Studies on Homologous Bone Marrow Transplantation in Irradiated Rabbits

By Sergio Piomelli and Marcus S. Brooke

Recovery after exposure to an otherwise lethal dose of total body x-irradiation can be achieved by the transfusion of viable bone marrow. It is now well established that this recovery results from colonization of the host's depleted cavities by transfused cells and their descendants, and the result is an animal with a mixture of cells, derived from two individuals—a so-called radiation chimera. Pioneer work in this field was done with small rodents, and successful bone marrow transplants have also been reported in the rabbit and the dog. The use of highly inbred mouse strains has shown that permanent recovery after irradiation can be achieved when donor cells are of animals of the same strain (isologous), whereas interstrain (homologous) combinations result in a temporary recovery of the irradiated animal, followed, after approximately 14 days, by death with cachexia and anorexia. The terms secondary disease, delayed mortality, homologous disease and runt disease have all been used to describe these late deaths.

Previous work in this laboratory had shown that in the rabbit, as well as in smaller rodents, bone marrow transplantation is followed by repopulation of host tissue spaces by donor cells, as evidenced by a cytologic difference in the heterophil leukocytes of the donor and the recipient (the drum-stick marker). A wave of delayed mortality following the immediate recovery was also observed, although the incidence was much lower than that obtained in many mouse strain combinations, thus arousing optimism that the results in the outbred rabbit might be more akin to those to be expected in man. The incidence and severity of secondary disease in the mouse appears to be related to the magnitude of the genetic difference at the H2 locus between the recipient and the donor; the less the disparity, the less the incidence of secondary disease.

Some features suggesting hemolytic anemia were noted in animals dying in the late period, and it was proposed that in these animals secondary disease was due to an immunologic reaction on the part of the graft against the host. Since the work reported in this communication was started, much experimental evidence has accumulated in favor of this hypothesis. Thus, in a situation in which the graft can react against the host, as when marrow of a parental strain is infused into F1 hybrids, secondary disease occurs, whereas in the reverse situations (F1 marrow into parental strains) where marrow cannot react against the host, permanent protection is achieved.

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The present investigation in the rabbit was planned in order to study the incidence and to clarify the genesis of secondary disease, and to investigate the hypothesis that immunologic incompatibility between the host and the graft results in the selective destruction of the cells of one, leading to hemolytic anemia and possibly the death of the chimera. To this latter end, the rate of disappearance of stored donor or recipient cells, labeled with Cr\(^{51}\) after thawing, was measured after infusion into chimeras, and the sera of the chimeras were examined for antibodies to these cells.

In addition to the use of the female heterophil drum-stick as a qualitative marker, female marrow always being infused into irradiated males, a serologic difference in donor and recipient erythrocytes was utilized as an additional marker.\(^{17}\) Donor-recipient combinations were paired so that they differed in at least one antigen on the basis of Cohen's Hg\(^{w}\) allelomorphic system for erythrocytes,\(^{16}\) and, by the use of the differential agglutination technic, a semiquantitative estimate of the number of donor and recipient erythrocytes in the chimeras could be made.

X-irradiation was delivered as a split dose of 600 r, followed 24 hours later by 500 r, as no significant survival has been observed in rabbits receiving homologous bone marrow after a single dose of between 900 r and 1200 r.\(^{19}\) Also, as there are conflicting views, but no systematic study, on the optimal interval between irradiation and transplantation of hematopoietic cells, the effect of different time intervals between X-irradiation and transplantation was compared.\(^{20,21}\)

**Materials and Methods**

**Animals.**—A total of 264 New Zealand albino rabbits was used in this study. They were purchased from commercial breeders and were not, in the genetic sense, inbred. The animals were individually caged, fed standard laboratory diet, and water ad libitum. Young adult males, weighing between 2.5 and 3.2 Kg. were used as recipients, and young weanling females weighing between 1.0 and 1.5 Kg. were used as marrow donors.

**X-irradiation.**—Whole body irradiation was administered as described by Wilson et al.\(^{22}\) An initial dose of 600 r (expressed as air dose at the center of the animal) was followed 24 hours later by 500 r, for a total of 1100 r. X-rays were produced by a 250 kVp. Philips machine operating at 15 mA, and delivering 35 R per min. in a field size of 20 x 20 cm. The target distance to center of animal was 50 cm. A T-3 filter gave an HVL in copper of 3.0 mm.

**Antibiotics.**—Procaine penicillin (100,000 units) and streptomycin sulfate (100 mg.)

| Table 1.—Recipient-Donor Pairings on the Basis of Their Erythrocytic Antigens |
|---|---|---|---|---|---|
| Number | Recipient type | Donor type | Antiserum used | Technic used | Unagglutinable cells | Number of animals |
| 1 | A | F | anti-A | direct | donor | 10 |
| 2 | AF | F | anti-A | direct | donor | 14 |
| 3 | F | AF | anti-A | indirect | recipient | 16 |
| 4 | F | A | anti-F | direct | donor | 15 |
| 5 | AF | A | anti-F | direct | donor | 5 |
| 6 | A | AF | anti-F | indirect | recipient | 13 |
| 7 | D | AF | anti-A | indirect | recipient | 3 |

For experimental details see text under Blood Typing and Differential Agglutination.
were injected intramuscularly twice weekly for 4 weeks, starting immediately after the second dose of x-irradiation.

Preparation of bone marrow.—Donors were killed by the intravenous injection of 20 ml. of air. The extremities were amputated, stripped of their skin and soaked in 50 per cent isopropyl alcohol for 15 minutes, after which they were rinsed three times in sterile saline. The next steps were carried out using sterile precautions. The femur, tibia, and humeri were freed from muscles and gently cracked longitudinally with pliers; the bone marrow was removed, suspended in Hank's balanced salt solution at pH 7.4, and the suspension filtered through a nylon filter (No. 9331 of Macalster Bicknell Co., Cambridge, Mass.), and centrifuged at 500 r.p.m. for 5 minutes, after which the upper layer of fat was removed by suction and the residue gently mixed to give a homogeneous suspension. Cell dilutions for counting were made in an ordinary red cell pipette using 3 per cent acetic acid as diluent and read in a hemocytometer. The yield of bone marrow per donor was between 0.6 and 1.5 x 10⁶ nucleated cells, in a total volume of 8-20 ml. The bone marrow suspensions were infused intravenously into the recipient between 2 and 4 hours after the death of the donor. In some instances the bone marrow suspension was immediately frozen at -20° C, and 24 hours later thawed, sonicated in a 10 KC Raytheon magnetostrictive oscillator for 5 minutes, and then lyophilized and stored at -20° C. for 2 weeks. For use, the lyophilized material was reconstituted to its original volume with sterile distilled water. (This preparation will be called lyophilized marrow.)

Hematology.—Leukocyte counts, microhematocrits and the percentage of neutrophils with female chromatin in the buffy coat were determined before the animals were irradiated or killed, twice weekly for the first 4 weeks following transplantation, and once per week thereafter. The animals were also weighed at these times.

Red cell preservation and Cr¹¹⁷-labeling.—Erythrocytes were collected in ACD, 24 hours before x-irradiation from the recipients, and from the donors, immediately before death. The cells were preserved in glycerol at -20° C. and after thawing and removing, the glycerol were labeled with Cr¹¹⁷. Such cells have a normal half-life and retain their antigenicity. Coombs' test.—The direct Coombs' test was done with fresh arterial blood, and the indirect test with preserved erythrocytes. Sera were always frozen immediately after collection, and those of any one animal were all tested simultaneously, using the same erythrocytes. Antiserum to rabbit gamma globulin was prepared in the goat.

Blood typing and differential agglutination.—Only Cohen's Hg¹⁰⁷ alleleomorphic system was used (hereafter referred to as ADF). Anti-A and anti-F sera were prepared in the rabbit. Animals whose cells were not agglutinated by either of these antisera were classified as homozygous type D. Blood typing was done according to the method of Cohen, except that whole blood instead of washed cells was used and no fresh rabbit serum was added. The mixtures were incubated for 3 hours at 37° C.

The distribution of blood types in 672 animals tested was as follows: A = 17.3 per cent; AF = 35.4 per cent; F = 44 per cent; and neither (type D) = 3.3 per cent. These figures are similar to other reports in the literature. Differential agglutination tests were done at approximately one week intervals, using the Dacie and Mollison modification of the Ashby technique. An 1:100 dilution of blood was mixed with an equal volume of diluted typing serum and incubated for 3 hours at 37° C. Appropriate controls with known blood types were included and blank counts of less than 50,000 free cells per cu.mm. were obtained. In some instances donor type cells were left unagglutinated in a donor-recipient mixture and counted (direct technic), whereas in others the recipient cells were left unagglutinated and were counted, the number of donor type cells being obtained by subtraction from the total red cell count (indirect technic). In still other cases both direct and indirect counts were done on the same sample.

*In the first 2 animals that were transfused, this step was omitted, and the result was the immediate death of the recipients, after transfusion, due to pulmonary embolism.
Experimental groups.—188 animals were irradiated; 25 (13 per cent) died after the first 600 r and 4 (2 per cent) after the second dose of irradiation and before they could be transplanted. The survivors were divided into the following experimental groups:

Group A—33 animals received x-irradiation and 24 hours later were infused intravenously with 25 ml. Hank's balanced salt solution.

Group B—50 animals received the same treatment as group A, and in addition were given a regimen of antibiotics.

Group C—13 animals were infused 72 hours after x-irradiation with lyophilized bone marrow and received antibiotics.

Group D—35 animals were infused 24 hours after x-irradiation with lyophilized bone marrow and fresh marrow, and also received antibiotics.

Group E—28 animals were infused 24 hours after x-irradiation with fresh marrow, and also received antibiotics.

Recipient-donor combinations.—The recipient-donor combinations used in groups B, C, and D are summarized in table 1. (It should be noted that naturally occurring ADF isoagglutinins do not occur in the rabbit.) In some combinations (Nos. 2 and 5) the donor ADF antigen was also present in the recipient, whereas in other combinations (Nos. 3 and 6) the recipient antigen was present in the donor.

RESULTS

Mortality

The mortality figures at 15 days and at 8 and 12 weeks are summarized in table 2. The animals that received no treatment other than antibiotics (group B) had a 15 day mortality of 72 per cent compared to 91 per cent for the control group A. No deaths in group B were observed after 15 days. Additional protection was not obtained by the use of lyophilized marrow (group C). When fresh marrow was given 24 hours after irradiation (group E) or 72 hours after irradiation (group D), the 15 day mortality was considerably reduced, but, in contrast to the other groups (A, B, C) in which death occurred only rarely after this time, there was a spate of deaths between the third and eighth weeks. The result was a higher 8 week mortality in group D than in the control groups B and C. In group E, however, in spite of delayed deaths, the 8 week mortality was 57 per cent as opposed to the 70 per cent in group C.

On the basis of the time of death after irradiation and transplantation the deaths in groups D and E may be defined as early—occurring within the first 15 days after irradiation and transplantation—or late or delayed—occurring after the 21st day after irradiation and transplantation. Clinical and pathologic

Table 2.—Mortality of X-irradiated Rabbits

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of animals</th>
<th>15 days</th>
<th>8 weeks</th>
<th>12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control animals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A (no treatment)</td>
<td>33</td>
<td>91</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>Group B (antibiotics)</td>
<td>50</td>
<td>72</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>Group C (antibiotics and treated marrow)</td>
<td>13</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Transplanted animals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group D (antibiotics + fresh marrow 72 hours post-irradiation)</td>
<td>35</td>
<td>54</td>
<td>83</td>
<td>83</td>
</tr>
<tr>
<td>Group E (antibiotics + fresh marrow 24 hours post-irradiation)</td>
<td>28</td>
<td>39</td>
<td>57</td>
<td>57</td>
</tr>
</tbody>
</table>
findings are very different in these two groups. In the group of early deaths the clinical events noted are very similar to those in irradiated rabbits which do not receive transplantation of bone marrow—severe weight loss, hemorrhages, diarrhea, and aplastic anemia. A prominent feature on autopsy is superficial mucosal necrosis and ulceration of the colon. In the animals dying after the third week, there is an apparent initial recovery, followed by a second loss of weight, frequently a dramatic drop of hematocrit, and occasional diarrhea. The pathologic findings in these late deaths are atrophic spleen and lymph nodes, with active bone marrow, occasional foci of pneumonia, but not obvious cause of death. These findings are similar to those observed in mice dying from the syndrome designated "secondary disease." Those animals which died between and second and third weeks had no definite symptoms, but had features characteristic of both the early and the late deaths. They are difficult to classify.

Transplant Results

In all irradiated rabbits, extreme leukopenia appeared within 48 hours after irradiation. At the end of the week hemorrhages, petechiae, and varying degrees of anemia were observed, together with marked anorexia and loss of weight. These signs persisted until the beginning of the third week, when evidence of hematopoietic regeneration could be recognized in most animals which had received fresh bone marrow (Groups D and E). This evidence consisted of a rise in peripheral nucleated cells, which were mostly red cells; the appearance of polychromatophilic red cells; a high percentage of reticulocytes; and a rise in hematocrit. Simultaneously with hematopoietic recovery there was a return of appetite, a steady increase in body weight and the cessation of bleeding. These signs of recovery occurred approximately one week later in those animals which recovered without the infusion of fresh bone marrow (Groups A, B, and C).

The success of the transplant was judged by the proliferation of donor erythrocytes or leukocytes in the circulation of the recipient (table 3). (No evidence of donor cells was found in the survivors of group C, nor, obviously, in the survivors of groups A and B.)

Four different patterns of repopulation were observed:
(a) donor erythrocytes and donor neutrophils;
(b) donor erythrocytes, but no donor neutrophils;
(c) no donor erythrocytes, but donor neutrophils;
(d) no donor erythrocytes and no donor neutrophils.

<table>
<thead>
<tr>
<th>Ultimate fate of graft or animal</th>
<th>Erythrocytes + neutrophils</th>
<th>Number of animals with donor erythrocytes</th>
<th>Neutrophils</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permanent graft</td>
<td>8</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Temporary graft</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Delayed death</td>
<td>7</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Unsuccessful graft (animal lived)</td>
<td></td>
<td></td>
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<td>1</td>
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Patterns (b) and (c) will be referred to as incomplete transplants; (a) is a complete transplant; and (d) is an unsuccessful graft.

The donor erythrocytes, present in the circulation of the recipient, could not have been transfused with the marrow, as the volume of blood present in the marrow suspension never exceeded 1 ml—a mount which would not be detected in the host with our technics; and indeed no donor cells could be demonstrated for the first ten days postirradiation. The fact that living marrow is essential for a successful transplant was confirmed by the observation that neither donor erythrocytes nor leukocytes were present in animals that received lyophilized marrow (group C).

The grafts in the chimeras were either temporary or permanent. In the latter, the graft persisted throughout the course of the experiment; in the former, the graft was usually rejected between the second and tenth weeks after transplantation. Some animals died while still carrying the graft. Representatives of these various types are shown in figures 1, 2, and 3. It will be observed that donor erythrocytes are not found until about the tenth day post-transplantation, and the red cell population is not composed entirely of donor cells until the tenth week (fig. 1). At no time after 10 weeks were both donor and recipient erythrocytes present simultaneously; there is apparently an entire conversion to donor erythrocytes. (Our technic, however, will not permit the detection of a minority population of below 5 per cent.)

Female heterophil leukocytes were found only to the extent of about 4 per cent.
Fig. 2.—Clinical findings in an x-irradiated transplanted rabbit with a temporary bone marrow graft.

cent. This is below the number of drum-sticks found in the average female in our laboratory (7–9 per cent) and suggests a mixed population of donor and recipient leukocytes.

Figure 3 shows the findings in an animal which had a successful graft, and while still bearing the graft died at five weeks. It will be noted that immediately prior to death this animal had a marked drop in hematocrit and a leukocytosis. In addition, it never really recovered weight after the initial loss. These results are typical of all animals dying with secondary disease, except that in some instances the leukocytosis is absent. Although the animal represented in figure 1 was apparently healthy while carrying its graft, it will be observed that at about the fourth week postirradiation there was a marked weight loss and a fall in hematocrit. Such a loss of weight and decline in hematocrit is typical of nearly all animals which had a successful graft, and suggests that at this period the animal goes through a hematologic crisis, resulting in either recovery and a successful graft, or death due to secondary disease.

Delayed deaths occurred among animals that showed an incomplete, as well as complete, transplant and also occurred in those donor-recipient combinations where the recipient contained the ADF antigen present in the donor. There was no correlation between the number of transfused cells and the incidence of secondary disease, or between the time interval allowed to elapse between irradiation and transfusion and the incidence of secondary disease. However, in all of these comparisons, the number of animals which was studied may be too few to be of valid significance.
Immunologic Studies

The results of immunologic studies with donor and recipient erythrocytes are summarized in table 4.

All but one transplanted animal had a weakly positive direct Coombs' test at both three and seven weeks. However, as the arterial blood of these animals frequently contained a mixture of both donor and recipient cells, together with a high percentage of reticulocytes known to cause nonspecific agglutination, their sera were tested with stored donor and recipient erythrocytes. Such erythrocytes, stored in glycerol at -20° C., retain their antigenicity. The sera of 27 animals were tested in this manner. Positive results were obtained in all cases but one with recipient erythrocytes. That animal (No. 37), whose serum gave a negative result, at no time showed evidence of a successful transplant. When donor erythrocytes were used the test was positive in only three instances; these 3 animals died between the third and eighth weeks postirradiation.

Three to four weeks after they had been transplanted 22 of the above 27 animals were infused with stored donor or recipient erythrocytes, which had been thawed and labeled with Cr51. The reason for this delay and the conse-
Table 4.—Immunologic Studies on Animals in Groups D and E

<table>
<thead>
<tr>
<th>Ultimate fate of graft or animal</th>
<th>Animal number</th>
<th>Nature of graft</th>
<th>Coombs’ test</th>
<th>Cr¹⁹⁵ half-life (days)*</th>
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<tr>
<td></td>
<td></td>
<td>erythrocyte</td>
<td>recipient</td>
<td>donor</td>
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<tr>
<td>Permanent graft</td>
<td>31</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<td>58</td>
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<td>14</td>
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<td>48</td>
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<td>Unsuccessful graft (animal lived)</td>
<td>37</td>
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*Cr¹⁹⁵-labeled erythrocytes infused into chimeras at about 3 weeks postirradiation.

quent use of stored cells was that in previous work in this laboratory it had been shown that when Cr¹⁹⁵-labeled erythrocytes are infused into heavily irradiated rabbits in the first three weeks after irradiation, they have an abnormal survival.²⁷ For approximately seven days after irradiation no disappearance slope is observed (the cells appear to be immortal), but in the following week there is a sharp, although variable, drop in radioactivity, accompanied by hemorrhages. At the end of this time nontransplanted animals died, whereas those which had received bone marrow transplant showed a hematologic recovery.

Therefore, stored donor cells were infused into 14 animals: a normal survival time (in our laboratory this is 18 ± 2 days) was observed in all but one animal. In this animal, which was one of the 3 rabbits (No. 84) which showed a positive indirect Coombs’ test with donor erythrocytes, and which died 35 days postirradiation, the donor red cells had a less than normal half-life.

Recipient erythrocytes were infused into 8 other rabbits, and in all cases but one a greatly shortened half-life of 8–12 days, commensurate with that of
a hemolytic anemia, was observed. A normal half-life of recipient cells was noted in one animal (No. 37), that which had given a negative indirect Coombs' test with recipient cells, and which was found not to have accepted its graft.

Twelve weeks after transplantation, 3 chimeras (Nos. 16, 41, and 47) that had previously received an infusion of donor erythrocytes which had a normal half-life, and one animal (No. 13) that had received an infusion of recipient cells which had a shortened half-life were infused with labeled recipient cells. (By this time the residual chromium count from the first infusion was low enough to cause no interference with the counting of the newly infused cells.) A normal half-life* was noted in each instance and all the animals were found to have rejected their grafts, both on the basis of the leukocyte and the erythrocyte marker.

DISCUSSION

The X-irradiated rabbit that has received a transplant of donor marrow presents an ideal experimental subject for the study of the fate of both donor and host cells, and indeed the fate of the animal itself. The genetic heterogeneity of a rabbit population makes the rabbit much more akin to man with his biological individuality, than do the pure bred homogeneous strains of mice usually used in such studies. Further, evaluation of the success of the transplant in each rabbit and the clinical fate of the animal is easier to follow than in the case of smaller rodents.

Differential agglutination of rabbit erythrocytes has enabled us to quantitate the success of homologous transplants. When the donor erythrocyte is of different antigenic composition than the erythrocyte of the recipient, it is possible to follow the repopulation of the host and the persistence of the marrow graft. Donor erythrocytes were usually first detected about ten days after transplantation, at which time cells of both donor and recipient were present. The period required for complete repopulation with donor cells was about ten weeks, a figure which agrees well with the time necessary for mature circulating erythrocytes present at the time of X-irradiation to disappear (the life of the rabbit erythrocyte is about 60 days), but which fails to be consistent with the observation that the apparent Cr51 half-life of recipient erythrocytes infused into the chimeras is approximately half the normal. A possible explanation for this anomaly is that (a) in the week immediately following x-irradiation, destruction of erythrocytes is not normal,27 and (b) the immune response on the part of the graft against the host is not a hyperimmune one, and a percentage of erythrocytes may persist for their normal life span.

In mouse chimeras, Owen, using the technic of differential immune hemolysis, found donor cells appeared about the seventh day post-transplantation, and completely repopulated the chimera towards the end of the third week, although in some strain combinations both donor and recipient cells were present

*It should be stated that although frozen cells kept for as long as 12 weeks were not tested in normal isologous rabbits, the above results strongly suggest that such cells behave as normal cells. Cells kept frozen for seven weeks had been found to behave in a normal manner.23
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as late as 93 days after irradiation.26 Welling et al.26 using electrophoresis of hemoglobin as their marker, and a different mouse strain combination from that used by Owen, found that complete repopulation of the host did not occur till between the 45th and 50th days after transplantation. If the life span of the mouse erythrocyte is taken as 20–30 days,1 then in most of Owen’s chimeras complete repopulation with donor cells occurred earlier than in this study and that of Welling et al. This difference could be due to the fact that in mouse strain combinations, differing markedly at the H2 locus, there is a hyperimmune response on the part of the graft against the host, resulting in all host erythrocytes being destroyed by antibody (although see Goodman and Owen31) and that the technic of immune hemolysis does not take into account the nonlysable fraction of test cells.

A surprising finding was that in some animals only leukocytes, in other, only erythrocytes were present, rather than both leukocytes and erythrocytes of donor origin. It is difficult at this time to present a satisfactory explanation for this phenomenon, unless it be a difference in the antigenic constitution of leukocytes and erythrocytes which are derived from the same stem cell, and that in the face of incomplete immunologic suppression of the host, one or other series may be eliminated. Or, the occurrence of one cell type in the absence of another may be due to physiologic factors: a lack of “lebensraum,” one cell type finding the room to survive and multiply, another not. In either case it should be possible to obtain more complete (i.e., both erythrocyte and leukocyte) grafts by increased irradiation, and indeed exposure of rabbits to 1400 r has resulted in complete rather than incomplete grafts.32 In rat-mouse, although not in homologous chimeras, Welling et al.26 have observed similar results: incomplete grafts at lower doses of x-irradiation and complete grafts with higher doses.

The incompleteness of some marrow transplants should be borne in mind in determining the radiation dose for patients with blood dyscrasias. It is clear that the proliferation of erythrocytes will be of little value to the patient with leukemia, and the successful transplant of leukocytes will scarcely help the patient with Cooley’s anemia. There is no method at present of predicting which cell type will multiply in the host, but it is possible that with larger doses of x-irradiation both types may be accepted.

In many chimeras the transplanted marrow persisted for only a limited time (temporary “take”), but the number of such animals that survived, even after the graft rejection, showed that a temporary proliferation of donor marrow at the critical moment of acute aplasia can support the animal for a time long enough to permit its own bone marrow to regenerate completely. This may have been the situation in the victims of the Yugoslavian reactor accident.52 On the other hand, it has been shown that sublethal irradiation together with homologous or heterologous bone marrow transplantation may cause an increased mortality due to the reaction of the host’s residual immunologic capacity against the grafted marrow.34-36

Bone marrow transfused to rabbits exposed to lethal doses of x-irradiation produces its protective effect as a result of proliferation within the tissues of the host, and not, as was formerly suggested, through the mediation of a
"humoral factor." Marrow rendered nonviable by freezing, sonication, and then lyophilization (a process most likely to give a minimum of denaturation), and injected with antibiotics, did not result in better survival figures than those obtained with antibiotics alone. It is of interest to note, parenthetically, that the buffy coat of x-irradiated males which had received treated female marrow at no time showed any drum-stick heterophils, indicating that the appearance of female leukocytes in x-irradiated males is not an artifact; neither is it the result of transformation similar to that observed in bacteria.

The proliferated bone marrow does, however, have the ability to act as a two-edged sword, or not only are the cells capable of proliferating and thus protecting the host, but they are capable of immunologic reactivity, resulting in a large number of animals that survive the acute early phase postirradiation period, succumbing at a later date to the so-called secondary disease. That an immunologic factor is responsible for this phenomenon was shown by following the fate of Cr-labeled erythrocytes infused into chimeras at three to four weeks after transplantation, and by looking for antibodies in the sera of the chimeras using stored host and donor erythrocytes as antigens. When the cells infused were of donor origin a normal disappearance slope was usually obtained, whereas it was found that infused recipient erythrocytes had, in all instances where a graft could be demonstrated, a much shorter half-life. This is compatible with an immune response on the part of the graft versus the host.

Further light on the immunologic nature of secondary disease is shed by the invariable finding of antibodies against host erythrocytes, but rarely against donor erythrocytes, in the circulation of chimeras between the third and seventh weeks after transplantation. These antibodies are detectable only with antiserum to rabbit gamma globulin. The frequent occurrence of antibodies, detectable only by the indirect Coombs' test, may appear surprising in a species in which incomplete hemagglutinins are rare and difficult to detect. However, it is not clear whether these are in fact incomplete antibodies, or whether the Coombs' serum merely permits the detection of extremely low concentrations of complete antibodies.

From the results it is clear that immunologically competent cells transfused with the donor marrow form antibodies to host antigens. When these antibodies are formed against erythrocytes, the result is an immunologically specific hemolytic anemia which is probably an indicator of a more generalized reaction on the part of the graft against the host. That there are antigens responsible for incompatibility reactions other than those controlled by the ADF locus is evident from the observation that recipient cells have a shorter than normal half-life, even when the donor has the same ADF gene as the recipient (groups 3 and 6 in table 1).

The hemolytic anemia which was observed in x-irradiated rabbits, and which has also been noted by Porter, is similar to that seen in newborn animals injected with foreign cells, and in rodents in parabiotic union. In newborn chickens injected before hatching with adult homologous splenic cells, Simonsen has demonstrated antibodies against the host cells detectable by the Coombs' test. In mice that received homologous bone marrow, the presence of circulating antibodies could not be detected directly, although they could
be demonstrated by transferring the spleens of chimeras to an isologous host.\textsuperscript{41} Oliner et al.\textsuperscript{42} observed that when adult hybrid mice were injected with parental splenic cells of mice that had been preimmunized against the hybrid, a positive Coombs' test resulted and Cr\textsuperscript{51}-labeled host cells had a markedly shortened half-life. The ease of detection of erythrocytic antibodies in different species will obviously vary, depending on the readiness with which they are formed and absorbed and on the differences in this physical characteristic. The physiopathological situation described above is strikingly similar to that observed in human disease in patients whose immunologic system, for unknown reasons, produces antibodies against the circulating red cells—the acquired hemolytic anemias.\textsuperscript{43}

It had been hoped that in a heterogeneous population of rabbits, less genetic disparity and consequently a lower incidence of secondary disease might occur than in the mouse, in which instance one is dealing with highly inbred populations, each with a constant strength of antigenic difference. Recently, Uphoff and Law have shown that the incidence of delayed mortality is proportional to the antigenic difference at the H\textsubscript{2} locus between mouse strains.\textsuperscript{15} However, the present results augur poorly for the absence of secondary disease in man. It appears that the reaction of the graft versus the host is a constant, yet not necessarily lethal, phenomenon. Some animals, in this series about 35 per cent, succumb to the disease, whereas in others the symptoms of weight loss and anemia are but temporary. In the latter case there are two alternatives: (a) The donor marrow completely takes over the hematopoietic function, at least as judged by erythrocytes, and the animal lives to become a chimera. It is possible that the rate of proliferation of transplanted marrow has a critical value for the continued survival of a chimera, as, when host cells are destroyed by an immunologic reaction on the part of the graft, the resultant degree of anemia will, in the absence of a reaction on the part of the host against the graft, be directly proportional to the proliferation of donor cells. (b) If the host marrow regenerates, and if there is an immunologic recovery on the part of the host, then the graft may be rejected. Such will be the case if the dose of irradiation is too low. This incomplete suppression of the host's immunologic reaction is thought to be responsible for the deaths\textsuperscript{34} occurring between the third and fifth weeks after transplantation. Trentin has shown, using F\textsubscript{1} hybrids as donors and parental strains as recipients, that a reaction of the host's residual immunologic capacity against the grafted marrow is the explanation for increased mortality observed in animals receiving sublethal x-irradiation and bone marrow transplants.\textsuperscript{34} Evidence for this hypothesis is the finding of antibodies against both host and graft. The hypothesis could not be tested by the infusion of labeled erythrocytes of the donor, because we had earlier shown that a normal disappearance slope is not obtained in rabbits exposed to 1100 r until at least three weeks after irradiation.\textsuperscript{27} No graft rejections are observed at 1400 r.\textsuperscript{32}

Some of the lethal effects of heavy doses of x-irradiation are the result of severe infections. The importance of infection as a cause of death is reflected in the fact that the LD\textsubscript{50} dose of x-irradiation is higher in germ-free chickens than in normals.\textsuperscript{44} On the other hand, in the germ-free animal exposed to
heavy irradiation, death is only delayed, compared to non-germ-free controls, but not prevented. In the heavily irradiated non-germ-free mouse given antibiotics, Lorenz and Congdon\textsuperscript{15} find that death is delayed but that the 30 day mortality is unchanged. Our results differ from these in that when penicillin and streptomycin are given prophylactically not only is the time of death delayed, but the 30 day mortality is reduced. The divergence in these results may be attributable to differences in (a) animal species; (b) regimen of antibiotics; (c) the flora of the animals; or (d) dose of irradiation—the dose in this study may be closer to the threshold for the LD\textsubscript{100}. The reduction of deaths by the prophylactic use of antibiotics shows that although there may be strains of bacteria within the animal which are resistant to antibiotic therapy, they play a lesser part in causing death than do the sensitive microorganisms. This experimental finding should be considered in planning germ-free life for totally irradiated persons.

The importance of the duration of the interval between irradiation and transplantation was shown by the fewer successful permanent grafts in group D (animals receiving a graft 72 hours after x-irradiation) than in group E (animals receiving a graft 24 hours after x-irradiation). Similar results to those obtained in group E had been observed by one of us in a previous study in which the marrow was given immediately after irradiation.\textsuperscript{14} It therefore appears that if bone marrow is to be of value it must be administered soon after irradiation. A possible explanation is that the heavily irradiated rabbit resembles immunologically a neonatal animal and is in the "null" period. Consequently, when marrow is presented to such an animal at this time no immune response is elicited, the animal regarding the marrow as "self." Further, the large amount of antigen (approximately $10^9$ marrow cells) given at this time might be partly responsible for the absence of an immune response by causing immunologic unresponsiveness. Giving marrow 72 hours after irradiation, rather than immediately or 24 hours after, may be comparable to injecting cells 24 hours, rather than immediately, after birth, in the neonatal mouse.

**Summary**

White New Zealand rabbits were exposed to 1100 r as a split dose of 600 r followed 24 hours later by 500 r and transplanted with fresh or frozen, lyophilized, sonicated homologous bone marrow. Fresh marrow was infused 24 hours or 72 hours after x-irradiation. All these animals received antibiotics and, in addition, another group received antibiotics only. Donors were females differing, on the basis of Cohen's Hg"\textsuperscript{\textsuperscript{a1}}" allelomorphic system for erythrocytes, from the recipient males. It was therefore possible to follow the success of the graft qualitatively by the occurrence of female heterophil leukocytes, and semiquantitatively by the presence of donor erythrocytes in the circulation of the recipient. Complete correlation between these two parameters did not occur: sometimes leukocytes, sometimes erythrocytes, and sometimes both elements of the donor were found in the circulation of the recipient. If animals retained their graft, complete repopulation with donor erythrocytes occurred at about the tenth week postirradiation and transplantation. The number of donor leukocytes present in the blood of the chimeras suggested a mixed population.
Not only were the grafts often incomplete but in many instances they were not permanent. Initially, all but one animal had a successful graft, but of the 18 animals which had received fresh marrow and survived at least 12 weeks 12 retained their graft.

Much better results in terms of permanent takes were obtained when the marrow was infused 24 hours, rather than 72 hours, after x-irradiation. Non-viable marrow had no protective effect, whereas antibiotics did decrease the mortality.

An immune hemolytic anemia was shown to be part of the secondary disease syndrome. Antibodies specific to stored recipient erythrocytes were found in the sera of all chimeras between the third and seven weeks after transplantation. When donor erythrocytes were used, the test was positive on only 3 animals. These 3 animals died between the third and eighth weeks postirradiation with secondary disease. When stored recipient erythrocytes were thawed, labeled with Cr\(^{51}\), and infused into chimeras three or four weeks after irradiation and transplantation, they had in every case a greatly shortened half-life commensurate with a hemolytic anemia. Donor erythrocytes were infused into other chimeras and in all but one instance had a normal half-life. It is suggested that all rabbit chimeras develop secondary disease to some extent, as indicated by a weight loss and fall in hematocrit, but that the situation is not necessarily fatal, except when recovery of the host's immune mechanism occurs in the presence of a graft which is actively producing antibodies against the host.

**Summario in Interlingua**

Conilios blanc de Nove Zelanda—exponite a 1100 r administrate in le forma de duo doses, un de 600 r sequite 24 horas plus tarde per un secunde de 500 r—recipeva transplantationes de fresc o refrigerate medulla ossee homologue que habeva essite lyophilisate e sonicate. Medulla ossee fresc esseva infundite 24 o 72 horas post le irradiation. In plus, omne iste animales recipeva antibioticos. Un altere gruppo recipeva solmente antibioticos. Le donatores esseva feminas que differeva del masculos in lor erythrocytos secundo le systema allelomorphic Hg\(^{nm}\) de Cohen. Consequentemente il esseva possibile observar le successo del transplantation qualitativemente per le occurrentia de leucocytos heterophile ab le femininas e semiquantitativamente per le presentia de erythrocytos del donatores in le circulation del recipiente. Un correlation complete inter iste duo parametros non occurreva: esseva trovate in le circulation del recipiente a vices solmente leucocytos del donator, a vices solmente erythrocytos, e a vices ambe elementos. Quando le animales reteneva lor graffo, un repopulation complete con erythrocytos de donator occurreva a circa le decime septimana post le irradiation e transplantation. Le numero de leucocytos de donator presente in le sanguine del conilios-chimeras pareva indicar un population de cellulas mixte.

Non solmente esseva le graffos frequentemente in-complete, sed in multe casos illos non esseva permanente. Initialmente omne le animales—con un exception—habeva un graffo successose, sed del 18 animales que habeva recipte medulla ossee fresc e que habeva supervivite al minus 12 septimanas, 12 reteneva lor graffo.
Resultanatos mucho mejor, con respecto al permanencia del gráficos, esseva obtenite quando le medulla esseva infundite 24 horas in loco de 72 horas post le irradiation. Medulla non-viabile habeva nulle effecto protective, durante que antibiioticos reduceva le mortalitate.

Esseva demonstrate un anemia immuno-hemolytic como parte del syndrome pathologic secundari. Anticorpore specific pro magasinate erythrocytos del recipientes esseva trovate in le seros de omne le conilio-chimerae inter le tertie e le septie septime septimana post le transplantation. Quando erythrocytos de donator esseva usate, le resultados del test esseva positive in solmente 3 animales. Iste 3 animales moriva de un maladia secundari inter le tertie e le octave septime septimana post le irradiation. Quando magasinate erythrocytos del recipientes esseva disgelate, marcate con Cr51, e infundite a in conilio-chimera tres o quatro septimanas post le irradiation e transplantation, illos habeva in omne caso le grandemente reducite periodo de medie valor a expectar in un anemia hemolytic. Erythrocytos de donator esseva infundite a in altore conilio-chimerae, e in omne casos, con un sol exception, illos habeva un normal periodo de medie valor. Es sugerite que omne conilio-chimerae disveloppa un certain grado de maladia secundari, a judicar per un reduction in peso e le declino del valor hematoctic, sed que le situation non es necessarimente letal, excepte quando le recuperation del mechanismo immunologic del hospite occurre in le presentia de un graffo que activemente produce anticorpore contra le hospite.

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d'irradiation totale aiguë accidentelle.


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Studies on Homologous Bone Marrow Transplantation in Irradiated Rabbits

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