Regeneration of Locally Irradiated Bone Marrow.  
II. Induction of Regeneration in Permanently Aplastic Medullary Cavities  

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A previous report described the late events following localized irradiation damage to rat bone marrow. A secondary wave of late aplasia was correlated with radiation-induced loss of sinusoidal structures and hemopoietic recovery depended upon sinusoidal regeneration. Those studies demonstrated that a single dose of 4,000 r or greater to a localized segment of marrow produced permanent aplasia.

The present experiments were performed to determine whether treatment might alter what would otherwise be a permanent aplasia. Previous experiments have demonstrated that mechanical ablation of bone marrow is followed by complete marrow regeneration. This technic offered a possibility of restoring severely aplastic and dysplastic bone marrow in which increased amounts of connective tissue or an altered microcirculation prevents hemopoietic recovery.

Methods

Two types of experiment were performed: intravenous transfusion of isologous bone marrow and mechanical disruption of the tissue in the medullary cavity.

A. Marrow Infusion Studies

Young adult female rats of the Lewis inbred strain weighing 150-175 grams received local irradiation to one leg distal to the midfemur with single doses of 2000, 4000, and 6000 r. The technic of radiation exposure was previously described. After three months suspensions of bone marrow obtained from isogenic donors of the same inbred strain containing 20-100 x 10^6 nucleated marrow cells were injected into the tail veins of irradiated and nonirradiated animals. Previous experiments showed that three months represented the nadir of postirradiation hemopoietic activity. Five animals from each irradiated group and nonirradiated control groups were killed at 1, 2, 5, 14, 30, 60 and 90 days after bone marrow transfusion. Femurs and tibias were removed and fixed in neutral, buffered formalin, sectioned at 6-8 µ and stained with hematoxylin and eosin.
B. Mechanical Ablation Studies

A second group of experiments was performed utilizing 150–175 gram females of the Walter Reed Carworth Farms variety of Wistar strain locally bred. The technic of irradiation was as previously described. All irradiated animals received 4000 r to a single limb in one dose.

Three months later, the medial plateau of the tibia was surgically exposed and a 1.0 mm. diameter hole drilled into the medullary cavity of the diaphysis with an air-powered dental drill. A barbed dental pulp cavity broach was then inserted into the medullary cavity of the tibia as far as the distal epiphysis. With linear and rotating movements of the broach, we disrupted and removed as much of the marrow as possible. In half of the irradiated animals so treated, bone marrow obtained from the amputated tibia of the opposite, unirradiated leg was injected into the disrupted marrow cavity. The hole leading to the cavity was plugged with bone wax and the incisions were closed with 4-0 silk sutures. The incisions in the irradiated leg and amputated opposite leg healed rapidly, and the animals suffered no significant disability. Unirradiated control animals were subjected to identical treatment. Two or three animals from irradiated and control groups were killed at 4, 9, 14, 30, 60 and 90 days after mechanical disruption of marrow. Femurs and tibiae were removed and processed as in Part A.

Results

A. Marrow Infusion Studies

There is no evidence of induced regeneration of any of the marrow irradiated with 4000 and 6000 r, followed by bone marrow transfusion. The lesions observed were identical to those reported previously.1 Diffuse and spontaneous marrow regeneration was present 3–6 months after irradiation with 2000 r as observed in previous studies.
Fig. 2.—Tibia irradiate 4000 r; after three months the marrow was mechanically disrupted. Two months later the animal was killed. This section shows fibrosis and reticular elements. There is no hemopoiesis or regeneration of sinusoidal circulation. × 200 H & E.

B. Mechanical Ablation Studies

Controls. Animals killed during the first week after mechanical disruption showed extensive hemorrhage with occasional patches of normal marrow. Then the medullary cavity was filled with a tissue of primitive-appearing reticulum cells. Cancellous bone developed in some of the disrupted cavities. Between two weeks and one month, most of the marrow architecture was restored with good representation of megakaryocytes, erythropoietic and granulopoietic cells. Vascular structures also regenerated, including the median long sinus, arterioles and marrow sinusoids. At two months the marrow seemed normal (Fig. 1).

Irradiated Groups. (4000 r 3 months before disruption and transplant). For the first two weeks after disruption and transplant, the lesions of the marrow resembled those observed in the control groups. Diffuse hemorrhage was followed by the appearance of a primitive reticulum tissue. Minimal hemopoietic regeneration occurred at one month only in those animals in which autologous marrow was injected into the marrow cavity. Although the primitive reticulum predominated in these animals, scattered aplastic areas typical of irradiated marrow persisted together with diffuse hemorrhage, fat and reticular tissue. Further hemopoietic regeneration had occurred at two and three months and a more normal vascular architectural pattern replaced the primitive reticulum as in the control animals (Fig. 3 and 4).
Fig. 3.—Tibia irradiated 4000 r; after three months the marrow was mechanically disrupted and into the cavity was injected marrow from the contralateral unirradiated leg. Two months later the animal was killed. This section shows scattered sites of hemopoiesis and dilated sinusoids. × 200 H & E.

Animals treated with mechanical disruption alone without injection of normal marrow into the irradiated cavity demonstrated little or no evidence of marrow regeneration (Fig. 2). In all animals the irradiated and undisrupted segment of the distal femur was aplastic.

Discussion

Steinberg and Martin demonstrated that complete mechanical ablation of bone marrow in adult animals was followed by complete marrow regeneration. They suggested that the sequence of marrow repair resembled the development of bone marrow during fetal life. A primitive reticulum budded from the endosteum, filling the medullary cavity. This primitive mesenchym soon formed sinuoids, larger blood vessels and hemopoietic foci.

It is evident that two varieties of stem cell are essential to the restoration of destroyed marrow: the hemopoietic cells and the cells which reconstruct the microcirculation. In a previous report, we described the pattern of the marrow's reaction to 4,000 r of x-irradiation applied to a small area. Acute aplasia was rapidly repaired by hemopoietic stem cells which came from unirradiated areas. (Four thousand r of total body irradiation destroys all stem cells everywhere. Therefore, when this dose is administered locally, it destroys all stem cells in the local area. Replacements must come from outside.) Three months after irradiation the marrow was once again aplastic, this time because the
Fig. 4.—As in Figure 3 except the animal was killed three months after mechanical disruption of the cavity and injection of normal marrow from his unirradiated leg. This section demonstrates further recovery of the hemopoietic marrow. × 200 H & E.

microcirculation of the marrow had deteriorated. The sinusoidal structure initially retained its integrity but, evidently, the ability to maintain and restore itself was destroyed. As stromal cells died during the ensuing three months they were not replaced. We suspect that the stem cells which normally provide replacements for the stromal cells at the end of their life span are destroyed by 4,000 r. The resulting aplasia is permanent, perhaps because this kind of stem cell does not circulate; therefore it is not replaced by migration from unirradiated areas as are the hemopoietic cells. For the same reason the transfusion of marrow is ineffective (experiments of Group A) because stromal stem cells do not reach the affected marrow. But after such cells had been injected directly into the marrow cavity, both the structure and the function of the marrow were restored (experiments of Group B).

In these experiments, it seemed advisable to remove or disrupt the aplastic marrow before injecting the normal marrow into the cavity. In our early experiments it was noted that the stroma of normal marrow is not “aggressive.” When the distal half of the femur was irradiated and the rest was not there was no extension of normal marrow into the adjacent aplastic area, even after a year’s time. Mechanical disruption of aplastic marrow permits the injected normal marrow to be mixed with the disrupted elements and “seeded” into all areas of the marrow cavity.

These experiments demonstrate that aplastic disease of the marrow can
exist on the basis of a lesion of the marrow's microcirculation and that the aplasia can be corrected by introducing into the marrow cavity tissue which is capable of proliferating to restore the unique sinusoidal structure of hematopoietic bone marrow.

**Summary**

Marrow cavities permanently aplastic after irradiation were mechanically disrupted and locally injected with bone marrow obtained from an opposite leg. Hemopoietic bone marrow regenerated with restoration of normal parenchymal and sinusoidal architecture. Mechanical disruption alone or intravenous transfusions of isogeneic bone marrow alone failed to induce any evidence of regeneration of the aplastic marrow.

**SUMMARIO IN INTERLINGUA**

Cavitates medullari rendite permanentemente aplastic per irradiation esseva disrumpite mechanicamente e replenate per injectiones local de medulla ossee obtenite ab le gamba contralateral del mesme animal. Medulla ossee a potentia hematopoietic se regenerava, e un normal architectura parenchymal e sinusoidal eseva restaurate. Disruption mechanic sol e transfusion intravenose sol de isogenee medulla ossee non induceva ulle evidentia de un regeration del medulla aplastic.

**REFERENCES**

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