\) tagged cells can be estimated following both auto- and homotransfusion.

**Interpretation of Cr\(^{51}\) measurements**

In contrast to its technical advantages, interpretation of the Cr\(^{51}\) measurements present considerable difficulties because of "elution" of Cr\(^{51}\) from tagged cells. The concept of Cr\(^{51}\) elution developed from simultaneous measurements of red cell survival by differential agglutination and with Cr\(^{51}\). The decline in Cr\(^{51}\) activity was always faster than could be ascribed to red cell loss, as determined by the differential agglutination.\(^{22, 23, 24, 17, 26}\) The excess loss has been reported to be a random process independent of the age of the cells, and has been explained in the past by elution or leakage of Cr\(^{51}\) at a steady rate from the surviving tagged cells.

Mollison\(^{24}\) has drawn attention to the more rapid loss of Cr\(^{51}\) during the first 24 hours, which is not consistent with a steady random loss and which suggests selective destruction. However, since there is no experimental evidence to decide between these alternatives, and since the concept of elution is well established, it has been retained throughout this paper.

In the absence of a simultaneous measurement of differential agglutination, the loss of red cells due to senescence may be estimated by drawing a straight line on arithmetic paper from the Cr\(^{51}\) activity at time 0 to the time where Cr\(^{51}\) activity has disappeared. The loss of Cr\(^{51}\) in excess of that due to senescence, however, includes the elution of Cr\(^{51}\) from individual cells, and any loss of red cells due to hemolysis or blood loss. Because of the variation among individuals, there is at present no way in which the exact contribution of elution and hemolysis can be apportioned when estimates of cell loss by differential agglutination are not made; e.g. an overall apparent excess loss of 2 per cent a day could be due to elution at the rate of 2 per cent per day without hemolysis or due to elution at the rate of 1 per cent combined with a hemolytic process accounting for a red cell loss of 1 per cent per day.

Notwithstanding this uncertainty, it is possible to describe the range of normal Cr\(^{51}\) measurements. This is conveniently done by the use of an apparent elution rate (i.e., the loss in excess of senescence) and by the initial apparent half time.

As has been noted previously, the curve of Cr\(^{51}\) decline has two components, one due to red cell loss from senescence, described by a straight line on arithmetic paper, and one due to apparent elution, described by a straight line on semilogarithmic paper. The resultant of these two components is not exponential, and therefore does not have a true half time. It is convenient, however, to refer to the decline from the original Cr\(^{51}\) activity to half its value as the initial apparent half time. In normal dogs this value is in excess of 21 days, so that an initial apparent half time of less than 21 days can be considered definitely abnormal and indicative of a hemolytic process or red cell damage.

We have so far assumed that elution from individual red cells and loss of Cr\(^{51}\) due to hemolysis proceed at a steady rate. Changes in the apparent elution rate have been observed, however, following red cell injury.\(^{21}\) Similarly in the present
experiments a marked change in apparent elution rate was observed in dogs receiving whole body irradiation.

In these experiments the dose of radiation used was sufficient to cause cessation of red cell production. In the absence of new red cell production, the ratio of non-tagged to tagged cells should remain constant—provided both tagged and non-tagged cells have the same age distribution. However if the tagged cells have a higher proportion of older cells at the time of irradiation as occurs when animals are observed for some time (20–55 days) after transfusion and before irradiation, then the ratio of non-tagged to tagged cells will not remain constant. A larger proportion of the tagged cells will die off each day, thus shifting the ratio upward. The magnitude of this shift can be computed from the following considerations.

For untagged cells

\[ N_t = N_0 \left( 1 - \frac{t}{T} \right) \]

where \( N_t \) is the number of cells remaining at time \( t \) days after cessation of red cell production

\( N_0 \) = number of cells at the outset

\( T \) = normal potential life span of cells.

For tagged cells

\[ N'_t = N'_0 \left( 1 - \frac{t}{T} \times \frac{1}{T - x} \right) \]

where the primes indicate tagged cells, \( x \) is the number of days between transfusion and irradiation, so that

\[ \frac{N_t}{N'_t} = \frac{N_0}{N'_0} \left( \frac{T - t}{T} \right) \left( \frac{T - x}{T} \right) \]

From this relation the change in the ratio \( \frac{N_t}{N'_t} \) can be computed. (It can be seen that if \( x = 0 \), i.e., no lag between transfusion and irradiation, then this equation reduces to \( \frac{N_t}{N'_t} = \frac{N_0}{N'_0} \), which is constant)

For example, for various values of \( x \) and \( t \), the ratio \( \frac{N_t}{N'_t} \) is greater than \( \frac{N_0}{N'_0} \) by the factors in table 3.

It may be seen from the table that changes induced by even very long delays between transfusion and irradiation are rather small. It should also be noted that the factors increase with time after irradiation. On either arithmetic or semi-logarithmic paper this will produce a curve which is concave upward. This concavity is in the opposite direction from the appearance of the curves in the dogs in which irradiation was delayed following transfusion. In addition, the increases accounted for by the different age distributions are much less than actually observed in the dogs. For example in animal number 997, irradiated on the 55th day following transfusion, the elution “constant” prior to irradiation was .014;
The factors by which the ratio of tagged:untagged cells change with time in the absence of red cell production when the age distribution of the 2 populations at the time of cessation of red cell production (irradiation) was different. X represents the number of days between transfusions and irradiation and t the time after cessation of red cell production (irradiation). for the 5 day period following irradiation this constant was .077. In the formula above, the x = 55 and t = 5, the expected rate would have been only 1.069 times the pre-irradiation rate, or .015, if the increase was due to age distribution inequalities alone.

Since a random hemolytic process would affect both the tagged and non-tagged cells equally, it would not result in an increase in apparent elution such as was observed. These observations could be explained by an actual increase in elution of Cr\textsuperscript{51} from individual cells or by destruction of some of the tagged cells. Neither of these processes can be represented by a steady rate. One may visualize that Cr\textsuperscript{51} is bound to the stroma of the red cell by more than one type of linkage and that “more lightly” bound Cr could be eluted selectively. Similarly, one might assume that during the process of tagging a different number of Cr atoms attach themselves to different cells, and that the more heavily tagged cells may be selectively destroyed following irradiation. The selective destruction of more heavily tagged red cell is more plausible, since there is experimental evidence to indicate that heavy tagging shortens the survival of normal cells\textsuperscript{21,23,26}. At present no experimental evidence is available to decide between the hypothesis of selective destruction and selective elution. Nevertheless, the changing apparent elution rate per se indicates a non-random, i.e., selective process. The deviation from the usually observed constant elution rate can be ascribed only to a change in the red cell itself, i.e., red cell damage.

In summary, the observation of a decline in Cr\textsuperscript{51} activity faster than expected from a simultaneously performed differential agglutination led to the concept of an elution of Cr\textsuperscript{51}, from individual red cells. This elution is of the order of 1–2 per cent in normal dogs. The variation between individuals, though small, is sufficient to preclude application of a standard correction for elution. An apparent elution rate of more than 2% or an initial apparent half-time of less than 21 days can be taken as evidence of a red cell damage. Moreover, a change in elution rate per se is indicative of red cell injury. All of these abnormalities indicating damaged red cells have been observed following whole body irradiation.

**Shortened red-cell survival on homologous transfusion**

When red cells were taken from dogs irradiated 1–8 days previously, and transfused into normal recipients, unequivocal alteration of the survival characteristics of these cells could not be demonstrated. Survival of cells collected on the 3rd
post-irradiation day was entirely normal as determined by this technic. Survival of cells collected between the 5th and 10th post-irradiation days fell in all instances in the lower ranges observed in apparently normal dogs. With the Cr\textsuperscript{51} technic minor hemolysis of the order of .5 to 1% may be masked, due to the variability of elution rate.\textsuperscript{17} The fact that in all instances the values fell within the low range of normal suggests that there was in fact mild red cell injury. This was supported by the observation of a changing elution rate. After the 10th post-irradiation day, the shortening of red cell survival was unequivocal. The injury to the red cells was not a direct effect of the radiations since normal cells transfused into irradiated dogs shortly after exposure also had a shortened survival time.

Red cell damage demonstrated by change in previously established elution rate

In these experiments the elution rate was first established by observing auto-transfused dogs for 20-55 days prior to irradiation. Between the 1st and 3rd day after irradiation there was a marked decrease in the Cr\textsuperscript{51} activity per unit of cells in all of fifteen dogs studied. This was reflected in the elution curve by a striking increase in the rate of Cr\textsuperscript{51} elution (figs. 5-7). The change in elution rate always occurred during the first 3 days following exposure to the ionizing radiations. In vivo and in vitro labeled cells behaved similarly. Though it is impossible to quantify the changes it appeared that there was a greater loss in the Cr\textsuperscript{51} activity after irradiation when a concentration of 15 gamma of chromium per ml. of cells was used for tagging than when 5 gamma per ml. was used. A difference could not be detected between concentrations of 0.1 and 5 gamma. The loss of Cr\textsuperscript{51} from normal cells transfused immediately after irradiation showed a similar alteration in the pattern of elution (fig. 8).

Additive damage of red cells due to extravasation

Since the onset of red cell damage recorded in the past occurred between the 7th and 10th day and coincided with the development of thrombocytopenia, the possible role of thrombocytopenia and hemorrhage in the production of red cell damage was investigated. In thrombocytopenic states many of the cells extravasated into the tissues are picked up by the lymphatics and returned through the thoracic duct into the general circulation. The survival of red cells collected from the thoracic duct on the 8th and 12th days after irradiation was compared with the survival of cells collected from the same animal from a peripheral vein. Following transfusion into normal recipients there was an accelerated rate of destruction of both the cells collected from the thoracic duct and the peripheral vein. The rate of destruction of cells collected from the thoracic duct, however, was substantially greater than was that of peripheral vein blood (fig. 12).

Since Hollingsworth\textsuperscript{10} had previously reported that normal cells injected intraperitoneally in rabbits survived normally on the return to the general circulation, a comparison was made of the survival of cells from normal animals and irradiated animals when injected extravascularly into normal recipients. Normal cells, when injected intraperitoneally or intramuscularly had a normal survival upon return to the general circulation. When normal cells were injected subcutaneously only about 1 per cent of the injected cells were returned to the general circula-
tion and these had an accelerated rate of red cell destruction. It would appear, therefore, that the tortuosity of the path traversed by these cells prior to reaching the general circulation in part determines the degree of damage and the rate of destruction of these cells in the vascular system.

In contrast to normal cells, cells from irradiated donors injected intramuscularly into normal dogs were destroyed rapidly. This departure from the normal was observed with cells collected as early as the 1st day after irradiation. Cells collected on the 4th day were destroyed even more rapidly than those collected on the 1st day. When normal cells were injected intramuscularly into irradiated dogs, it was evident that there was selective damage to the infused cells (fig. 11).

These studies indicate that the extravascular cycle produces an additive damage to red cells of the irradiated animal. Moreover, they would support the hypothesis of early intravascular red cell injury which progresses until irreversible damage occurs. Presumably there is sufficient red cell damage by the 1st post-irradiation day so that the additional trauma of passage through an extravascular cycle, perhaps aided by chromation, produces irreversible changes in the red cell.

Additive damage due to Cr$^{51}$

It is of interest to note that chromium apparently produced additive damage to cells when present during exposure to the indirect effects of ionizing radiation. When cells from irradiated donors passed through the extravascular cycle prior to chromation as in the studies of blood collected from the thoracic lymph and then tagged with chromium, the rate of loss of Cr$^{51}$ was significantly less than when chromation preceded the exposure to extravascular cycle as in those studies in which cells from irradiated donors were tagged with chromium on the 1st or 4th post-irradiation day and then injected intramuscularly into normal dogs. Moreover, in dogs transfused prior to irradiation there was a marked increase in loss of chromium between the 1st and 3rd day following irradiation, the result either of increased elution or selective destruction, whereas cells collected on the 3rd post-irradiation day, tagged and transfused into normal recipients survived normally. An alteration in the survival characteristics of normal cells on transfusion into normal recipients has been observed by ourselves$^{26}$ and others$^{22, 25}$ when a concentration of chromium metal in excess of 20 gamma per ml. of packed cells is used in tagging the cells. The magnitude of this increase in the rate of destruction of chromated cells is quite striking when 100 gamma per ml. are used. It is reasonable to expect, therefore, that some types of cell damage may be enhanced by the chromium metal itself even though the concentrations used are not normally toxic. In this study it was not possible to establish a quantitative relationship between the concentration of chromium metal and the effects of radiation although it did appear that the red cell damage incident to radiation was more marked when 15 gamma of chromium per ml. of cells were used in tagging than when 0.1–5 gamma were used. It was not possible to reduce the degree of red cell damage by using concentrations of 0.1 gamma of chromium per ml. The alternative possibility that the small amount of x and γ rays incident to the disintegration of chromium to vanadium would account for this apparent selective damage to the red cells would seem unlikely, since it has been previously
demonstrated that in vitro radiation does not affect survival characteristics of red cells.\textsuperscript{4}

These observations do not necessarily imply that chromium produces additive damage following other types of red cell injury (antibodies, etc.) but they do raise the question of the applicability of chromium for quantitative estimates of red cell loss. It appears that following irradiation injury the chromium technic permits a qualitative study of red cell injury and enables us to establish the presence of red cell damage but does not allow its quantitation.

The studies reported were done following whole body exposure to ionizing radiations. Whether similar effects might occur following partial body irradiation was not established. Sheets and his associates\textsuperscript{3} have reported a hemolytic process appearing between the 7th and 10th day after partial body exposure of patients with malignancies. It may be that the hemolytic mechanism described by these authors is similar to that observed by us following whole body irradiation and that early red cell damage did indeed occur. On the other hand red cell damage under these circumstances may be related to the effects of the radiations on the malignancy.

**Summary**

Post-irradiation anemia is primarily the consequence of red cell aplasia and of hemorrhage secondary to thrombocytopenia. Data have been presented which indicate that in addition there is intravascular red cell damage. Of particular interest was the observation herein presented that the damage to the red cells produced by ionizing radiations is of an indirect nature. Using the chromium technic it was possible to demonstrate red cell damage as early as the 1st–3rd post-irradiation day. In these studies tagged cells were transfused 15–55 days prior to irradiation in order to establish the elution rate of Cr\textsuperscript{51}. On the 1st–3rd day following irradiation there was an abrupt increase in the rate of Cr\textsuperscript{51} loss. When normal cells were transfused immediately after irradiation there was also a striking increase in the rate of Cr\textsuperscript{51} loss above that observed in normal animals, indicating that radiation damage to the red cell was indirect. When cells were transfused from irradiated donors into normal recipients, it was not possible to demonstrate red cell damage prior to the 10th post-irradiation day, indicating that the damage was progressive.

Since there was a selective loss of Cr\textsuperscript{51} it is suggested that chromium produced an additive damage to the cell. The possible implication of this observation in the use and interpretation of Cr\textsuperscript{51} survival curves is discussed.

It was also determined that red cells which were recirculated following extravasation during the thrombocytopenic phase of radiation injury had a shortened survival, whereas normal cells, tagged and injected intramuscularly into normal recipients survived normally on return to the general circulation. Cells collected from dogs one day after irradiation, tagged with Cr\textsuperscript{51} and then injected intramuscularly into normal recipients, showed a striking increase in the rate of destruction. Cells collected on the 4th post-irradiation day were destroyed even more rapidly. These studies confirmed the early onset of radiation injury to the cells, its progressive nature, and demonstrated the damage incurred as a result.
of passage through the extravascular cycle. As might be expected there was also a shortened survival of red cells collected from the thoracic lymph of thrombocytopenic irradiated animals.

Conclusions: 1. Ionizing radiations produce intravascular red cell damage in addition to the known loss incident to hemorrhage. This damage is evident within 24–72 hrs. of irradiation.

2. The red cell damage is an indirect and progressive effect of irradiation.

3. Those cells which are returned to the general circulation, following passage through an extravascular cycle, are damaged. The extent of injury to normal cells is such that it is not detectable by current methods. However, the addition of a second injury, such as that following irradiation, enables the detection of this damage.

4. Chromium “elution” is altered by irradiation injury.

SUMMARIO IN INTERLINGUA

Anemia post-radiatio es primariemente le consequentia de aplasia erythrocytic e de hemorrhagia secundari a thrombocytopenia. Es presentate datos que indica que il occurre in plus un vulneration del erythrocytos intravascular. Esseva specialmente interessante observar que le vulneration del erythrocytos effectuate per radiation ionisante es de natura indirecte. Per medio del technica a chrom il esseva possibile demonstrar lesiones in le erythrocytos post intervallos post-irradiational de non plus que 1 a 3 dies. In iste studios, cellulas marcate per radioisotopos esseva transfundite 15 a 55 dies ante le irradiation, con le objectivo de determinar le rapiditate del processo de elution de Cr⁴¹. Un a tres dies post le irradiation, il occurreva un perdita abruptemente accelerate de Cr⁴¹. Quando cellulas normal esseva transfundite immediatemente post le irradiation, il etiam occurreva un acceleration frappante del perdita de Cr⁴¹ in excesso de lo que es observate in animales normal. Isto indica que le lesion radiacional del erythrocytos esseva indirecte. Quando cellulas esseva transfundite ab donatores irradiate a in recipientes normal, il non esseva possibile demonstrar erythrocytos vulnerate ante le decime die post-irradiational. Isto indica que le vulneration esseva progressive.

Proque il occurreva un perdita selective de Cr⁴¹, il es suggerite que le chro mos produceva damnlos additional in le cellulas. Es discutite le implicationes possibile de iste observation pro le uso e le interpretation de curvas de superviventia a Cr⁴¹.

Esseva etiam trovate que erythrocytos recirculate post extravasation durante le phase thrombocytopenic del vulneration radiational habeva un reduce superviventia, durante que cellulas normal que esseva marcate e injicite intramuscularmente in recipientes normal habeva periodos de superviventia normal post lor retorno al circulation general. Cellulas colligite ab canes un die post le irradiation monstrava, quando illos esseva marcate con Cr⁴¹ e alora injicite intramuscularmente in recipientes normal, un augmento frappante in le rapiditate de lor destruction. Cellulas colligite le quarte die post le irradiation esseva destruite ancora plus rapidemente. Iste studios confirmava le prompte declaration del vulneration radiational in le cellulas, su natura progressive, e demonstrava le
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insulto sufriría in consequentia del passage per le ciclo extravascular. Il non eseva surprendente constatar un reduction del superviventia de erythrocytos colligite ab le lympa thoracic de irradiate animales in stato thrombocytopenic.

Conclusions: 1. Radiation ionisante produce lesiones del erythrocytos intra-vascular, a parte le cogmioscite facto ciue le hentorrhagias causate per illo resulta in perditas de erythrocytos. Iste lesiones es evidente intra 24 e 72 horas post le irradiation.

2. Le lesions erythrocytic es un effecto indirecte e progressive del irradiation.

3. Cellulas que es retornate al circulation general post lor passage per un cyclo extravascular suifre un certe vulmteration. Le grado del vulneration in be caso de cellulas miorntal es Si leve que illo non poter esser detegite per be methodos currentemente disponihile. Tamen, le addition de un secunde vulneration—per exemplo illo que resulta de irradiatiomt—rende le detection de ille vulneration possibile.

4. Le elution de chromo es alterate per vulneres de irradiatiomt.

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The Hemolytic Effect of Ionizing Radiations and Its Relationship to the Hemorrhagic Phase of Radiation Injury

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