Effect of Homologous Bone Marrow–Spleen Cell Suspension on Survival of Swine Exposed to Radiation from a Nuclear Weapon

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It has been demonstrated that under certain conditions administration of bone marrow or spleen cell suspensions can prevent death in otherwise fatally irradiated animals. Several species of small animals can be treated successfully in this manner. Tissue from isologous, homologous and heterologous sources has been administered intact or in cell suspensions and by many routes. In most studies x- or gamma irradiation has been used. Responses after other types of exposure are now being investigated. Available reports indicate that the results of such therapy depend upon multiple factors, including the irradiated animals’ size, species and strain as well as upon intensity of irradiation and weight, charge and speed of irradiating particles. Eventual success of similar therapy in treatment of human casualties exposed to excessive radiation may well be dependent upon experiments on larger animals (comparable to man in size), under controlled laboratory conditions. It is not known whether the relatively hematopoietic organ of a large mammal (perhaps 1000 Gm. for a 120 pound pig or man) can regenerate as readily after exposure and subsequent therapy as can the smaller organ (approximately 0.15 Gm.) of a mouse. During the weapons tests completed by the Atomic Energy Commission at their Nevada Test Site in 1957, an opportunity was presented to evaluate the effect of homologous cell suspensions on survival of swine exposed to mixed neutron and gamma irradiation from a nuclear weapon. This paper reports the results of these studies.

METHODS

Sixty-nine swine of a Hampshire-Landrace strain, 4 to 6 months of age and weighing 35 to 60 Kg., were exposed to the mixed gamma and neutron irradiation from a nuclear weapon. The dose of irradiation could not be controlled so that a wide range of doses in this series resulted. Four to 8 hours after exposure, 3 to 4 x 10^6 nucleated cells of a bone marrow-spleen mixture were administered to each animal by the intravenous, intracardiac or intraperitoneal route. No further therapy was given. Treated animals were compared with similarly exposed, untreated animals, in terms of signs, symptoms and ultimate survival. Spleen weight as well as spleen and bone marrow cytology and cellularity were examined for comparison in those animals that did not survive. Six groups of swine were studied (Table 1). Two of these groups (groups E and F) received from the weapon burns, wounds or both in addition to irradiation. Similar trauma was inflicted upon the animals designated as “controls” for these groups. Doses of gamma irradiation were measured in air with Sigoloff chemical dosimeters. Neutron irradiation was measured by the fission-product system of Hurst, as the first collision air dose measured in rep’s. Cell suspensions were obtained from donor pigs (of the same stock) weighing 17 to 35 Kg. and prepared as

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Submitted Mar. 20, 1959; accepted for publication Oct. 31, 1959.
SURVIVAL FROM NUCLEAR WEAPON RADIATION

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Gamma rep</th>
<th>Neutron rep</th>
<th>No. of cells</th>
<th>Therapy route*</th>
<th>30 day survivals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>treated % animals N</td>
</tr>
<tr>
<td>A</td>
<td>10</td>
<td>10</td>
<td>$3 \times 10^9$</td>
<td>IV</td>
<td>100 10 100 10</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>10</td>
<td>$3 \times 10^9$</td>
<td>IV</td>
<td>100 10 100 10</td>
</tr>
<tr>
<td>C</td>
<td>725</td>
<td>715</td>
<td>$3 \times 10^9$</td>
<td>IV or IC</td>
<td>0 9 0 10</td>
</tr>
<tr>
<td>D</td>
<td>660</td>
<td>640</td>
<td>$3 \times 10^9$</td>
<td>IV or IC</td>
<td>0 9 0 10</td>
</tr>
<tr>
<td>E</td>
<td>435</td>
<td>180</td>
<td>$4 \times 10^9$</td>
<td>IP</td>
<td>0 13 4 142</td>
</tr>
<tr>
<td>F</td>
<td>197</td>
<td>71</td>
<td>$4 \times 10^9$</td>
<td>IP</td>
<td>65 18 65 107</td>
</tr>
</tbody>
</table>

*IV = intravenous; IC = intracardiac; IP = intraperitoneal.

follows: Each donor was bled several hundred milliliters, then killed by means of intracardiac injection of air. Long bones from the animal were split lengthwise, and the marrow scooped out and suspended in a solution of 10 per cent serum in saline. Long bones of very young pigs were filled with cancellous bone, and those from older pigs contained much fat, rendering each difficult to prepare for injection. The spine was removed intact through a dorsal incision (fig. 1). A 1.4 inch square steel rod was driven through the spinal canal and anchored at both ends to a wooden fixture to hold the spine rigidly in place. The vertebral bodies were shaved away with a 6 inch jack plane, (fig. 2) and the planings dropped into a solution of 10 per cent autologous serum in saline, shaken and allowed to sediment briefly so that the particles of bone fell to the bottom. The supernatant was re-

Fig. 1.—Removal of intact spine from donor pig. Scalpel blade indicates spleen, easily removed through same incision.
placed and the flask was shaken again. After two such suspensions, most of the marrow cells had been shaken loose from the bony fragments, and the cell suspension was concentrated by centrifugation. The spleen was cut into large pieces, which were placed in a mortar with a stainless steel screen at its bottom (fig. 3). By means of a syringe handle (used as a pestle) the spleen pulp was forced through the screen, then shaken in solution, and strained through the screen once more, prior to final suspension in serum-saline. Cell concentration in each solution was determined by counting in a hemocytometer chamber, and all three suspensions were mixed together prior to injection. Contributions from each of the three sources (long bones, vertebral column and spleen) were nearly equal in the final suspension. Less than two hours passed between death of donor pigs and administration of cell suspension to recipient animals. Each donor pig supplied cells for 8 to 10 recipient pigs. Cell suspension was administered by intravenous (marginal ear veins), intracardiac or intraperitoneal route (table 1). Volume of solution was 10 to 25 ml. Each animal received 3.0 to 4.0 × 10⁸ nucleated cells. Animals were offered Pillsbury’s Hog Feast No. 1 and No. 2 ad libitum after irradiation. No therapy other than the cell transfusion was given. The clinical course of all animals was followed carefully, but no laboratory examinations of blood were done because pilot studies had indicated that the struggle entailed in drawing blood samples from thrombocytopenic irradiated pigs affected survival adversely. Postmortem examinations were conducted on all animals within a few hours of death.

Results

Approximately 15 per cent of the animals treated by intravenous or intracardiac injections exhibited mild ataxia, lasting from a few seconds to 10 minutes. One animal died promptly after intracardiac injection, apparently of cerebral embolism.

Fig. 2 (at left).—Vertebral column has been anchored in wooden fixture by means of a pointed steel rod in the spinal canal driven into end block and anchored by short length of chain at the other.

Fig. 3 (at right).—Apparatus for homogenizing splenic tissue consisting of mortar with stainless steel screen bottom and syringe handle.
All animals receiving minimal doses of radiation survived and showed no other ill effects from either the radiation or the therapy.

All animals receiving intermediate or supralethal doses of irradiation exhibited clinical signs of radiation illness (hemorrhage, anorexia, diarrhea, fever). Treated animals did not differ significantly in their survival or clinical course from untreated control animals.

All fatally irradiated animals revealed prominent signs of radiation damage on necropsy (lymphoid and hematopoietic atrophy and hemorrhage), and swine which had received intravascular injections demonstrated many small emboli in numerous tissues, as well. Bone marrow smears and sections from treated and untreated swine dying at identical intervals after irradiation did not differ in cellularity, composition or cytology. The few specimens obtained during the first four days following exposure were characterized by lysis, pyknosis and fragmentation of hematopoietic cells with rapid disappearance of all cell forms other than a few phagocytes, reticulum cells and occasional plasma cells. By 10 days post-irradiation many of the animals that died exhibited small islands of erythropoietic cells and a few early cells of the myelopoietic series. Thirteen days after irradiation all phases of hematopoiesis had prominently reappeared and many marrows were either normocellular or hypercellular. Survival data are summarized in table 1 and figure 4.

DISCUSSION

The cell homogenates used in previous similar studies have been obtained from several sources. Most frequently, the long bones have served as a source of marrow, although both ribs and ilia have also been used. We are unaware of other reports indicating that marrow can be obtained in considerable quantities from the vertebral column and successfully prepared for injection. In our experience with swine the number of cells obtained from this source is similar in amount to that obtainable from all of the long bones from the same animal (10 × 10^6 in a 20 Kg. pig). The method is relatively simple, and
may very well be of further use in studies on other large experimental animals. 

The beneficial effect of marrow and spleen cell suspensions on survival of 
irradiated animals has been clearly demonstrated on a number of species 
including mice, rats, guinea pigs and rabbits. Improved survival following cell- 
homogenate therapy has been closely related to accelerated repopulation of the 
hematopoietic and lymphatic tissues. In our study on swine, no evidence of 
accelerated repopulation was found. Failure to improve survival or marrow 
repopulation in our studies may have resulted from differences in the animal, 
the quality of radiation, the type of therapy, the technic of therapy or a com- 
bination of these.

In small mammals, genetic similarity between donor and recipient animals 
have been closely correlated with improved survival. It seems possible that the 
genetic heterogenicity of swine, or perhaps the increased mass of marrow 
tissue to be regenerated, or a combination of these factors may make demon- 
stration of such a beneficial effect difficult.

The type and dose of radiation in our study may not be optimal for such 
a demonstration. In other animals maximal beneficial effect has been demon- 
strated at doses of irradiation near the LD$\text{95/30}$ to LD$\text{100/30}$ range. Of our 
animals, groups A and B were irradiated with doses well below the lethal 
range, and groups C and D probably received several times the lethal dose. 
Lethality of the dosages received by groups E and F cannot be determined, 
due to the presence of wounds and burns which affected survival adversely. 
However, the survival of 4 per cent of control animals in group E, which 
received 435 rep of gamma irradiation and 180 rep of neutron irradiation, 
suggests that they may have fallen in the range in which an effect is most 
likely to be demonstrable.

Vogel et al.\cite{1-4} have recently reviewed evidence indicating that death after 
fission neutron irradiation may be of a different nature from the hematopoietic 
death following just-lethal exposure to gamma, beta or x-irradiation. These 
authors were unable to demonstrate a beneficial effect of transplanted spleens 
in mice previously exposed to LD$\text{20/30}$ doses of fission neutrons, whereas 
similar therapy in mice exposed to LD$\text{20/30}$ doses of Co$\text{60}$ gamma 
rays produced a significant improvement in survival. Repeated demonstration 
of a beneficial effect by homologous marrow therapy after exposure to faster 
(14 Mev) neutrons by the same authors\cite{1} and by Randolph et al.\cite{2} indicated 
that the energy, in addition to mass and electrical charge of the bombarding 
particles, may be responsible for this difference.

Some evidence suggests that the neutron irradiation received by our animals, 
rather than the gamma irradiation, was the dominant force in their radiati:ion 
injury. Mean survival time of dying animals in each experiment was 6 to 10 
days, the same period reported by Vogel et al.\cite{4} in mice irradiated with varying 
doses of fission neutrons and in contrast to the mean survival time of 13 to 15 
days in mice receiving equally lethal doses of gamma rays. With survival as 
the common parameter, RBE (relative biologic effectiveness) of fission neu- 
trons, as compared with gamma rays, is between 2 and 7 in mice.\cite{4} Although 
these values can certainly not be transposed to swine, it seems likely that
neutrons are more effective biologically than gamma rays (when rep are compared), perhaps by a factor near 4.

Both spleen and bone marrow have been shown to have a beneficial therapeutic effect in the post-irradiation period. Rarely have they been used together. Since our study, however, Schwartz et al.3 have reported that injection of parental splenic homogenate into F-1 mice previously given an LD100/30 dose of radiation resulted in a fatal reaction within 8 to 16 days. Nonirradiated animals were not affected by the homogenate, and the reaction occurred whether or not “protective” doses of bone marrow homogenate had also been given. This fatal reaction was ascribed to formation by the implanted cells of antibodies against the recipient tissues. Although one cannot extrapolate results obtained in mice to predict what might happen in the pig, it is possible that the addition of splenic tissue to our cell homogenates is responsible for the failure to demonstrate improved survival in the treated animals.

The cell homogenate that we injected into recipient animals seemed adequate from a technical viewpoint. All reasonable precautions regarding sterility were observed, and the tissues were not exposed to excessive temperature changes. The homogenates contained large numbers of intact cells. Time intervals between sacrifice of donor animals and injection of cell homogenates were kept within limits accepted as satisfactory under laboratory conditions. Volume of cell suspension was small (less than 25 ml.), and injected cells were given in large numbers (3 to 4 X 10^9).

Previous experience7 has indicated that larger numbers of transfused cells are required for a beneficial effect on survival in genetically heterogenous animals and when cells are given intraperitoneally rather than by intravenous or intracardiac routes. These factors may have contributed significantly to the lack of benefit observed in groups E and F.

Summary

1. Swine exposed to mixed gamma and neutron irradiation from a nuclear weapon were treated by intravenous, intracardiac or intraperitoneal injection of homologous marrow-spleen cell suspensions.

2. This therapy did not affect survival of animals receiving sublethal, intermediate or supralethal doses of irradiation. No evidence of accelerated repopulation of hematopoietic or lymphatic tissues was found in the treated animals that died, compared with untreated animals dying at the same time. Possible reasons for failure to demonstrate a beneficial effect on survival are discussed.

3. A new, simple method for obtaining large numbers of marrow cells from the vertebral column of large mammals is described.

Summario in Interlingua

1. Porcos exponite al irradiation miscite de radios gamma e de neutrones ab un arma nucleari esseva tractate con injectiones intravenose, intracardiac, o intraperitonee de suspensiones de homologe cellulas de medulla ossee e de splen.

2. Iste terapia non afficeva le superviventia del animales, tanto post doses
subletal como etiam post doses intermediari e supraletal de irradiation. Nulle evidentia de un accelerate repopulation del tissus hematopoietic o lymphatic esseva trovate in le tractate animales que moriva in comparation con le non-tractate animales que moriva al mesme tempore. Es discutite le rationes possibile pro le non-obtention de un effecto benefic super le longevitate del porcos.

3. Un nove, simple methodo pro obtener grande quantitates de cellulas de medulla ab le columna vertebral de grande mammiferos es describite.

REFERENCES

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