Influence of Time of X-Irradiation on the Regenerative Process after Localized Bone Marrow Depletion[1] (34532)

HARVEY M. PATT AND MARY A. MALONEY

Laboratory of Radiobiology, University of California Medical Center, San Francisco, California 94122

In previous studies (1, 2), we examined the radiosensitivity of the elements responsible for initiation of bone marrow restoration in a mechanically depleted femur shaft. It was found that X-irradiation of a femur with a few hundred R just prior to its depopulation was sufficient to impair the regenerative process. In contrast, similar irradiation of the entire body with exception of the femur was ineffectual. Regeneration of marrow in the evacuated shaft is associated with a series of events which lead first to reconstruction of the microenvironment and then to restoration of hemic cells (3–5). Because of the sequential nature of the process, the intervention of radiation at various times after marrow removal may provide important clues about the underlying mechanism. The studies reported here suggest that a given dose of X-irradiation is more effective at the time of marrow depletion than after onset of hemic cell repopulation.

Materials and Methods. The experiments were performed with New Zealand white female rabbits, age 10 weeks, and weighing 2–2.5 kg. The procedure for bone marrow removal was identical to that described earlier (5). Turkel needles were inserted into the proximal and distal ends of the right femur diaphysis under pentobarbital anesthesia, and the cavity was aspirated and simultaneously perfused, first with a dextran solution and then with physiological saline. All radiation exposures were confined to the right femur, the remainder of the body being protected by lead shielding. The X-ray source was a G.E. Maxitron operated at 300 kVp, 20 mA, HVL 1.65 Cu, TSD 30 cm, and dose rate 360 R/min. The radiation exposure was 1000 R as determined by placement of lithium fluoride dosimeters in the femur shaft. Femur irradiation occurred either just before or after depopulation, (*i.e.*, within 0.5 hr) or 9 days later. In some experiments, the femur was X-irradiated without subsequent marrow extrusion.

Two criteria were employed for evaluation of the status of marrow regeneration: (1) the incorporation of tritiated thymidine (^{3}HTdR) into DNA and (2) the number of hemic cells per unit weight of marrow. Tritiated thymidine uptake, which was based on all nucleated cells, signaled the onset of regeneration before obvious hemic cell repopulation. Hemic cells included nucleated erythroid elements and myeloblasts through metamyelocytes. These parameters were related in each case to similar indices in the normal contralateral femur. Rabbits were injected with 0.5 μCi/g of ^{3}HTdR (sp act 11 Ci/mmole) 1 hr before sacrifice. Right (experimental) and left (undisturbed) femora were removed and the marrow from each was divided into two pieces, weighed, and dispersed in 0.9% saline. An aliquot of the marrow suspension was used for enumeration of nucleated cells with a Coulter-Counter, Model A, and for differential counts of smears treated with Wright's stain. The remainder of the suspension was used for DNA extraction and measurement of its tritium activity in a Packard scintillation spectrometer. Details of the methods are given elsewhere (5).

Rabbits in which the right femoral marrow was mechanically depleted were sacrificed either 21 or 30 days later. The corresponding times after irradiation differed depending on whether the X-ray exposure occurred imme-

[1] Work performed under the auspices of the U. S. Atomic Energy Commission.

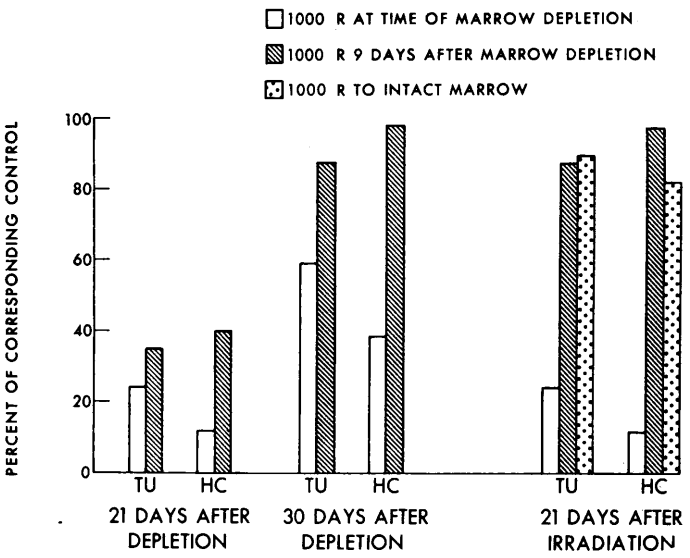

FIG. 1. Effect of local X-irradiation before and after onset of femoral marrow regeneration. (TU refers to ^{3}HTdR uptake and HC to hemic cells.)

diately before or after marrow extrusion or after a 9-day interval. Thus, in the latter instance, the times of sacrifice corresponded to 12 and 21 days after X-irradiation. In those cases in which the right femur was irradiated without subsequent marrow extrusion, the sampling times were 1, 12, and 21 days after the exposure. Since the possible influence of time of X-irradiation on the regenerative process involves temporal variables which pertain to both marrow removal and irradiation, the results were compared at similar times after marrow removal as well as at similar times after irradiation.

TABLE I. Thymidine Uptake and Hemic Cell Repopulation after Femoral Marrow Ablation and/or Irradiation.

Group[a]	N[a]	Days after depletion	X-ray	^{3}HTdR uptake[b]	Hemic cells[b]
I	2	—	1	7	40
I	2	—	12	20	5
I	4	—	21	89	82
D	2	21	—	75	41
D-I_0	5	21	21	18	5
D-I_9	6	21	12	26	16
D	2	30	—	114	74
D-I_0	3	30	30	67	28
D-I_9	2	30	21	99	72

[a] I (irradiation), I_0 (irradiation at time of depopulation), I_9 (irradiation 9 days after depopulation), D (depopulation), N (number of rabbits).

[b] Expressed as percentage of contralateral femur.

Results and Discussion. The data are summarized in Table I, and the magnitude of the several radiation responses is shown in the histogram in Fig. 1. Marrow activity is severely depressed during the first 2 weeks after local X-irradiation with 1000 R. Recovery, however, occurs rather promptly thereafter as indicated by the nearly normal uptake of ^{3}HTdR and the number of hemic cells at 21 days. The picture in the unirradiated mechanically depopulated femur is similar to that described previously (5) in that recovery is well underway at 21 days. Recovery of proliferative activity as revealed by thymidine uptake precedes the increase in number of hemic cells which is to be expected, since thymidine uptake reflects the activity of a variety of all types, *e.g.*, fibroblasts and osteoblasts as well as hemic cells.

Regeneration is delayed by irradiation immediately before or after marrow extrusion. The effect of the former, which was observed in earlier work (1, 2), is the same as that of the latter. This finding is of more than passing interest. The tissue in the bone

cavity after marrow removal is essentially a blood coagulum due to breakage of the nutrient vessels. Studies with radioactive microspheres indicate that there is no blood flow to the interior of the cavity 0.5 hr after marrow extrusion or after ligation of vessels which enter the nutrient foramen of the diaphysis.[2] Although blood flow is resumed during the next few days in each instance, the bone cavity must be hypoxic soon after the marrow is removed. If initiation of regeneration were due to residual cells in the bone marrow cavity, irradiation should be less effective immediately after than before marrow removal because of the relative radioresistance of hypoxic cells. The similar effect of irradiation just before and after the depopulation procedure thus provides further evidence for the completeness of marrow removal.

As shown in Table I and Fig. 1, the radiation-induced delay of regeneration is decreased when irradiation takes place 9 days after mechanical ablation of a femur shaft. In contrast to the blood coagulum which occupies the marrow cavity immediately after depopulation, the histologic picture during the second week reveals the reestablishment of a connective tissue matrix and blood vessels interspersed with cancellous bone (Fig. 2). Very few recognizable hemic cells are evident at this stage. It is noteworthy that recovery of the irradiated 9-day regenerating system is about the same as that of normal bone marrow after 1000 R (Fig. 1).

If seeding of circulating hemic stem cells were a major factor in recovery, restoration of a receptive microenvironment during the second week after femur depopulation could account for the similar response of the irradiated 9-day regenerating marrow and the irradiated normal marrow. There is, however, reason to believe from other radiation studies that migration of hematopoietic stem cells via the circulation is not a leading factor in regeneration of a small volume of depleted marrow as described here (1). Further evidence for the local origin of the repopulating cells has also been provided in recent experiments with heterologous marrow transplants; the regenerating mechanically ablated marrow was "host-type" although donor marrow was evident elsewhere.[3] Although seeding of hematopoietic stem cells is known to occur under certain circumstances (6), it is possible that recovery of marrow even in the locally irradiated normal (*i.e.*, undisturbed) femur is due mainly to surviving cells *in situ*. If this were so, we might conclude that the 9-day regenerating marrow closely resembled the intact marrow in its response to 1000 R because the local pool of available hematopoietic stem cells had been restored to essentially normal levels by this time. The similar radiation response could also be fortuitous and attributable to the interaction of possible differences in cell radiosensitivity and cell population dynamics in the normal and regenerating marrow.

The basis for the greater effect of 1000 R at the time of marrow removal than at 9 days after the ablation is also a matter of conjecture. Normal marrow tissue may contain several types of stem cells, *i.e.*, stromal, hematopoietic (7), but it is not known whether the regeneration after complete marrow removal emanates from one or more than one type of progenitor cell. There are two obvious alternatives: (1) one cell type that can be transformed directly or through a branching differentiation into cells responsible for matrix formation and hematopoiesis; (2) two or more cell types already committed to a given stem line. The greater response with irradiation before regeneration is underway need not signify a differential radiosensitivity at the cellular level. It may merely reflect an additivity of sequential effects on impairment of matrix formation and hematopoiesis. Alternatively, the difference may be due simply to the larger number of progenitor or stem-type cells in the 9-day regenerating marrow.

Summary. Studies were made of the effect of X-irradiation with 1000 R on marrow restoration after localized depletion of the rabbit femur. The femur was X-irradiated immediately before or after depopulation by

[2] Maloney, M. A., Forsyth, R., and Patt, H. M. Unpublished observations.

[3] Fong, P., Maloney, M. A., and Patt, H. M. Unpublished observations.

dextran perfusion or 9 days later. In some experiments, the femur was X-irradiated without subsequent marrow extrusion. Regeneration was evaluated by determining the uptake of tritiated thymidine into DNA and the number of hemic cells in the depopulated and/or irradiated femoral marrow relative to the contralateral marrow. Impairment, *i.e.*, delay of regeneration, was similar whether the femur was irradiated just before or after marrow removal. However, the radiation effect at this time was greater than that when

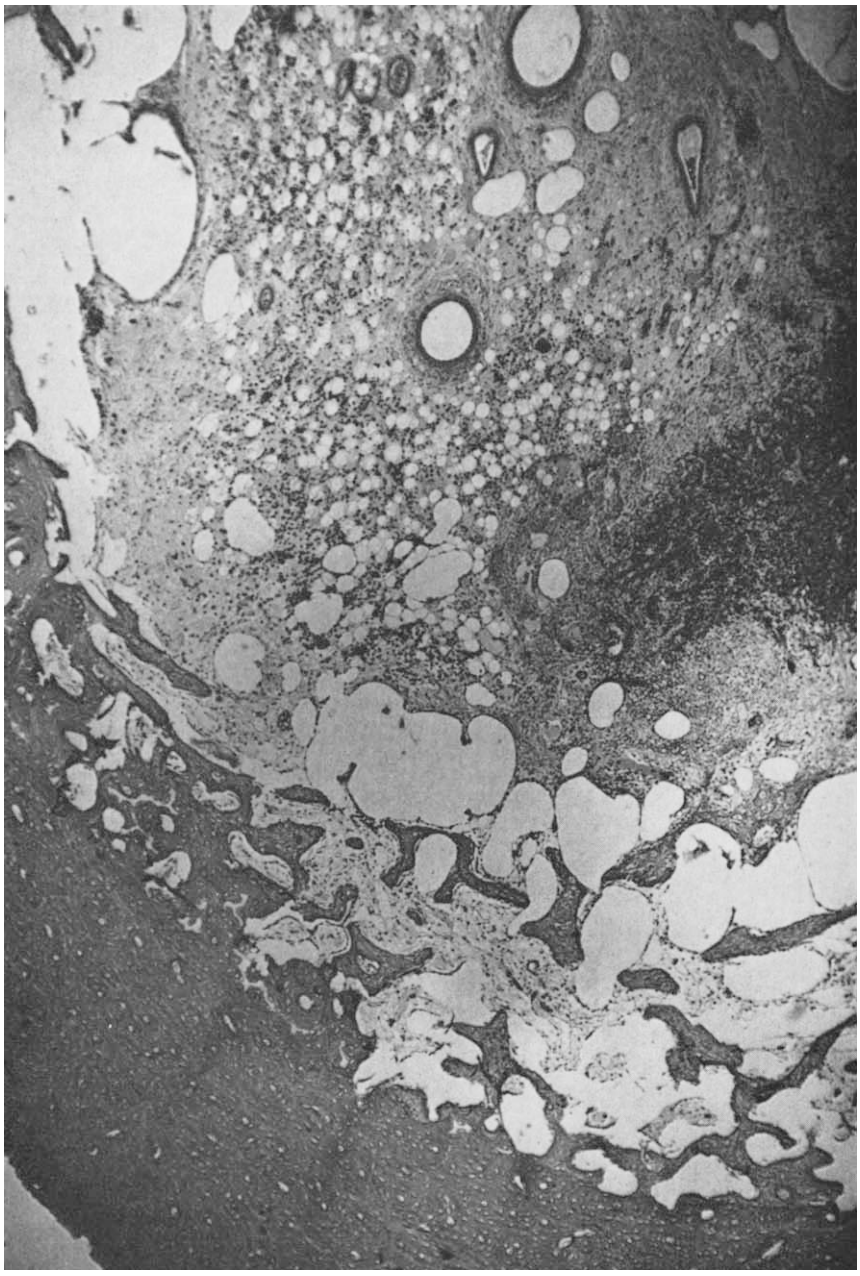

FIG. 2. Development of stroma, vasculature, and cancellous bone 8 days after marrow extrusion; ×50.

regeneration was in progress. The response of the 9-day regenerating system resembled closely that of normal marrow. Reconstitution of the microenvironment precedes hemic cell repopulation, and it is noteworthy that a connective tissue matrix and vasculature have already been formed by 9 days after marrow removal. The difference in degree of effect with irradiation before and after regeneration has been initiated can be explained in several ways. It does not necessarily indicate a differential radiosensitivity at the cellular level.

The authors gratefully acknowledge the excellent technical assistance of Miss Marilyn Haley.

1. Maloney, M. A. and Patt, H. M., Science **165**, 71 (1969).

2. Patt, H. M. and Maloney, M. A., Radiation Res., in press.

3. Röhlich, K., Z. Mikrosk.-Anat. Forsch. **49**, 425 (1941).

4. Steinberg, B. and Hufford, V., Arch. Pathol. **43**, 117 (1947).

5. Maloney, M. A. and Patt, H. M., Cell Tissue Kinet. **2**, 29 (1969).

6. Ford, C. I., Micklen, H. S., Evans, E. P., Gray, J. G., and Ogden, D. A., Ann. N. Y. Acad. Sci. **129**, 283 (1966).

7. Knospe, W. H., Blom, J., and Crosby, W. H., Blood **31**, 400 (1968).

Received Sept. 9, 1969. P.S.E.B.M., 1970, Vol. 133.