Regeneration of Locally Irradiated Bone Marrow
I. Dose Dependent, Long-Term Changes in the Rat, with Particular Emphasis upon Vascular and Stromal Reaction

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This investigation was begun to learn what factors contribute to the recovery, or the failure to recover, of bone marrow following localized injury by x-irradiation in amounts which would be supralethal if administered to the whole body. It describes the effects of 2000 to 10,000 rads upon segments of rat marrow observed from 24 hours to 1 year following irradiation. An estimate is presented of the regenerative capacity of locally irradiated bone marrow and its dependence upon regeneration of marrow sinusoids.

Methods

Eighty-three pathogen-free, female Wistar rats bred at Walter Reed were utilized in this study.* The radiation source was a C. E. maxitron supervoltage therapy unit. The quality of the x-ray beam was as follows: 300 KEV; M.A. 20; dose rate 229 r/min.; target/skin distance 85 cm.; H.V.L. 1.65 mm. cu. The following doses were administered in a single session; 2000 r, 4000 r, 6000 r and 10,000 r (mid-line air dose). Dosimetry was performed with Victoreen Ionization Chambers and total dose administered was checked by an ionization integrator device. The animals were placed under a 3 mm. lead shield in such a way that the left hind leg was extended by means of a taut string tied to the foot. The distal one-half of the femur and the entire tibia were included in the radiation field. The unirradiated right leg served as a control. The animals were housed in individual wire-mesh cages and fed on a diet of D & C Rat-Mouse pellets and water ad lib. End points were selected at 24 hours, 48 hours, 72 hours, 4 days, 7 days, 10 days, 2 weeks, 1 month, 2 months, 3 months, 6 months and 1 year. with 2 animals killed at each point. Only animals receiving 2000 rads were studied during the period between 24 hours and 10 days after irradiation. Beginning at 14 days all dose levels were examined at each end point. At the time of death the animals were anesthetized with intraperitoneal Na nembutal, 15 mg./Kg. Both femora and both tibiae were disarticulated and cleaned of adherent soft tissue. The bones were fixed in 10 per cent formalin and subsequently processed for paraffin embedding, histologic sectioning at 6-8 μ, and staining with hematoxylin and eosin. The bones

*The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.
LOCALLY IRRADIATED BONE MARROW

Fig. 1.—2000 rads, 4 days, femur (140×). Fat is increased and hemopoiesis is severely depressed. Sinusoids (arrows) are dilated and often disrupted with extravasation of erythrocytes into parenchymal areas.

were sectioned in toto along the longitudinal axis to preserve as completely as possible the intramedullary architectural relations of marrow and vascular structures with the surrounding bone.

**Results**

Marrow cellularity refers to clearly recognized hemopoietic elements of the erythrocytic, granulocytic and megakaryocytic series.

**2000 Rads**

*Twenty-four Hours.* Slight dilatation of sinusoids, some decrease in cellularity, increased numbers of pigment-laden macrophages and slight extravasation of erythrocytes were seen.

*Forty-eight Hours.* There was maximum dilatation of sinusoids with further extravasation of erythrocytes and further decline in cellularity. Pigment laden macrophages were markedly diminished compared with 24 hours.

*Four Days (Fig. 1).* The sinusoidal pattern was well-defined and contracted. This suggested that some repair with reconstitution of sinusoidal integrity had
occurred. There was complete absence of hemopoietic elements. Pigment laden macrophages were absent.

Seven Days. Hemopoietic activity of erythrocytic, granulocytic and megakaryocytic elements was generally evident.

Ten Days. The activity was the same as at 7 days, but more intense.

Fourteen Days (Figs. 2 and 3). Marrow cellularity was further increased. Most of the erythrocytes were contained within sinusoidal areas, although some erythrocytes were seen outside sinusoids. Sinusoids were reduced in number as compared to controls. The endosteal layer was widened and a thin layer of collagen was present adjacent to it.

One Month. Marrow cellularity and sinusoids were slightly reduced as compared to 14 days. Endosteal fibrosis was slightly increased. Pigment laden macrophages were again prominent.

Two Months. A marked decrease in hemopoietic cellularity was seen. The number of sinusoids was markedly reduced. Sinusoids were disrupted in many areas and erythrocytes appeared to be flowing freely through parenchymal areas. Endosteal fibrosis was somewhat greater than the previous month. In-
Increased reticulin deposition was evident throughout the medullary cavity. Macrophages were still present.

Three Months (Figs. 4 and 5). There was almost complete absence of hemopoietic elements. Sinusoidal structures were not present. Many reticulum cells were seen scattered throughout the marrow cavities and appeared to be larger than usual. Transitional forms, present in areas of reticulum cell proliferation, varied in structure from spindle-shaped forms resembling fibroblasts to thin-walled endothelial cells or fat cells, and were frequently seen “aligned” along walls of fat spaces. Endosteal fibrosis was maximal at this point.

Six Months (Fig. 6). Diffuse and scattered islands of hemopoietic regeneration were present. Reticulum cell activity was still increased throughout the entire marrow. Scattered regeneration of sinusoid-like structures was seen. Erythrocytes were confined to well-defined, thin-walled channels at sites where fat spaces were directly adjacent. Reticulin and collagen deposition were either unchanged or somewhat reduced.
4000 Rads

One Year (Figs. 7 and 8). Diffuse but sparse hemopoietic proliferation was noted throughout the marrow, including epiphysis, metaphysis, and diaphysis, and was equal in tibia and femur. There was no enhancement of regenerative activity at the margin between the irradiated and unirradiated areas. Sinusoidal regeneration had progressed beyond that evident at 6 months. Around most areas of hemopoiesis there were numerous sinusoids which contained a rich supply of blood within well-defined channels. Although the walls of sinusoids were not always clearly visible, virtually no pools of free red blood cells were seen within areas of hemopoietic regeneration. The configuration of individual sinusoids, as well as their overall distribution, was irregular as compared to controls. Reticulum cell activity was not increased. Reticulin and collagen deposition was definitely less than at 6 months.

4000 Rads

Fourteen Days and 30 Days. The changes seen at these points were similar to those described at the 2000 rad level. Hemopoietic cellularity and sinusoids
Fig. 5.—2000 rads, 3 months, femur (520 x). Hemopoiesis is absent and erythrocytes are scattered through areas of fat. Sinusoids have disappeared.

were both diminished to a somewhat greater extent than at 2000 rads. Sinusoidal disruption and collagen deposition were both increased somewhat over 2000 rads.

Two Months. Markedly decreased marrow cellularity and reduction of sinusoids were seen.

Three and 6 Months. Complete disappearance of hemopoiesis and loss of sinusoidal structures were noted. Increased reticulum cell activity occurred. No evidence of hemopoietic or sinusoidal regeneration was seen at 6 months.

One Year (Fig. 9). Only rare foci of hemopoietic regeneration were seen and these were restricted to large lacunar-like areas adjacent to epiphyseal plates. Scattered areas of reticulum cell activity were present. Regeneration of sinusoid-like structures could be seen but many of these were surrounded by dense, eosinophilic material.

6000 Rads

Fifteen Days–6 Months. The changes were qualitatively similar to those
described at 2000 and 4000 rad levels. At each point a dose dependent effect was evident with greater decline in marrow cellularity and loss of sinusoids. It should be emphasized, however, that at 14 and 30 days a remarkable degree of cellularity was preserved, considering the very large doses of radiation administered to the involved segments of marrow. No regeneration of hemopoietic elements or sinusoids was seen at 6 months. Collagen formation overshadowed reticulin deposition at 6 months and was scattered throughout the marrow cavities.

One Year (Fig. 10). No foci of hemopoietic regeneration could be seen. Collagen and reticulin deposition was further increased as compared to 6 months. No sinusoidal regeneration occurred. There was an irregular distribution of blood vessels varying in size from large capillaries to arterioles. These thick-walled vessels were present throughout areas of dense fibrosis, reticulin fibers or fat. No evidence of infiltrative repair was seen at the border between irradiated and unirradiated segments of the femur.

Fig. 6.—2000 rads, 6 months, tibia (520×). Diffuse activity of all hemopoietic elements is present but fat remains increased and the marrow remains hypocellular.
Fig. 7.—2000 rads, 1 year, femur (13x). Scattered hemopoietic activity is present throughout most of the marrow although cellularity has not returned to normal. Several areas in the diaphysis and epiphysis remain severely hypoplastic. There is marked disorganization and reduplication of the epiphyseal plate.

10,000 Rads

Fourteen Days–3 Months (Figs. 11 and 12). The changes were similar but more intense and more rapid in their progression at each of the points. It is again emphasized that a remarkable degree of marrow cellularity, as well as sinusoidal architecture, was present at 14 days and 1 month, although only about 50 per cent of that observed at 2000 rads. Endosteal fibrosis occurred at 14 days and was progressively increased at 1, 2 and 3 months. Increased reticulum cell activity appeared at 3 months.

Six Months and 1 Year. No foci of hemopoietic or sinusoidal regeneration occurred, although scattered groups of reticulum cells were seen. Massive fibrosis interspersed with areas of fat and reticulin occurred throughout large areas of the marrow. A scattering of irregularly dispersed capillaries, arterioles and venules was seen (Fig. 13).
Fig. 8.—2000 rads, 1 year, tibia (520×). Diffuse hemopoietic activity is present. Many sinusoids filled with erythrocytes are seen (arrows).

A peculiar and unexplained observation at the 6000 and 10,000 rad levels was the presence of a ridge of bony proliferation into the lumen of the marrow cavity at the line of demarcation between irradiated and shielded portions of the femur (Fig. 14).

Unirradiated Marrow

The marrow of the shielded portion of the femur and of the unirradiated opposite limb was normal at all levels of irradiation studied (Figs. 15 and 16).

Discussion

Previous investigators of radiation effects upon bone marrow of animals as well as man have described severe aplasia of marrow following doses larger than 2000 rads, but they limited their observations to periods of less than 4 months postirradiation.1,16

Our experimental material indicated that early loss of cellularity with sinusoidal dilatation and hemorrhage occurred between 1 and 4 days. These
changes were almost identical to those reported by Bond, Fliedner et al.7,8 in animals given whole body irradiation, except for the much more rapid regeneration of hemopoietic elements and reconstitution of sinusoidal architecture. In locally irradiated marrow, these events were well-established by 7 days, which was about 1 week before similar events in animals that received whole body irradiation. Although the mechanism of regeneration was not demonstrated, the fact that many groups of hemopoietic elements were seen even at very high doses of radiation—10,000 r—pointed toward an influx of cells from remote unirradiated areas. An alternative explanation would be that regeneration occurred from stem cells surviving in situ, which did not seem likely in view of the very large dose used in our experiments. However, following partial regeneration of hemopoietic elements at 1 to 4 weeks, a secondary late decrease in cellularity occurred, paralleled by a concomitant reduction in sinusoidal structure. Progressive disruption of sinusoids was seen at 2 to 3 months with
Fig. 10.—6000 rads, 1 year, femur (13×). Severe hypoplasia is present without evidence of hemopoietic regeneration. Hyaline, eosinophilic material is extensively deposited between fat spaces.

Blood flowing through a mesh of disrupted sinusoids and fat-reticulum structures. Evidence of erythrocyte stasis and breakdown was manifested by an abundance of siderin-laden macrophages.

The maximum reduction in cellularity seen at 2 to 3 months following all doses of irradiation correlated well with the disappearance of the marrow sinusoidal architecture. Whether this reduction in sinusoidal structure was a late expression of primary injury by ionizing radiation or was related to injury of larger components of the vasculature cannot be determined from these studies. When regeneration of hemopoietic elements occurred, and this was largely restricted to the 2000 r level, it was preceded by intense reticulum cell activity which appeared to develop into a uniform regeneration of sinusoidal structures. It was of interest that this regeneration of sinusoids proceeded in close relationship to the reticulum cells lining the fat spaces. Branemark has speculated that the apparent hexagonal arrangement of sinusoids may have its origin in the alignment of reticulum cells along fat spaces. In many areas,
Fig. 11.—10,000 rads, 30 days, femur (13×). The distal irradiated segment is severely hypoplastic. A narrow transition zone merges into the unirradiated marrow (arrow) of the proximal bone.

Before sinusoidal regeneration occurred, red cells appeared to have penetrated the fat spaces and flowed through broad areas of the marrow. These fat spaces might provide primitive channels from which further differentiation into a sinusoidal system could occur.

Casarett has proposed that a significant portion of late radiation injury of parenchymal elements had its origin in primary injury to fine vasculature. With reduction in fine vasculature, perfusion was inadequate to support parenchymal structure. Fibrosis developed in the presence of hypoxia and this led to further reduction in perfusion, hypoxia and loss of parenchyma. Our observations of the changes seen between 2 and 6 months were in accordance with Casarett’s concepts of late radiation injury being dependent upon radiation-induced vascular changes. Also pertinent to this was Maier’s investigation of late vascular changes in dog kidneys exposed to 500, 1000 and 2000 rads. Following 1000 and 2000 rads he observed changes in capillaries and arterioles which led to a significant reduction in numbers of glomeruli and renal function.

Regeneration of hemopoietic elements seems to require the development of
a sinusoidal system approximating that of normal marrow. The regenerated sinusoidal system seen at the 2000 r level at one year had an irregular pattern which lacked the normal arrangement of small and rectal sinusoids draining into a large central collecting vein. However, there was an abundance of very thin-walled channels throughout all parts of the marrow, in intimate relationship to the interstices of the fat spaces which provided an abundant supply of blood on all sides of the foci of hemopoietic activity. It is of interest to look at the circumstances under which hemopoiesis can occur from early embryonic life throughout ontogenesis. Blood cell formation at its earliest stage develops in intimate relationship to primitive vascular channels developing upon the yolk sac. Later, hemopoiesis takes place in organs that have a well-developed sinusoidal system—e.g., bone marrow, spleen and liver. In teleologic terms, it is evident that a rapidly proliferating tissue such as bone marrow would require a sinusoidal system of abundant, thin-walled vessels, permitting a rapid exchange of $O_2$, $CO_2$, metabolites, regulator substances, etc.
If one may infer that regeneration of the marrow sinusoids originates from the reticulum cells, then the radiosensitivity of these cells would be a limiting factor in permanent regeneration of the marrow following local irradiation. Some reticulum cells might be pluripotential stem cells and give rise to sinusoidal cells as well as hemopoietic cells. Bloom reported that reticulum cells (histiocytes) demonstrated functional activity by the removal of cellular debris following local irradiation of lymph nodes and bone marrow up to doses of 3000 rads. This does not necessarily mean that such histiocytes have stem cell activity. Our studies did not indicate whether repopulation of the irradiated marrow came from primitive pluripotential reticulum cells or whether more differentiated hemopoietic stem cells migrated into the areas where the sinusoidal system was restored. The appearance of the material at the 4000 rad level at 1 year indicated many areas with an “active, primitive” reticulum. There were also areas at the 4000 rad level which developed a sinusoidal system almost as extensive as the segments of marrow that received 2000 rad. It differed...
in the presence of an eosinophilic hyaline material surrounding the sinusoids. This material could conceivably have interfered with the required exchange of nutrients, metabolites, etc. The inability to remove material such as this might represent injury to the reticulum cell population itself. Whether a longer period of observation would see the reestablishment not only of a sinusoidal system, but of parenchymal elements as well at 4000 r levels (or even higher levels), must remain conjectural. Our studies indicated that late radiation damage of bone marrow was intimately related to the changes produced in the sinusoidal circulation.

Initial recovery may be speeded by the availability of stem cells from other parts of the body; hence after localized radiation the marrow recovered more promptly than it did when animals received whole-body irradiation. In the later phases after the sinusoids had disappeared and there was failure to recover, the availability of stem cells counted for nothing. One end of the

**Fig. 14.—6000 rads, 1 year, femur (50×).** A narrow flange of bone (arrow) protrudes into the medullary cavity between irradiated and unirradiated marrow. Extensive deposits of hyaline, eosinophilic material are in the irradiated areas.
Locally irradiated bone marrow was uniformly depressed during the first week after radiation doses of 2000 to 10,000 rads. The early appearance of hemopoietic regeneration between 7 and 14 days at all dose levels suggested an influx of stem cells from unirradiated sites. Between 2 and 6 months postirradiation a second wave of hemopoietic depression occurred at all dose levels and was correlated with the disappearance of the sinusoidal microcirculation. Between 6 and 12 months diffuse hemopoietic regeneration occurred only at the 2000 rad level and could be correlated with regeneration of the sinusoidal microcirculation. No significant hemopoiesis or sinusoidal regeneration was observed at the higher dose levels after 6 months or 1 year. Although large vascular channels developed, indicating circulation through the marrow cavity,
Fig. 16.—Unirradiated limb, femur (340×). The cellularity and sinusoids (arrows) are normal.

the characteristic and essential anatomy of the microcirculation was not restored.

**Summario in Interlingua**

Localmente irradiaente medulla ossea esseva uniformemente deprimite durante le prime septimana post doses de radiation de 2000 a 10000 rad. Le precoce apparition del regeneration hematopoietic inter 7 de 14 dies a omne nivellos de dosage suggestionava un influxo de cellulas primordial ab sitos non irradiiae Inter 2 e 6 menses post le irradiation, un secunde unda de depression hematopoietic occurreva a omne nivellos de dosage e esseva correlative con le disparition del microcirculation sinusoidal. Inter 6 e 12 menses, diffuse regeneration hematopoietic occurreva solmente al nivello de 2000 rad e non poteva esser correlative con le regeneration del microcirculation sinusoidal. Nulle grado significative de hematopoiese o de regeneration sinusoidal esseva observate al plus alte nivellos de dosage post 6 menses o mesmo post 1 anno. Ben que grande canales vascular se disveloppava—indicante
le presentia de un circulation a transverso le cavitate medullari—le anatomia characteristic e essential del microcirculation non esseva restaurate.

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Regeneration of Locally Irradiated Bone Marrow: I. Dose Dependent, Long-Term Changes in the Rat, with Particular Emphasis upon Vascular and Stromal Reaction

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