Evidence for a Humoral Factor (or Factors) Concerned in Recovery from Radiation Injury: *A Review*

LEON O. JACOBSON

(Division of Biological and Medical Research, Argonne National Laboratory, Department of Medicine and Institute of Radiobiology and Biophysics of the University of Chicago, Chicago, Ill.)

The death of animals exposed to single total-body doses of ionizing radiations in the lethal range is assumed to be due to the failure of functional reconstitution of one or more of the tissues in the body (e.g., the hematopoietic system), and until recently the possibility of a specific approach to the management of the severe injury produced by such an exposure seemed far-fetched.

Prophylactic measures, such as pretreatment with estrogen (53) or cysteine (43), have been used to reduce radiation morbidity and mortality in experimental animals, but, while of great fundamental importance, they are not effective in enhancing recovery when given after the radiation exposure has been sustained. Experiments which may be termed “therapeutic approaches” to the problem have yielded results that have changed this rather discouraging picture to one of restrained optimism; these studies—some of which have been reported previously and others which are herein reported in the open literature for the first time—are briefly reviewed in the paragraphs to follow.

The effects of lead-shielding the exteriorized spleen compared to shielding other parts of the body

Lead-shielding of the surgically exteriorized spleen (average weight, 0.1 gm.) of adult mice during exposure to 1,025 r total-body x-radiation markedly enhances survival (77.0 per cent1), compared to the same exposure without spleen-shielding (1.1 per cent1); none survive exposure to 1,100 r; but 55 per cent survive this dose if the spleen is shielded (23, 32). After exposure to 1,025 r, no anemia and only a transient leukopenia and thrombocytopenia appear in spleen-shielded mice, whereas pancytopenia and death follow exposure without spleen-shielding. Recovery of hematopoietic tissue in spleen-shielded mice occurs by 8 days, but no hematopoietic recovery is noted during this interval in unshielded mice (21). Recovery of the lymphatic tissue in the wall of the gastrointestinal tract in spleen-shielded mice parallels recovery of the hematopoietic tissue elsewhere. These observations prompted the author to postulate that the mechanism of recovery from radiation injury under these conditions was on a humoral basis and that the factor (or factors) responsible was produced by the cells of the protected tissue (17, 29, 33).

The survival of mice exposed to 1,025 r total-body x-radiation is approximately 30 per cent if part of the exteriorized liver (0.8 gm.), the exteriorized intestine (2.5 gm.), the entire head (3.0 gm.), or one entire hind leg up to the thigh (1.5 gm.) is lead-shielded during exposure. Without shielding, only 0.8 per cent survive this dose; with spleen-shielding, at least 76 per cent survive. Shielding one exteriorized kidney (average weight, 0.19 gm.) does not enhance survival. Recovery of the hematopoietic tissue, as judged by histopathologic study, is under way by 8 days in liver- or intestine-shielded animals, whereas after lead-shielding of the head, recovery of these tissues is delayed even longer, and the recovery of hematopoietic tissue is nil with kidney-shielding (21, 29, 30, 32).

1 These percentage figures vary throughout manuscript, because survival varies from one experiment to another.

Received for publication February 5, 1952.
The amount of tissue shielded in the intestine and liver experiments is greater, respectively, by factors of 25 and 8 than in the spleen-shielding experiments. These findings cannot be interpreted with any certainty but do suggest that the potential production of a factor (or factors) by the intestine and the liver is not so great as that by splenic tissue but yet is sufficient to institute recovery early enough in a sufficient number of cells of the body to have a definite effect on survival.

It is conceivable that nearly all tissues of the body are capable of producing the factor (or factors) concerned in recovery from radiation injury, but certain tissues and, in particular, the hematopoietic system have a greater potential production per unit volume.

There are several differences between head- or limb-shielding and spleen implantation (to be described later) and spleen-shielding. According to generally accepted concepts, the reduction in volume dose when the head, limb, or intestine is shielded must be considered as playing a role in the reduction of mortality, since these structures represent a fairly large proportion of the body weight (15 per cent, 7.5 per cent, and 12.5 per cent, respectively). On the other hand, the shielded spleen weighs only 0.1 gm. (0.005 per cent body weight), and spleen implants weigh 0.010 gm. (0.0005 per cent body weight), which eliminates the volume-dose factor of shielded spleen or implanted spleen from consideration. The fact that the head, hind limbs, and intestine contain reticulo-endothelial tissue and other tissue of mesenchymal origin may be as important or more important than the volume-dose consideration.

The work of other investigators who have studied the effect of shielding various parts of the body on radiation mortality (1, 2, 6, 7, 10, 12, 49) will be discussed in the paragraphs on species differences.

**Effect of Clamping Off Splenic Circulation during the Irradiation-Shielding Procedure**

The survival of mice in which the circulation to the shielded spleen is clamped off during exposure of the animal to 1,025 r and in which the clamp is released immediately after irradiation is approximately the same as survival in animals with spleen-shielding without clamping (30–32). Histologic recovery of the hematopoietic system is the same as in the spleen-shielded animals without clamping on the splenic pedicle. This observation was convincing evidence that the presence of shielded tissue in the circulation was not required during the period of irradiation in order for survival to be enhanced and hematopoietic regeneration to proceed. Re-introduction of the spleen into the circulation after irradiation could thus be considered an effective postirradiation "therapeutic approach" to the problem.

None of eight mice survived which had the circulation to the spleen clamped off without simultaneous spleen-shielding during exposure to 1,025 r (16). In this experiment, however, the clamp was applied immediately before irradiation of the animals and released immediately after the completion of irradiation. In view of the observations of Jolly (33) and Dowdy (9), one might expect temporary clamping of the splenic pedicle during exposure of the mouse to 1,025 r to enhance survival if indeed the radiation injury sustained by the splenic tissue is sufficiently reduced by this procedure.

Further data, including histologic study of the spleens which have been clamped off during irradiation, are necessary before the reason for the ineffectiveness of this procedure can be resolved.

**Effect of Splenectomy after Spleen-Shielding**

Surgical extirpation of the initially shielded spleen at intervals after exposure of mice to 1,025 r total-body x-radiation shows that a beneficial effect (survival of greater than 70 per cent and early regeneration of hematopoietic tissue) has already been exerted if the shielded spleen is left intact in the circulation for 1 hour (32). Leaving the spleen in the circulation for longer periods, such as 6 hours, 24 hours, or 2 or more days, does not increase the percentage of animals surviving. In a previous communication (30) it was reported that, if splenectomy was performed within 10 minutes after the irradiation–spleen-shielding procedure, none survived. Further work, however, has shown that leaving the spleen in the circulation for as little as 5 minutes is sufficient to increase significantly the survival of mice exposed to 1,025 r (24). Full recovery of the blood-forming tissues is delayed longer in mice splenectomized 5 minutes after irradiation than in mice splenectomized 24 hours after irradiation. If the originally shielded spleen (whether or not the pedicle is clamped during the irradiation) is not removed, complete regeneration of hematopoietic tissue occurs earlier than in mice with splenectomy 24 hours after the shielding procedure (32). These facts indicate that the intact spleen may release enough of the factor in a few moments significantly to enhance survival but that, if left in the circulation longer, a greater and more rapid regeneration of hematopoietic tissue occurs.
OBSERVATIONS ON RELATIONSHIP OF AGE OF MICE TO EFFECT OF SPLEEN-SHIELDING ON SURVIVAL

Lorenz and associates (40) reported that 4-week-old strain A mice died within the first 8 days after exposure to 800 r x-radiation with spleen-shielding. The survival of adult mice (10 weeks of age or more) of the same strain, exposed to 900 r with spleen-shielding, was 95 per cent. Simmons and associates (48) investigated this problem in Carworth (CF #1) female mice exposed to 1,025 r with spleen-shielding and found that, whereas the survival of adult mice (10-12 weeks) was 76 per cent, the survival of younger mice was as follows:

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>With shielding (per cent survival)</th>
<th>Without shielding (per cent survival)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>4-5</td>
<td>38</td>
</tr>
<tr>
<td>18</td>
<td>4-5</td>
<td>0</td>
</tr>
<tr>
<td>188</td>
<td>5-6</td>
<td>9.4</td>
</tr>
<tr>
<td>14</td>
<td>5-9</td>
<td>64</td>
</tr>
<tr>
<td>170</td>
<td>10-12</td>
<td>70</td>
</tr>
<tr>
<td>192</td>
<td>10-12</td>
<td>0.8</td>
</tr>
</tbody>
</table>

These observations of Lorenz (40) and Simmons (48) suggest (a) that physiological aspects characteristic of the age influence the effectiveness of the shielded spleen to enhance survival or (b) that the shielded spleen produces less of the factor under discussion before maturity. The former suggestion seems the more likely, since the transplantation of spleens from immature mice of these same age levels to adult mice exposed to 1,025 r total-body x-radiation is about equally effective in enhancing survival (30-32).

EFFECT OF TOTAL-BODY EXPOSURE TO 1,025 R WITH 200 R TO THE SPLEEN ON SURVIVAL AND ON REGENERATION OF THE HEMATOPOIETIC SYSTEM

Mice have been exposed to 1,025 r total-body x-radiation and the spleen has been given various increments of the total-body dose. Doses up to and including 200 r may be given to the spleen at the same time as 1,025 r total-body exposure without reducing survival below the 75 per cent, which is expected from the earlier spleen-shielding studies (32, 34). In contrast to animals with spleen-shielding, and thus no irradiation of the spleen, these animals become moderately anemic and develop a severe leukopenia that persists beyond the twelfth day. Histological studies show that recovery of the blood-forming tissue is qualitatively complete by 10-12 days (32). Even with dosages of 400 r, 500 r, or 600 r to the spleen and 1,025 r to the body, survival is significantly higher (59, 50, and 34 per cent, respectively) than in mice exposed to this dose without spleen-shielding (1.1 per cent). These data indicate that the capacity of the splenic tissue to elaborate the factor is still partially retained or recovery of the tissue in the spleen that produces the factor occurs early enough to enhance survival, even with doses as high as 600 r to the spleen and 1,025 r to the body. These observations tend to add support to the hypothesis that the factor (or factors) responsible for recovery from radiation injury under these circumstances is derived from more primitive but more "radio-resistant" cells, such as reticular cells, rather than from "free" cells such as lymphocytes, granulocytes, and the like. The spleen is, for all practical purposes, devoid of these "free cells" after a dose of 500 r, whereas the basic reticular network remains "histologically" intact. It is interesting that with a lethal dose to the body (1,025 r) and an LD_{50} (500-600 r) to the spleen circa 50 per cent of the animals survive.

EFFECT OF POSTIRRADIATION SPLEEN TRANSPLANTATION ON RECOVERY FROM IRRADIATION

Transplantation of spleens (total weight, 10-100 mg.) from baby or adult mice into the peritoneal cavity of mice within 2 hours after exposure of the recipient adult mice to 1,025 r total-body x-radiation significantly increases the survival (circa 50 per cent) of the irradiated mice and hastens regeneration of hematopoietic tissue (30-32). The donor spleens are simply dropped free into the peritoneal cavity. Transplantation of spleens into the peritoneal cavity of mice 1 or 2 days after exposure to 1,025 r total-body x-radiation likewise enhances survival (circa 25 per cent) but not as effectively as earlier transplantation (30, 34). Implantation of muscle into the peritoneal cavity after exposure of mice to 1,025 r total-body x-radiation has no beneficial effect on survival.

If splenectomy is performed in mice prior to irradiation, followed by the implantation of fresh spleens into the peritoneal cavity after irradiation, survival is enhanced, indicating that the animal's own spleen is not required to make the transplant effective (18). Surgical removal of the transplanted spleens from the peritoneal cavity of mice 1 and 2 days after the irradiation-transplant procedure has invariably been followed by death of the animals. Gross and microscopic observations on mice surviving the irradiation-transplant procedure reveal that the implanted spleen or spleens have vascularized and eventually appear as normal splenic tissue. Revascularization and reconstitution of the implanted spleen is usually well under way by the sixth day after implantation. Transplantation of splenic tissue 2
days after irradiation is admittedly less effective in enhancing survival of mice exposed to 1,025 r than earlier transplantation, but it has not been determined when a state of irreversibility has been reached. Actually, if establishment of a vascular supply to the transplant is essential to the manufacture and transport of the factor (or factors) in question, one might venture a conservative guess that supplying an optimum amount of the factor to mice as late as 6 days after exposure to 1,025 r would still significantly increase the survival.

**Effect of Suspension of Mashed Embryos on Recovery from Radiation Injury**

Intraperitoneal administration of a suspension of 12-day-old mouse embryos, prepared by forcing the embryos through an 18-gauge stainless steel mesh, and of such a consistency as to permit delivery through an 18-gauge hypodermic needle, is effective in enhancing survival of mice exposed to 1,025 r total-body x-radiation (19). This suspension, prepared in the cold with or without the addition of normal physiological saline or buffered saline, when given intraperitoneally in a dosage of from 0.5 to 1.0 ml. 2-6 hours after irradiation of the recipient resulted in 30 per cent survival in 95 animals. Suspensions of baby or adult spleens, prepared and administered in a similar manner, have thus far been ineffective in enhancing survival of mice exposed to 1,025 r but have been effective in enhancing the survival of mice exposed to 800 r (20). Chick embryo suspensions (age of embryos, 11-14 days), prepared and administered in a similar manner to the mouse embryo suspensions, have been reported by Marks and Brues* to be ineffective in enhancing the survival of mice exposed to dosages of x-radiation in the LD50 range or above.

The factor (or factors) in the embryo or spleen suspensions responsible for recovery from radiation is probably the same as that which is responsible for the effectiveness of the spleen-shielding and spleen implants. Two possible explanations for the effectiveness of cell suspensions are obvious: (a) that the cells in the suspension quickly implant and begin elaboration of the factor or factors effective in initiating tissue regeneration throughout the body or (b) that the peritoneal cavity serves as an incubator which allows the cell suspension to remain alive and to elaborate the factor or factors responsible for increased survival and tissue regeneration in the irradiated mice.

* Unpublished data of E. K. Marks and A. M. Brues.

**Effect of Postirradiation Parabiosis on Survival from Radiation Injury**

Brecher et al. (8) have recently reported that approximately 50 per cent of rats exposed to 700 r total-body x-radiation survive if joined to normal nonirradiated litter-mates within a few hours after irradiation. None of the controls given the same dose of irradiation survived the 28-day period of observation. Hematopoietic regeneration was more rapid in the irradiated rats with a parabiont than in the irradiated controls. These findings, like those of Lorenz (42) reported below, corroborate Jacobson's previously reported observation that effective postirradiation "therapy" is a reality. Like the embryo suspension experiments described above, however, the experiments of Brecher do not point out the cellular source or the identity of the effective factor (or factors) concerned.

**Relationship of the Quantity of Shielded or Implanted Splenic Tissue and Survival of Irradiated Mice**

Two separate observations indicate that a definite relationship exists between the quantity of implanted or shielded tissue and the effect as measured by survival from irradiation (32).

1. The transplantation of two spleens (wt., circa 5 mg.) from 7- to 12-day baby mice into the peritoneal cavity of mice immediately after irradiation (1,025 r) does not enhance survival, although the transplantation of four spleens (wt., circa 10 mg.) is effective in reversing the process in time to allow recovery of the animal (32).

In the regular spleen-shielding technic the main splenic pedicle is left intact, but a small blood vessel at the distal tip of the spleen is severed to facilitate exteriorization and lead-shielding of the spleen. Invariably, from one-fourth to one-half of the spleen proximal to the severed vessel becomes infarcted and undergoes liquefaction necrosis. If this vessel is not cut so that the whole spleen is shielded and remains intact, 100 per cent of the animals survive exposure to 1,025 r rather than the expected 77 per cent. In fact, with a total-body exposure to 1,300 r, only 3.4 per cent of animals survive if the distal vessel of the spleen is cut during the shielding procedure, whereas 26.9 per cent survive this exposure with spleen-shielding if the distal vessel is not cut (32). This observation, like the one described above, indicates that the quantity of the "factor" being produced is directly related to the number of cells available in the shielded or implanted tissue, and the amount of the "factor" available determines the survival of the animal. It would appear that the repair...
process must be initiated in a minimum number of cells in the body of the irradiated animal to insure survival of the animal exposed to dosages of 1,000 r or more.

**Evidence from Histologic Studies Supporting Humoral Theory of Cell Regeneration**

Rabbits exposed to 800 r or 1,000 r with spleen-shielding or appendix-shielding show histologic evidence of beginning recovery of hematopoietic tissues on the fourth postirradiation day, whereas in animals thus exposed without shielding evidence of regeneration is delayed for 8 or more days (26, 28). Mice exposed to 1,025 r with spleen-shielding likewise show histologic evidence of recovery of the hematopoietic tissue on the fourth postirradiation day; in fact, recovery is essentially complete by 8 days. Mice irradiated without shielding have no evidence of recovery or show "spotty foci" of beginning regeneration in the bone marrow about on the tenth to twelfth day. Death of all mice without spleen-shielding occurs by the fourteenth day. In none of the animals examined, including those on the fourteenth day, has hematopoietic regeneration been other than focal. In these mice and rabbits which had spleen-shielding, regeneration may occur from the scattered "free cells" in the lymphatic tissues and bone marrow that survive the radiation, but heteroplastic regeneration from reticular cells is prominent. Thus, colonization from the shielded tissue, followed by repopulation by multiplication of these colonized cells, if a factor at all, is only one aspect of the recovery process. The shielded tissue in some way restores the functional capacity of the reticular cells to repopulate the hematopoietic tissues. The shielded tissue may likewise restore the functional capacity of the residual "free cells," which are not destroyed by irradiation, to multiply and thus repopulate the hematopoietic tissues. Cells coming from the shielded or implanted tissue cannot at the moment be distinguished from these residual free cells. If the cells which migrate out from the shielded tissue do "lodge" in hematopoietic tissue, then it is possible that they also contribute by division and multiplication and also by elaboration of the factor (or factors) under discussion in this dissertation. In a previous report (26, 28) it was shown that recovery of hematopoietic tissue was more rapid in rabbits which had shielding of the exteriorized appendix during exposure to 800 or 1,000 r total-body x-radiation than in animals similarly exposed without appendix-shielding. The shielded appendix does not become the site of ectopic formation of such cells as erythroblasts, megakaryocytes, or granulocytes. If recovery of the hematopoietic system could be attributed primarily to migration of cells from the shielded tissue and subsequent multiplication of these cells, then the general concept of the unitarian school of hematology would have additional support, since the appendix is exclusively a lymphatic organ.

**Species Differences in Effect of Spleen-Shielding on Recovery from Radiation Injury**

A significant percentage of mice (31 per cent) with spleen-shielding survives a dose of 1,300 r total-body x-radiation, which is more than 500 r above the 30-day LD<sub>50</sub>. Recovery of the blood-forming tissue is rapid in these mice and by hematologic examination is shown to be well under way by 8 days (25). The effect of spleen-shielding on the survival of rats is also definite but less spectacular than in mice (34). The effect of spleen- or appendix-shielding on the survival of irradiated rabbits has not been studied carefully, but it is clear that no such enhancement of survival occurs as is observed in spleen-shielded mice or rats. Spleen- or appendix-shielding in this species (rabbit) during exposure to 800 r or 1,000 r appears not to affect survival appreciably, even though regeneration of blood-forming tissue precedes the recovery of this tissue in the animals without spleen- or appendix-shielding (32). These species differences cannot be adequately evaluated at the present time, since, for example, considerable differences may exist between rabbit and mouse spleen in terms of the potential production of the factor (or factors) involved in survival or early regeneration of hematopoietic tissue.

Allen (9) has reported that 450 r total-body x-radiation is invariably lethal to dogs. With head-shielding, however, the mortality of this dose is reduced to 75 per cent, and other aspects of the usual postirradiation syndrome, such as hemorrhage and evidences of infection, are greatly reduced or absent. Further work on the dog, comparing the relative effectiveness of shielding such parts as the head, spleen, intestine, limbs, and liver, will be of interest if for no other reason than to obtain base-lines on the potential effectiveness of these tissues for comparison with mice, rats, and rabbits and to accumulate some facts on the potential production of the factor (or factors) involved in survival or early regeneration of hematopoietic tissue.
precisely to determine the relative importance of the various abdominal tissues in enhancing survival on a weight basis and more adequately to assess the volume-dose factor. This has been done to some extent by Gershon-Cohen and associates in rats (12) and by Jacobson et al. (21, 29, 30, 32) in mice, but further data in all species are needed. As was pointed out by Bond (7), the radiosensitivity of the part of the body irradiated may be more important than the gram-roentgens sustained by the balance of the body. To this must be added the fact that the actual or potential production of the factor (under consideration in this paper) by the shielded or nonirradiated tissue may be more important in determining survival of the animal than the radiosensitivity of the tissue in the radiation field and, within certain limits, more important than the gram-roentgens sustained by the balance of the body.

**Effect of Postirradiation Injection of Homologous Bone Marrow on Survival of Irradiated Mice**

Lorenz et al. (37, 41, 42) have recently shown that, whereas 900 r is the LD₉₀ for genetically homogeneous hybrid LAF₁ mice, approximately 75 per cent survive this dose if bone marrow from normal nonirradiated mice of the same strain is injected intravenously within an hour after the irradiation. If the bone marrow is administered intraperitoneally, survival is slightly less (circa 50 per cent). The author estimates that the total weight of the injected marrow is approximately 1.5 mg. The recovery of the hematopoietic tissue of the bone marrow-treated mice as in the spleen-shielded, spleen-implanted, or embryo suspension-injected mice is hastened. Jacobson et al. (16) have corroborated Lorenz's finding. The survival of Carworth (CF #1) female mice exposed to 900 r and given homologous bone marrow intravenously immediately after irradiation was approximately 50 per cent. Rekers (44, 45) and Talbot (51, 52) have reported negative or equivocal beneficial effects on the radiation syndrome in dogs and rats, respectively, after bone marrow administration. In a preliminary report by Hilfinger et al. (14), normal rabbit bone marrow was emulsified in serum and injected intravenously into rabbits after exposure to dosages from 1,000 r to 1,400 r total-body x-radiation. A more transient leukopenia was observed in the bone marrow-treated rabbits than in the control irradiated rabbits.

The suspension of bone marrow which Lorenz has injected contained mature and immature free cells, such as granulocytes and megakaryocytes, as well as free and fixed macrophages, reticular cells, and endothelial tissue. It seems likely that the cells injected establish themselves as scattered foci of hematopoietic tissue and produce a factor (or factors) responsible for survival of the animal which is identical with that postulated in the spleen-shielding, spleen-implantation, and embryo-suspension experiments. No data are as yet available to compare adequately the relative effectiveness of splenic tissue and bone marrow in enhancing survival from radiation injury.

**Effect of Heterologous Transplants and Cell Suspensions on Recovery from Radiation Injury**

Jacobson and associates (23) have reported a preliminary experiment in which rabbits were exposed to 900 r total-body x-radiation and, following exposure, from four to thirteen spleens, freshly obtained from young mice, were inserted into the peritoneal cavity of the rabbits. No attempt was made to study the effect of this procedure on the survival of the irradiated rabbits. At intervals after the irradiation-spleen-transplant procedure the rabbits were sacrificed, and the hematopoietic tissues as well as the donor spleens were removed for histologic study. The postirradiation intervals studied were 6, 8, 10, and 12 days. At the time of sacrifice it was obvious on gross examination that the donor spleens were firmly attached to the spleen of the rabbit or the surrounding omentum, and many or all of the donor spleens were viable. Microscopic study of the donor spleens revealed even at the 12-day interval that vascularization had occurred, viable cells including lymphocytes and megakaryocytes were in abundance, and in some of the donor spleens the basic splenic architecture was still clearly evident. On comparison of the extent of regeneration of the hematopoietic tissues of the irradiated rabbits which had intraperitoneal mouse spleen transplants with the irradiated controls, it was found that only on the twelfth postirradiation day was a difference demonstrated. At this interval regeneration of hematopoietic tissues was normal or hyperplastic in two of four rabbits which had spleen transplants, whereas only relatively little evidence of regeneration was found in four control irradiated rabbits. This finding may be a coincidence or a reaction to the foreign transplant and should therefore not be considered as evidence indicating the effectiveness of heterologous tissue to hasten recovery from radiation injury.

Lorenz has reported evidence of the effectiveness of heterologous tissue transplants on recovery from radiation injury (38). Within an hour after exposure of mice to 900 r (LD₉₀) of total-body x-
radiation, approximately 25 mg. of freshly aspirated guinea pig bone marrow (in buffered saline) was injected intravenously into the irradiated mice. None of the control irradiated mice survived, but 40 per cent of the irradiated mice which received intravenous guinea pig bone marrow suspension survived the 28-day period of observation. The number of animals used by Lorenz was small, and the results must be considered preliminary. The survival of this strain of mice injected with homologous bone marrow (1.5 mg.) after exposure to 900 r is circa 75 per cent.

The report of Lorenz (38), if it can be corroborated, probably should be considered as conclusive evidence that the factor (or factors) supplied by spleen or bone marrow, which so significantly enhances survival, is indeed a humoral substance (or substances).

It is not likely that heterologous spleen transplants or heterologous bone marrow injection, if indeed they are effective at all, produces its effect by seeding the mouse or rabbit hematopoietic tissue with cells which by multiplication repopulate the bone marrow. It seems more likely that this heterologous tissue lives, at least temporarily, in its new environment and produces a substance (or substances) that aids in recovery from the radiation injury.

**Effect of Combined Prophylactic and Therapeutic Measures on Survival from Radiation Injury**

The fact that a reduction in the mortality of animals exposed to lethal dosages of total-body x-radiation could be effected by pretreatment with estrogens (62) as well as by spleen-shielding (21) suggested to Simmons (47) that these two measures might produce an additive effect on survival. Accordingly, he tested this hypothesis and found that (a) mortality of mice exposed to 1,025 r total-body x-radiation was 100 per cent, (b) 61.5 per cent of mice survived this dose if estrogens were given prior to irradiation, (c) 82.8 per cent survived if the spleen was shielded during exposure to 1,025 r, and (d) 100 per cent survived 1,025 r if the techniques of pretreatment with estrogens as well as spleen-shielding were employed. Bethard (4), employing the same general approach, found that cysteine and spleen-shielding similarly had an additive effect on survival of mice exposed to x-radiation. Furthermore, it was found that, when the techniques of pretreatment with estrogens and cysteine and spleen-shielding during irradiation were all combined, an additive effect of all three on survival was observed (5). Jacobson found that pretreatment with cysteine, followed by 1,025 r total-body x-radiation, and postirradiation intraperitoneal transplantation of normal spleen were also additive in enhancing survival (16). Cysteine fails to produce any enhancing effect on survival of irradiated mice when given after 1,100 r total-body x-radiation, whether or not the mice had spleen-shielding during irradiation. These studies, while very interesting, shed no light on the obvious question of whether or not the pretreatment or prophylactic technics are related to the "therapeutic" technics from the standpoint of mechanism.

**Studies on Antibody Formation**

The fact that a single total-body exposure to x-rays inhibits antibody formation is well documented (13). It was recently shown that, if the spleen or the appendix of the rabbit is surgically exteriorized and shielded with lead during total-body exposure to 800 r, the capacity to form antibodies to a particulate antigen injected 24 hours after irradiation is retained (38). In another series of experiments this observation was carried a step further (25, 27). Rabbits were exposed to dosages of 800 r or 500 r total-body x-radiation with spleen-shielding. Twenty-four hours later the spleen was removed surgically. After another 24-hour period (48 hours after irradiation) a particulate antigen (sheep red cells) was given intravenously. The capacity of these animals to form antibodies (anti-sheep cell hemolysin) was compared to that of various control groups given the same antigen at the same time relative to the irradiation as the experimental animals described above. The capacity to form antibodies to the injected antigen was retained in the rabbits given 800 r or 500 r total-body x-radiation which had spleen-shielding during irradiation, the spleen left intact in the circulation for 24 hours and then removed surgically even though the antigen was given 24 hours after splenectomy and 48 hours after irradiation. The facts that these rabbits retained the capacity to form antibodies, even though hematopoietic tissues in the body were as yet atrophic, and that control rabbits exposed to the same dose did not retain this capacity are considered to be results of the functional restoration of cells in the body (such as free and fixed macrophages and reticular cells) by a humoral (noncellular) substance entering the general circulation from the originally shielded spleen during the 24 hours prior to splenectomy.

**General Discussion**

In view of these observations, it seems extremely unlikely that cell migration from the shielded or transplanted tissue and subsequent
proliferation of these cells account for the reconstitution of hematopoietic tissues and increased survival of irradiated animals or that neutralization of some "toxin" produced by irradiation can account for these findings. Perhaps neither of these possibilities can be positively excluded on the basis of the evidence presented, but the evidence strongly suggests that the factor (or factors) responsible for recovery from radiation under these circumstances is noncellular and may be required only for the initiation of the repair process. The factor (or factors) may be quite labile or, as is more likely, may be produced in an effective quantity only by living cells. These cells may be present in shielded or implanted tissue or may have migrated out, but wherever they are they are probably producing the factor under discussion. The factor may be a single substance such as an enzyme or coenzyme necessary for the functional reconstitution of many different cell types in the several organ systems or several different factors may be concerned.

Salisbury (46) found in a small number of dogs that early direct cross-transfusion between irradiated (LD₉₀ x-ray) and nonirradiated dogs significantly reduced mortality, reduced the severity of the expected hematopoietic depression, and reduced the severity of the usual clinical signs of irradiation sickness. Salisbury's experiments (46) should be expanded in numbers of animals and more adequately controlled to make the significance of the experiment more clear-cut.

The fact that 75 per cent of mice that have lead-shielding of the spleen during exposure to 1,025 r and then splenectomy 1 hour after the irradiation–spleen-shielding procedure survive would lead one to expect early administration of whole blood to be effective as well. This seems logical since whatever the spleen accomplishes under these circumstances must have been via the blood stream. That the factor (or factors) must be supplied early is also strongly supported by the fact that in mice given a lethal dose of radiation spleen transplants are more effective on the day of irradiation than on the second day after irradiation. In other words, supplying "the factor" responsible for initiating the functional repair in some unknown minimum number of cells throughout the body must be accomplished early enough to reverse the processes that ordinarily end in death of the animal. Once the factor is adequately supplied, as is clearly demonstrated by the splenectomy experiments, the process of repair is initiated, yet histologic evidence of repair or regeneration is not apparent for 4 or more days.

Indirect transfusion initiated on the fourth postirradiation day has been reported to be unsuccessful in significantly increasing survival of x-radiated dogs (3). This apparent failure of whole blood transfusions to enhance survival effectively and reduce morbidity is understandable if one assumes that (a) the amount of the "factor" (or factors) present in the blood per unit volume is small, and therefore relatively large amounts of blood would be necessary to initiate effectively the recovery process, or (b) the factor (or factors) was administered too late after exposure of the recipient to initiate the repair process in time and widely enough in cells of the body to have a critical effect on morbidity and survival. The preliminary experiments of Swisher et al. (50) indicate that the morbidity of dogs exposed to dosages of x-irradiation in the mid-lethal range is reduced if as little as 250 cc. of whole blood collected in ACD solution from a compatible donor is administered to irradiated dogs shortly after exposure. The lability of the factor is as yet unknown. The ineffectiveness of cell-free extracts obtained from extirpated splenic tissue or embryos may only indicate that too small an amount of the factor is present or too small an amount of the factor is obtained in the extracts from these tissues by present methods. A method of preservation or concentration of the factor (or factors) or a more sensitive method of assay may be necessary before a positive result can be obtained by cell-free extracts. If the factor is present in whole blood in a concentration sufficient to alter the radiation syndrome even when given in relatively small amounts soon after irradiation of the recipient, then varying the conditions and methods of the administration of whole blood may supply important clues to the identification of the factor (or factors).

Any attempt to relate these findings to problems other than radiation injury would be premature at this time, but speculation is intriguing. It is a well known fact that in cases of myelogenous leukemia in the human being, x-irradiation of the spleen is often sufficient to produce a hematologic and clinical remission. One might therefore suggest that these findings in animals, as reported above, are at variance with those observed in human leukemia. However, the spleen in myelogenous leukemia cannot be considered normal, and further study will be required before this apparent paradox can be resolved. It has been reported by Furth (11) and by Lorenz et al. (59) that the incidence of ovarian tumors was markedly increased in mice that had been given total-body radiation. Lick (36) reported, however, that if one ovary is lead-shielded and the balance of the body including the other ovary is irradiated, the incidence of ovarian tumors is significantly reduced. He postu-
lated that some factor from the nonirradiated ovary prevents tumor formation in both the irradiated and nonirradiated ovary. Lick’s findings are undoubtedly on a hormonal basis and therefore may be quite different from the findings discussed concerning mice with spleen-shielding or implants. A recent report by Hollcroft and Lorenz (15) has perhaps more direct bearing on the problem related in this paper. These authors were studying the effect of irradiation on a transplanted lymphosarcoma in mice. The tumor grew slowly locally and killed the animal in 30–40 days. A dose of 400 r total-body x-radiation, including the tumor, failed to kill the tumor; local irradiation of the tumor alone with dosages up to a total of 1,500 r likewise was ineffective. A single dose of 800 r to the whole body (including the tumor) but with the spleen shielded effected a cure of the tumor in a high percentage of the animals so treated. These authors suggested that the “toxicity” produced by total-body irradiation was responsible for the effective suppression of the tumor; spleen-shielding merely made it possible for the mice to survive a dose that would ordinarily kill 100 per cent of animals irradiated without spleen-shielding.

CONCLUSIONS

A brief review of data is presented which conclusively shows that survival of laboratory animals exposed to a single lethal dose of total-body x-radiation can be significantly increased by measures instituted after the radiation injury has been sustained. These measures involve spleen-shielding, intraperitoneal implantation of splenic tissue, and intraperitoneal implantation of embryo suspensions, intravenous or intraperitoneal injection of homologous bone marrow suspensions, and related techniques. The evidence presented indicates that the factor (or factors) responsible for recovery from radiation injury under these conditions is likely a humoral (noncellular) substance (or substances) produced by the living cells of the shielded, implanted, or injected tissue that is capable of instituting recovery of certain tissues of the body vital to the survival of the animal. If further experiments indicate that heterologous transplants or injections of tissue suspensions are effective, the possibility of seeding or colonization by cells of the shielded or implanted tissue as a factor in the reconstitution of the depleted blood-forming tissues is reduced to one of secondary importance for the purposes of this discussion. It is admitted, however, that a possibility exists that the shielded or implanted tissue may produce its effect by some detoxification process.

The identity of the humoral factor (or factors) under discussion is as yet unknown. Nor is it known from which cells of the body the factor (or factors) originates. It is produced or made available in an effective quantity by minute amounts of hematopoietic tissue or so-called reticulo-endothelial tissue, whereas larger quantities of tissue such as kidney have thus far proved ineffective. With the assay methods now in use living cells appear to be necessary in the material administered to alter effectively the radiation syndrome and promote recovery. It is firmly established that the factor (or factors), when made available to irradiated animals, brings about an early recovery of the blood-forming tissue. This recovery may be directly as well as indirectly responsible for the enhanced survival of irradiated animals. It may be that the factor (or factors) is involved in the orderly regeneration or functional reconstitution of yet other cells, tissues, or organ systems of the body which are important, if not vital, to survival of the irradiated animal.

The implications of these findings for problems of radiation injury and radiation therapy are obvious, but their application to medicine in general and to neoplastic and non-neoplastic diseases of the blood-forming tissue in particular requires exploration.

REFERENCES

6. BIRNBOIM, M. S.; DOWDY, A. H.; BURLINGAME, L.; and LAMPERT, J. Systemic Effects of Irradiation of the Exter-teriorized Small Intestine in Rabbits. UCLA-128, 1951.4
7. BOND, V. P.; SWIFT, M. N.; ALLEN, A. C.; and FISHELER, M. C. Sensitivity of the Abdomen of the Rat to X-irradiation. Naval Radiological Defense, ADB-92, 1940.3
38. -----. Modifications of Acute Radiation Injury in Mice and Guinea Pigs by Bone Marrow Injections. Radiology (in press).


44. REKERS, P. E. Transplantation of Bone Marrow into Dogs That Have Received Total Body Single Dose Radiation. University of Rochester Atomic Energy Project, UR-11, 1948.


51. TALBOT, J. M., and GERSTNER, H. B. Bone Marrow Implants in the Treatment of Radiation Sickness. USAF School of Aviation Medicine, Project 21-47-001, 1951.


Evidence for a Humoral Factor (or Factors) Concerned in Recovery from Radiation Injury: A Review

Leon O. Jacobson