Extracorporeal Irradiation of the Blood in Humans: Effects upon Erythrocyte Survival

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The first successful experiments utilizing extracorporeal irradiation of the blood (ECIB) to deplete the body of lymphocytes were performed by Cronkite et al. using the cow as an experimental animal. It soon became evident that continuous prolonged ECIB produced hemolytic anemia and hemoglobinuria. Thus, despite the fact that erythrocytes are more radioresistant than leukocytes, it was shown that sufficient irradiation shortens their lifespan and produces hemolysis. Mathematical analysis designed to measure the accumulated dose distribution to the erythrocytes indicated that hemoglobinemia did not occur to any significant extent until at least 25 per cent of the circulating erythrocytes received over 100,000 rads. Further studies on bovine erythrocyte survival after in vitro gamma irradiation have been performed and will be considered later. The clinical application of ECIB in human beings as a method of reducing lymphocyte populations of the body and for destroying leukemic leukocytes has been initiated by at least two other groups besides ourselves. In view of the interest expressed by many investigators in the potential uses, and possible harmful effects, of ECIB, it was considered important to explore the effects of large doses of irradiation upon erythrocytes.

This study was designed to measure the in vivo survival of autologous erythrocytes after in vitro gamma radiation. We will present the dose effect curve between in vitro radiation dose and red cell survival. A computer program has been developed to estimate the total accumulated dose of radiation for various schedules of continuous or intermittent ECIB, and to try to correlate these findings with ECIB-produced red cell effects. Lastly, data on actual transfusion requirements of 11 patients who have received therapeutic ECIB will be reported.

Materials and Methods

Ten individuals were studied. all of whom were hematologically normal. Forty ml of venous blood, drawn into a plastic syringe containing 10 ml of special ACD* solution, was...
slowly injected into a small plastic bag. The bag was placed on a wire-supporting rack surrounding a $^{60}$Co extracorporeal irradiation source. Radiation doses of 35,000 rads, 50,000 rads, 100,000 rads or 200,000 rads were given to the blood at a dose rate of 1800 rads per minute. Immediately after irradiation, Na$_2$$^{51}$CrO$_4$ of specific activity 100 mc./mg., 0.25–0.5 μc./Kg. body weight, was added to the blood. After incubating the blood at room temperature for 30 minutes, 50 mg. of sodium ascorbate was added and the mixture injected intravenously into the donor. Samples of blood collected at 15, 30, 60 and 120 minutes after injection were washed three times with saline, hemolyzed, and counted in a NaI (TI) crystal scintillation counter with a pulse-height analyzer. Photopeak counts were used and excellent counting statistics (<2 per cent error) were obtained. Extrapolation to zero of the counts at these time periods was used as the 100 per cent value for retention of $^{51}$Cr-labeled erythrocytes. Frequent blood samples were obtained and counted to establish the labeled red cell disappearance times.

Irradiation and chromation of blood at 37 C. and irradiation of blood after chromation did not significantly alter the results in two individuals. In addition, repeated $^{51}$Cr red cell disappearance times without irradiation of the cells were normal in three subjects. The results are referred to as “apparent” red cell survival times, since the magnitude of chromium elution from irradiated red cells is unknown. In vitro elution studies, utilizing both saline and plasma, showed no significant elution after two days at 20 C. and 37 C.

The IBM 7090 Sanghai II program was designed to estimate the average dose distribution of irradiation to the erythrocytes. Since only a fraction of the cardiac output is diverted through the artificial shunt, there is a probability function for repetitive cycles through the irradiator. This function has been established by Slatkin et al. using a differential equations method. Very similar results are obtained using the statistical method developed by Marsaglia. For practical purposes the circulating blood volume is arbitrarily divided into quartiles, with the first quartile receiving the least dose and the fourth quartile the highest average dose. The variables that must be inserted into the program and their methods of measurement are noted in the following section.

1. **Blood Volume:** The blood volume was measured in one of two ways. In those individuals not receiving $^{51}$Cr for other reasons, the patient’s own erythrocytes were chromated, reinjected, and the blood volume calculated from the dilution equation. In patients who received $^{51}$Cr for other purposes (platelet survival studies) RISA-125I was injected and the total blood volume or plasma volume* was determined.

2. **Radiation Source:** The irradiation source consisted of 4000 curies of $^{137}$ cesium chloride in square compressed pellets within a stainless steel container, 33.7 cm. × 2.9 cm. × 0.95 cm. This source is held within a lead cylinder. Stainless steel tubing penetrates at an angle through the lead cylinder and passes around the cesium source at a constant distance of 1.6 cm. For irradiation of the blood, silicone rubber tubing† is threaded through the stainless steel tube, and the two ends connected to the patient’s arteriovenous shunt. The dose rate of the irradiator was calibrated by pumping a solution of ferrous sulfate through a similar silicone rubber tube and measuring the oxidation of Fe$^{+++}$ to Fe$^{+++}$. The intensity of the source under the stated conditions is 425,000 rads per hour.

3. **Transit Dose:** The transit dose is the number of rads received by a segment of blood as it passes once through the irradiator. It is calculated from the known intensity of the $^{137}$cesium source and flow rate of the blood through the irradiator. The flow rate is measured by the passage of a bubble of air introduced into the flowing blood. About 0.1 ml. of air is sterilely injected into the silicone rubber tubing on the arterial side and is timed as it passes into and back out of the radiator and into the venous segment of the shunt. The volume of the extracorporeal segment is calculated from the inside diameter and length of the silicone

*Volumetron, Ames Lab-Tek.
†Designed and produced by Brookhaven National Laboratory, Nuclear Engineering Department, High Intensity Radiation Development Laboratory.
‡I.D. 0.104 inches, O.D. 0.193 inches, supplied by Extracorporeal Medical Specialties Company, Medford, New Jersey.
rubber tubing. The total number of transits is calculated from flow rate, length of external
shunt, and time the patient is treated. Flow rates average about 100 ml/min. with extracor-
poreal volumes of about 10 to 15 ml.

Semipermanent Teflon-Silastic arteriovenous shunts, similar to the Quinton-Scribner type, are
made between the radial artery and a forearm vein several days before commencement
of ECIB.

The osmotic resistance of erythrocytes from 10 normal individuals was measured in
graded concentrations of hypotonic saline after incubation of the blood for 24 hours at 37 C.
Blood was drawn in special ACD solution for purposes of comparison with the in vitro
radiation-in vivo disappearance study.

RESULTS

Survival Time of Irradiated Labeled Erythrocytes

In Figure 1 the survival of \( ^{51}\text{Cr} \)-labeled red cells following different doses of
gamma radiation are presented and compared to the normal survival of red
cells. The survival time of irradiated red cells diminished as the dose increased
from 35,000 to 200,000 rads. At all doses each curve had two components.
There was a rapid initial loss of cells extending over a 24-hour period which
was followed by a slower disappearance of the labeled cells.

As shown in Figure 2 the 24-hour percentage loss of irradiated erythrocytes
is a linear function of the dose of radiation. In Figure 3 the “apparent”
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Fig. 2.—Twenty-four-hour loss of irradiated erythrocytes as a function of dose (in kilorads).

biological half-life, based upon the second component of the survival curves shown in Figure 1, is shown to be a negative exponential function of the radiation dose to the erythrocytes.

Computer Estimates of the Accumulated Dose of Radiation by Red Cells

Idealized data from the IBM 7090 digital computer and the accumulated dose to red cells for different patterns of irradiation is presented in Table 1. The results show that for equivalent fourth quartile (highest dosage quartile) dosages of 100,000 rads, less radiation is delivered to the first and other quartiles when the periods of irradiation are spaced further apart.

In Figure 4 the accumulated radiation dose to the four quartiles of the blood is shown in a typical clinical situation in which the patient receives 4 hours of ECIB daily. The variables are as indicated on the figure. If all newly produced erythrocytes survive for 100 days and the irradiation is continued daily for 100 days, the production and destruction rates of red cells will then remain equal. The distribution of the average quartile accumulated dose to the red cells varies from 40,000 to 300,000 rads.
Transfusion Requirements of Irradiated Patients

In Table 2 the transfusion requirements of 11 patients who have been treated with ECIB and the computer estimate of the accumulated radiation dose to the fourth quartile of blood are presented. Estimated blood replacement for defined bleeding episodes was not used in these calculations.

Osmotic Resistance of Irradiated Erythrocytes

Immediately after irradiation, red cells displayed no difference from normal with respect to their osmotic resistance. After 24 hours of incubation at 37 C., the osmotic resistance of samples given 50,000 and 100,000 rads actually increased above normal (Fig. 5), but after 200,000 rads markedly decreased.

DISCUSSION

Analysis of the disappearance rates of $^{51}$Cr labeled erythrocytes reveals the presence of two exponential curves. The first has a short and variable half-life of 12–24 hours and probably represents an acute loss of irradiated cells rather than elution of chromium from the erythrocytes.

External probe counting over organs in two individuals receiving erythrocytes exposed to 100,000 rads supports this view, since the liver/heart and
Table 1.—Computed Accumulated First Quartile Dose to Erythrocytes When Fourth Quartile Dose Reaches 100,000 Rads

<table>
<thead>
<tr>
<th>Pattern of ECIB</th>
<th>Total Hours of ECIB</th>
<th>Days Involved</th>
<th>Total Accumulated First Quartile Dose (Rads)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous</td>
<td>120</td>
<td>50.0</td>
<td>80,000</td>
</tr>
<tr>
<td>4 hours daily</td>
<td>144</td>
<td>36.0</td>
<td>52,000</td>
</tr>
<tr>
<td>4 hours every other day</td>
<td>144</td>
<td>72.0</td>
<td>32,000</td>
</tr>
<tr>
<td>8 hours daily</td>
<td>130</td>
<td>16.3</td>
<td>68,000</td>
</tr>
<tr>
<td>8 hours every other day</td>
<td>130</td>
<td>32.6</td>
<td>53,000</td>
</tr>
<tr>
<td>8 hours daily for 4 days</td>
<td>130</td>
<td>36.0</td>
<td>54,000</td>
</tr>
<tr>
<td>rest 4 days...</td>
<td>120</td>
<td>100.0</td>
<td>24,000</td>
</tr>
<tr>
<td>8 hours once a week</td>
<td>120</td>
<td></td>
<td>24,000</td>
</tr>
</tbody>
</table>


spleen/heart ratios increased significantly within the first day. Further evidence against elution is the fact that the in vitro studies demonstrated no significant elution during the two days of incubation at 37°C.

Irradiation of the magnitude we used experimentally for small quantities of blood was not used during therapy. Patients received daily treatments of four or more hours resulting in average accumulated fourth quartile doses of 1000 to 4000 rads per treatment. Clearly, Figure 1 shows that as little as 35,000 rads in a single dose shortens the red cell life span. It is probably fallacious to equate the experimentally performed acute irradiation with the repetitive prolonged clinical irradiations and expect the same biological responsiveness of the erythrocytes per rad, since repair mechanisms may be operative during the prolonged exposure period. From the data of Schnappau et al.4 on cows, it would appear that repetitive prolonged radiation had a lesser effect. Therefore, the data from a single radiation exposure probably represents an upper limit of effect.

Not only may the original red cells have long disappeared from causes not related to radiation (for example, hemolysis from other factors in the natural course of leukemia), but they also may have been replaced by transfused cells with unknown survival times. Thus, no direct comparison of red cell survival times have been attempted in our patients. In addition, many of the patients required transfusion before, during, and after treatment, and erythrocyte survival times have very limited value in these nonsteady states.11

The present computer program (Sangrad II) assumes an average erythrocyte life span of 100 days, but does not take into account the changes in erythrocyte loss due to radiation effects or possible changes in red cell production. Modifications are being incorporated to correct for these effects.

Although multiple factors determine transfusion requirements, one gains some insight into the problem from the data presented in Table 2. Three patients (D. O., P. O. and J. O.) accumulated 4th quartile doses to their red cells of 27,300, 52,400, and 32,000 rads, respectively, without requiring transfusion and without a significant decline in hemoglobin concentration. All of
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these patients had active erythropoiesis as measured by total reticulocyte counts and by bone marrow examination. Three other patients with normal or subnormal erythropoiesis (D. H., T. E. and S. K.) accumulated 4th quartile doses of 26,100, 51,325, and 47,300 rads, respectively, and transfusion requirements increased. Three individuals (R. O. M., C. I. and D. A.) received an amount of radiation to the 4th quartile from which one would expect severe hemolysis if given in a short period of time—that is, 100,000 to 150,000 rads. All three manifested some increase in transfusion requirements and all had depressed erythropoiesis.

Patient R. O. B. had three courses of ECIB. During the first there was a decrease in transfusion requirements, and in the second and third courses
Table 2.—Transfusion Requirements* of 11 Patients Being Treated with ECIB

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Transfusion Requirements (units/wk.)</th>
<th>Computed Accumulated Dose to 4th Quartile</th>
<th>ECIB</th>
<th>Time</th>
<th>Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before ECIB</td>
<td>During ECIB</td>
<td>After ECIB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. O.</td>
<td>Acute myelocytic leukemia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>27,300</td>
<td>26 days</td>
</tr>
<tr>
<td>D. H.</td>
<td>Acute myelocytic leukemia</td>
<td>0.75</td>
<td>1.5</td>
<td>0</td>
<td>26,100</td>
<td>17 days</td>
</tr>
<tr>
<td>R. O. M.</td>
<td>Acute myelocytic leukemia</td>
<td>0.28</td>
<td>2.1</td>
<td>0</td>
<td>92,954</td>
<td>85 days</td>
</tr>
<tr>
<td></td>
<td>A)</td>
<td>3.0</td>
<td>2.0</td>
<td>1.8</td>
<td>48,850</td>
<td>15 days</td>
</tr>
<tr>
<td>R. O. B.</td>
<td>Acute myelocytic leukemia</td>
<td>1.8</td>
<td>0</td>
<td>0</td>
<td>48,200</td>
<td>40 days</td>
</tr>
<tr>
<td></td>
<td>B)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25,890</td>
<td>41 days</td>
</tr>
<tr>
<td></td>
<td>C)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. I.</td>
<td>Acute myelocytic leukemia</td>
<td>1.1</td>
<td>1.6</td>
<td>0</td>
<td>97,790</td>
<td>52 days</td>
</tr>
<tr>
<td>D. A.</td>
<td>Acute myelocytic leukemia</td>
<td>0.3</td>
<td>0.9</td>
<td>0</td>
<td>150,000</td>
<td>90 days</td>
</tr>
<tr>
<td>T. E.</td>
<td>Acute myelocytic leukemia</td>
<td>0.25</td>
<td>1.3</td>
<td>0</td>
<td>51,325</td>
<td>51 days</td>
</tr>
<tr>
<td>S. K.</td>
<td>Acute myelocytic leukemia</td>
<td>0.33</td>
<td>0.83</td>
<td>0</td>
<td>47,300</td>
<td>35 days</td>
</tr>
<tr>
<td>P. O.</td>
<td>Chronic myelocytic leukemia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>52,400</td>
<td>31 days</td>
</tr>
<tr>
<td>H. U.</td>
<td>Chronic lymphatic leukemia</td>
<td>1.0</td>
<td>1.7</td>
<td>1.3</td>
<td>36,000</td>
<td>34 days</td>
</tr>
<tr>
<td>J. O.</td>
<td>Chronic glomerulonephritis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>32,000</td>
<td>24 days</td>
</tr>
</tbody>
</table>

*Not including replacement for defined bleeding episodes.
during partial remissions, no transfusions were necessary. Bone marrow aspirations were hypocellular, but unchanged throughout this period of time. Patient H. U., who received 36,000 rads to the 4th quartile of red cells, clearly had an increase in transfusion requirements during ECIB and a decline after ECIB was halted.

We have had the opportunity of performing ECIB upon 5 patients prior to and after renal homotransplants in collaboration with Dr. John P. Merrill of the Peter Bent Brigham Hospital, Boston, Massachusetts. Two of these patients had normal hemoglobins, and three were anemic. All patients except one from the latter group displayed a drop in hemoglobin of from 1.5 to 3.0 Gm. per cent after a minimum of six ECIB sessions. The one exception is patient J. O. in Table 2. Most (4 of 5) patients received 4th quartile accumulated radiation doses of 20,000 to 35,000 rads. The reticulocytes in all patients rose from 35 per

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**Fig. 5.—Osmotic fragility of irradiated erythrocytes. Average of 10 normal individuals.**

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cent to 275 per cent above their initial value, indicating increased erythropoiesis. No transfusions were administered. Although we were unable to perform valid autologous \(^{51}\text{Cr}\)-labeled red cell disappearance times, there is presumptive evidence to suggest that ECIB was producing a hemolytic syndrome in these hemopoietically abnormal, but not leukemic, patients.

From these data it is clear that accumulated red cell radiation is not the only factor that must be considered when anticipating red cell damage. We have probably produced hemolysis in some leukemic patients that remained undetected clinically except for increases in transfusion requirements. At the same time, however, equivalent doses of radiation to the blood of other leukemic patients, some of whom had little erythropoiesis, seemed to have had minor, if any, effects. There appears to be convincing evidence that hemolysis does result from ECIB in some patients with renal disease. This information leads us to presume that there are individual variations in red cell sensitivity to radiation, but ECIB in therapeutic use can be expected to produce red cell hemolysis in varying degrees.

Irradiation is probably not the only factor which must be considered in patients with arteriovenous shunts. The nonuniformity of Teflon and Silastic junctions, the plastics themselves, and mechanical damage due to trauma should be taken into account. That these factors are not very important is clear from the lack of reports from chronic renal hemodialysis units.

From the data of Schnappauf et al.,\(^4\) it is apparent that there are major species differences in red cell survival performed with chromated erythrocytes. In Holstein cows, 40 per cent of \(^{51}\text{Cr}\) tagged nonirradiated autologous erythrocytes either disappeared or lost their label by two days. Normal, nonirradiated Angus cows lost 70 per cent of their label in the same period. Canine erythrocytes appeared to be considerably more radiosensitive than bovine erythrocytes, but only in the initial days. Normal chromated human cells display no such acute disappearance characteristics.

Of great interest also is the finding that radiosensitivity appears to increase with erythrocyte age, as shown by irradiation of differently aged cohorts of \(^{59}\text{Fe}\) labeled erythrocytes transfused into identical twin calves. If one assumes that \(^{51}\text{Cr}\) labels red cells uniformly and that radiosensitivity is age-dependent, one would expect a nonexponential decline in the disappearance rate of labeled cells. In each of our studies the disappearance of \(^{51}\text{Cr}\) activity has followed a double exponential during the time period followed. The initial 24-hour loss of labeled cells may be related to more radiosensitive older erythrocytes, although Mollison\(^1\) has described a similar loss in normal unirradiated red cells. This preferential loss of chromated erythrocytes is clearly defined but not understood. In vivo elution of \(^{51}\text{Cr}\) from irradiated red cells of cows and humans must be investigated further before complete quantification of radiation effects can be made.

Stohlman et al.\(^1\) studied the disappearance of \(^{51}\text{Cr}\)-labeled red cells from dogs given several hundred rads of total body gamma irradiation. They concluded that ionizing radiation indirectly produced mild but progressive hemolysis, and that the amount of chromium used produced an additive effect. The
specific activity of $^{51}$Cr presently available is approximately 50 to 100 times that previously used, and two control studies performed by irradiation after chromation did not alter our results. The indirect hemolytic effect described by Stohlman was not noted if irradiated red cells were transfused into normal recipients. Although this is essentially what we did in our in vitro radiation-in vivo disappearance study, easily measurable results were obtained. It must be pointed out, however, that entirely different magnitudes of radiation dose were involved. Studies of indirect effects produced by irradiation upon large quantities of plasma are now in progress.

The actual mechanism of radiation damage to the red cells in vitro and during ECIB is unclear. The recognition of significant radiation effects upon blood dates back to Henri and Mayer’s description of radium-induced hemolysis in 1904. Loss of intracellular K$^+$ and influx of Na$^+$ and decreased glutathione, glyoxalase, and ATPase, have been described as occurring in response to in vitro irradiation. After 50,000 rads glucose, lactate, cholinesterase and ATP have been described as normal, and carbonic anhydrase activity was reported unchanged by 200,000 rads. Minor chemical changes in purified hemoglobin were produced by 100,000 rads and in purified cytochrome C by 10$^6$ rads. Two million rads caused disruption of polypeptide protein chains and either hemolysis or fixation of red cells, depending on the surrounding medium and concentration of cells. Sauerbier, reporting on in vitro irradiation of rabbit erythrocytes, in dose ranges similar to ours, found that 20–100 per cent of the cells were eliminated by the second day. He postulated a one-hit enzyme inactivation mechanism. Several studies of nucleated erythrocytes of amphibia indicated that these cells were considerably more radiosensitive than nonnucleated mammalian cells. Nucleated fowl red cells exhibited increased respiratory activity after 10$^6$ rads. The nucleated erythrocytes of ducks, when irradiated in vitro and labeled with $^{51}$Cr, were found to be more radiosensitive than human red cells, but not as radiosensitive as bovine or canine cells.

Studies upon osmotic resistance of irradiated red cells were undertaken to hopefully provide a measureable index of radiation-produced red cell damage. As demonstrated in Figure 5, we obtained the unexplained results of increased osmotic resistance with radiation doses up to 100,000 rads. These findings do not correlate with the observed smooth decline of in vivo red cell disappearances, nor does it agree with other reported in vitro studies. Aside from its intrinsic interest, this study points up the complexity of the problem of radiation effects upon erythrocytes.

Several investigators have described radiation effects seen by electron microscopy after 24,000 to 84,000 rads. Utilizing both intact cells and red cell ghosts, we have not been able to distinguish such lesions from artifacts in unirradiated cells.

It is quite apparent from the foregoing sampling of the literature that the etiology of radiation damage to red cells, in the dose ranges we have been using, is as yet unresolved.

ECIB is performed as an experimental form of therapy for leukemia and in
preparation for homotransplantation of tissues and organs. As such, the major objective is the reduction in the number of leukemic leukocytes or normal lymphocytes within the body. A therapeutic pattern of irradiation should be designed to exert the maximum effect upon the leukocytes and the minimum effect upon the rest of the blood, including the erythrocytes. Leukocytes have a comparatively short sojourn in the blood, whereas erythrocytes have a long residence in the blood. Theoretically, then, short intense periods of irradiation would be preferable to continuous irradiation. Further information about the lethality of radiation on specific types of leukocytes, the normal residence time in the blood, and the time that it takes for the irradiated leukocytes to be removed from the blood is urgently needed to design a more meaningful extracorporeal irradiation program on an individual basis.

**Summary**

Autologous erythrocytes were irradiated at doses of 35,000 to 200,000 rads, chromated, and red cell survival studied. The 24-hour loss of labeled cells and subsequent apparent erythrocyte survival times were found to be functions of the radiation dose. EC1B-produced red cell hemolysis of a mild degree is to be expected during courses of therapy, as demonstrated by clinical findings. However, there is no doubt that acute, severe, hemolysis could be produced by administering large doses to patients over a short period of time.

**SUMMARIO IN INTERLINGUA**

Autologe erythrocytos esseva irradiate con doses de 35000 a 200000 rad e postea chromate, e le superviventia erythrocytic esseva studiate. Esseva trovate que le perdita de marcate cellulas in 24 horas e le subsequente apparente tempore de superviventia erythrocytic esseva functiones del dose de irradia-tion. Leve grados de hemolyse erythrocytic resultante del irradiation extracorporee del sanguine debe esser expectate durante cursos de therapia, a judicar per constatationes clinic. Tamen, il non existe ulle dubita que acute e sever hemolyse pote esser producete per le administration de grande doses a patientes in le curso de un breve periodo de tempore.

**REFERENCES**

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