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Structure and Function of Bone Marrow Hemopoiesis: Mechanisms of Response to Ionizing Radiation Exposure*)**)

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ABSTRACT

It is the purpose of this presentation to review the unique structure and function of bone marrow anchored hematopoiesis in their significance for its response mechanisms to an exposure to ionizing radiation. The ultimate objective of bone marrow hematopoiesis is to maintain in the peripheral blood a constant level of the different blood cell types (erythrocytes, granulocytes, platelets, lymphocytes, etc.). All of them have their particular turnover kinetics (such as granulocytes 120×10^9/d, erythrocytes 200×10^9/d or thrombocytes 150×10^9/d), are semi-autonomous in their steady state regulatory mechanisms and dependent on a life-long supply of mature cells from a stem cell pool with unlimited replicative and pluripotent differentiative potential. The present knowledge of hematopoietic cellular renewal is the result of years of basic experimental and clinical studies using radionuclides in various metabolic forms including ^{59}Fe, ^{32}P (DF ^{32}P), ^{51}Cr, ^{131}I, ^{60}Co, ^{3}H ($^{3}HTdR$) and ^{14}C ($^{14}CTdR$). To understand the physiology but in particular the radiation-pathophysiology, it is essential to recognize in detail the infrastructure of the bone marrow as a distinct unit. Indispensable for a life-long cell production is the capsule of the marrow—the bone cortex—, the arterial supply of blood connected to the sinusoidal microvascular architecture with its sinusoids contorti and recti as well as the central (cell collecting) sinusoids. It is further of importance to recognize the significance of nerval regulation of blood flow, characterized by myelinated and unmyelinated nerve fibers. The type of unique lining cells of the sinusoids is the prerequisite for the cell traffic between the hemopoietic parenchyma and the blood. This in turn cannot be achieved without an alternative opening and closing of the sinusoidal segments which—in turn—requires a rigid long capsule to assure an—in toto—constant volume of each bone marrow unit. If a bone marrow unit is exposed to ionizing radiation, a perturbance of the balance between cellular growth pressure and blood flow dynamics can be observed, resulting in a special type of bone marrow hemorrhage and an "excess cell loss" that may result in an non-thrombopenic exhaustion of the stem cell pool. Of great importance is the question as to the mechanisms that allow the bone marrow hemopoiesis to act as one cell renewal system although the bone marrow units are distributed throughout more than 100 bone marrow areas or units in the skeleton. The observation that "the bone marrow" acts and reacts as "one organ" is due to the regulatory mechanisms: the humeral factors (such as erythropoietins, granulopoietins, thrombopoietins etc.), the nerval factors (central nervous regulation) and cellular factors (continuous migration of stem cells

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through the blood to assure a sufficient stem cell pool size in each bone marrow "sub-unit"). It should be recalled that the bone marrow functions as a physiological chimera and becomes established by the hematogeneic seeding of stem cells to a mesenchymal matrix during embryogenesis. The repopulation of the bone marrow after partial body irradiation, after strongly inhomogeneous radiation exposure or after total body exposure with stem cell transplantation can well be considered as a repetition of the embryogenesis of bone marrow hemopoiesis with the key element of stem cells migrating via the blood to stromal sites of the marrow prepared to accept stem cells to home and start their replication and differentiation if the micro-environmental quality permits. In summary, the radiation biology of bone marrow hemopoiesis requires a thorough understanding of the physiology and pathophysiology of structure, function and regulation not only of the process of cellular renewal but also of the intricate infrastructure.

1. PURPOSE AND SCOPE

It is the purpose of this contribution to provide a synopsis of the knowledge available on the structure, function and some regulatory mechanisms that characterize the hematopoietic cell renewal systems. These are rooted—under physiological conditions—in the bone marrow, distributed throughout the skeleton both in human beings as well as in other mammalian organisms. Such a review is appropriate, since most of the classical textbooks in hematology do not address in sufficient detail the unique interplay between bone marrow structure, function and regulation (1, 2), which, however, is essential to understand the responses of hemopoiesis to ionizing radiation exposure and regeneratory mechanisms. A more adequate treatise of the subject was published by Trubowitz and Davis in 1982 (3) and relevant monographs on the bone marrow were published (4, 5).

To achieve such a brief synthetic review, three aspects of bone marrow hemopoiesis are of importance. First of all, the dynamics of hematopoietic cell renewal as well as radiation induced perturbations of the homeostatic equilibrium between cell production and cell removal will be addressed. Secondly, it will be demonstrated that such a lifelong cell renewal is under physiological conditions firmly bound to a characteristic infrastructure which is available only in bone marrow units distributed throughout the skeletal bones. Under pathological conditions, hemopoiesis may "emigrate" from these bone marrow sites and may continue its cell production in other "extramedullary sites" (such as spleen, liver, lymphnodes) but always much less efficient. The underlying mechanisms are very important to understand possibilities and limitations in the diagnosis and treatment of radiation induced perturbations and of hematopoietic neoplasia. Thirdly, it appears essential to examine the question as to how bone marrow hematopoiesis acts and functions as one organ system, although its integral sub-units localized in skeletal parts are distributed throughout the body. This "organ system unity" can only be understood by considering regulatory mechanisms. In particular, it is important to consider the functional significances of the hemopoietic stem- and progenitor cells migrating continuously through the peripheral blood. They may well be considered to assure—at all times—a constant stem- and progenitor cell concentration in the bone marrow units in which lifelong cell production occurs.

This paper will also provide the reader with a selection of pertinent literature containing experimental or circumstantial evidence for the conclusions to be drawn.

2. DYNAMICS OF HEMATOPOIETIC CELL RENEWAL AND RADIATION INDUCED PERTURBATIONS

In order to appreciate the functionality of hematopoietic homeostasis, it is of importance to analyze the turnover kinetics of the various blood cell types under normal conditions. Each cell type in the blood is characterized by a life-expectancy given in Figure 1. It appears to be a characteristic property of hemopoietic cell renewal to achieve—whenever feasible and possible—a constant number of the different blood elements per unit volume. Under physiological conditions, the rate of cells entering the blood (K_{in}) equals the rate of leaving the peripheral blood (K_{out}). Thus, the turnover per day varies considerably between the major cell types, but is in the order of several to many billions per day. If one considers the annual cumulative cell production, one can calculate a total blood cell production rate of about 490×10^9 cells per day or about 88 kg per year (6). The fact that a human being can live a

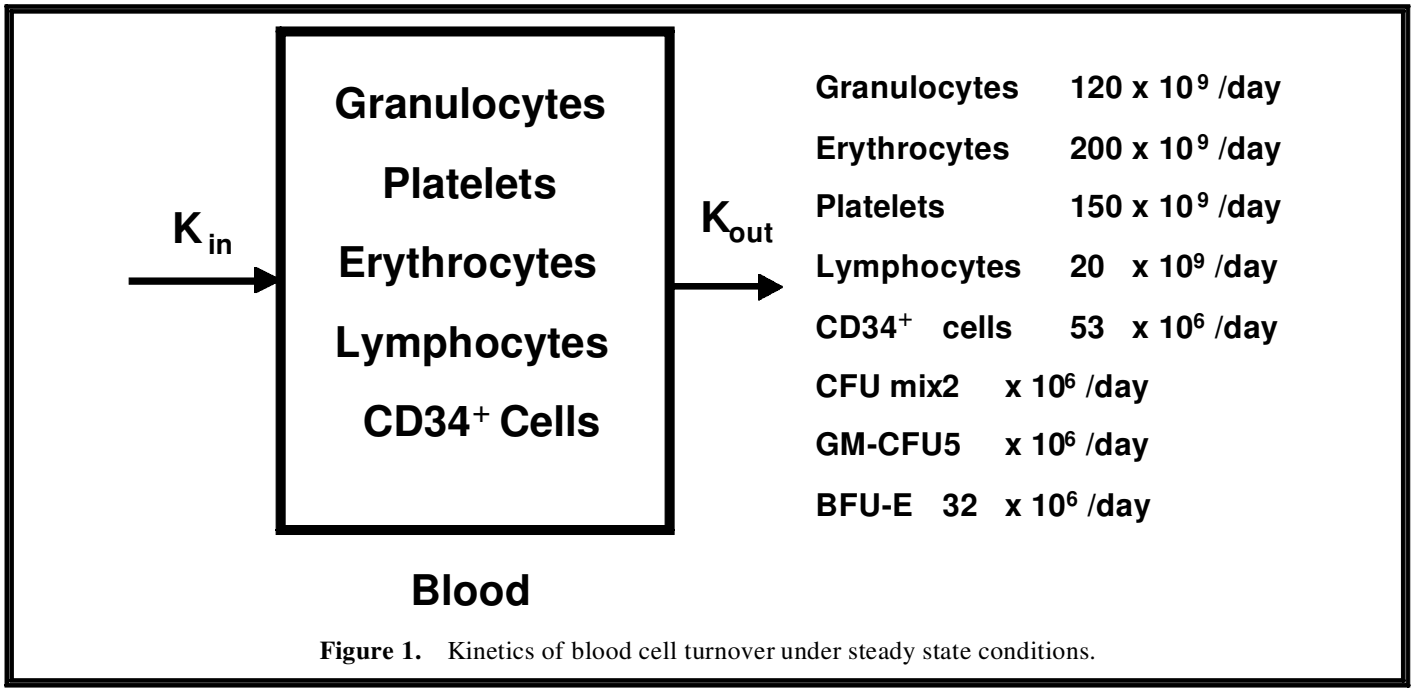

Figure 1. Kinetics of blood cell turnover under steady state conditions.

normal life and is not "drowned" within his own cell waste is due to the fact that for each cell produced in the hemopoietic system, one cell is lost due to senescence or emigration in order to maintain a homeostatic equilibrium between production and removal.

It should be mentioned that the "constancy" of blood cell concentrations in the blood from day to day is a relative one. All cell lines appear to have regular oscillations as determined in appropriate experimental setups (reviewed by King-Smith and Morley (7) and analyzed for pathological circumstances, for instance cyclic neutropenia, elsewhere (8, 9)).

In Figure 1 are also turnover rates for hemopoietic stem- and progenitor cells (1) measured as $CD34^+$ cells, as CFU mix, GM-CFU and BFU-E. These rates indicate a fairly extensive turnover. These data are relatively new: it is only since a few years that these "early" cells of the hemopoietic system are to be considered regular elements of the "blood leukocytes". They belong to cells commonly identified as "mononuclear cells". Their functional properties can only be established using appropriate cell culture or surface marker technologies (10, 11, 12). However, their regular presence in the blood and their rapid turnover is indicative of their apparent major role in allowing the bone marrow system to act and react as one singular organ as will be discussed later.

What is the source of knowledge about turnover kinetics of the hematopoietic cell renewal systems? It appears legitimate to stress the contribution of nuclear medicine and the utilization of radioactive tracer methods developed during the 1950s and 1960s. It was L.G. Lajtha—himself a pioneer in this field—who published in 1961 the first monograph entitled "The Use of Isotopes in Hematology" (13). At that time, the major radionuclides available and used were ^{32}P, ^{59}Fe, ^{51}Cr, ^{131}I, ^{60}Co, ^{3}H and ^{35}S. During this early period and in the subsequent years, the life span of blood cells was established as indicated in Table 1 (for details see (1) or (2)). As one can see, the neutrophilic granulocyte pool in the blood is renewed once every day, while the platelet pool is renewed once during 10 days, the erythrocytes once every 113 to 118 days. The cells subsumized under the term "lymphocytes"

Table 1. Life-Spans of Blood Cells as Determined by Radionuclide Labeling Techniques[1-4]

Erythrocytes	^{51}Cr	113 (108–120) days
	^{15}N-glycine	118 (109–127) days
	$DF^{32}P$	124 days
Granulocytes	$DF^{32}P$	t1/2 = 7 hrs
	$^{3}HTdR$	24–30 hrs
Platelets	^{51}Cr	9–12 days
	$DF^{32}P$	9–12 days
Lymphocytes	^{32}P, $^{3}HTdR$	4.4 years (average)

are considered to have an average life span of 4.4 years. One has to remember though that the cell types encompassed by this term are very heterogeneous such as the different classes identified in recent years by surface marker techniques (10).

These renewal characteristics of peripheral blood cells are the result of the actions of specific cell renewal systems, the nature and genealogy of which has been discussed on purely morphological ground in the early 20th century by the eminent hematologist in Europe as well as in the USA and summarized in a "historical text" by Maximow in 1927 (14). In the 1950s and 1960s, the utilization of radioactive compounds such as tritiated thymidine by the group of Cronkite at the Brookhaven National Laboratory (15) and of $DF^{32}P$ by the group of Wintrobe and Cartwright in Salt Lake City (16) paved the way for establishing compartment models of hemopoietic cell renewal system first summarized in 1959 in the monograph by F. Stohlman (17). A major contribution regarding cell formation kinetics came from kinematographic studies performed by I. Boll (18).

The major elements of hemopoietic cell renewal systems is given in Figure 2. Cells in the functional (blood) pool have been discussed above in their renewal characteristics. They are lost from the blood by senescence (e.g., erythrocytes, but in part also granulocytes) or by utilization (e.g., platelets) or by emigration (granulocytes) or in part by recirculation (lymphocytes). At the rate of cell loss from the functional pool, cells are entering the blood from a maturation/storage pool, which is relatively large in the case of granulocytes (19), small (if existent at all) in platelets (20) and apparently non-existent in erythrocytes, which are released into the blood after the nucleus at the stage of the orthochromatic normoblast has been expelled. The maturation compartment is fed continuously by cells from the proliferation and differentiation compartment. In this compartment, cells entering a specific cell line (erythrocytic, granulocytic, megakaryocytic etc.) multiply either by duplication of cells with a concomitant maturation process (granulocytopoiesis: myeloblast (M1), promyelocyte (M2), large and small myelocyte (M3, M4); erythropoiesis: proerythroblast (E1), macroblast (E2), basophic (E3), polychromatic (E4) and/or orthochromatic normoblasts (E5)) or by endoreplication (megakaryocytopoiesis: megakaryocytes with 2–32 nuclei). These dividing-maturing cells (in the bone marrow) are continuously fed by stem- and progenitor cell pools. The composition and mode of function of these regenerative cell pools is still under discussion. The existent knowledge was first derived from morphological observations (starting with A. Maximow in the 1920s), via intravital, cytochemical and electromicroscopical studies (summarized by M. Bessis (21)), cell transplantation and cell culture tech-

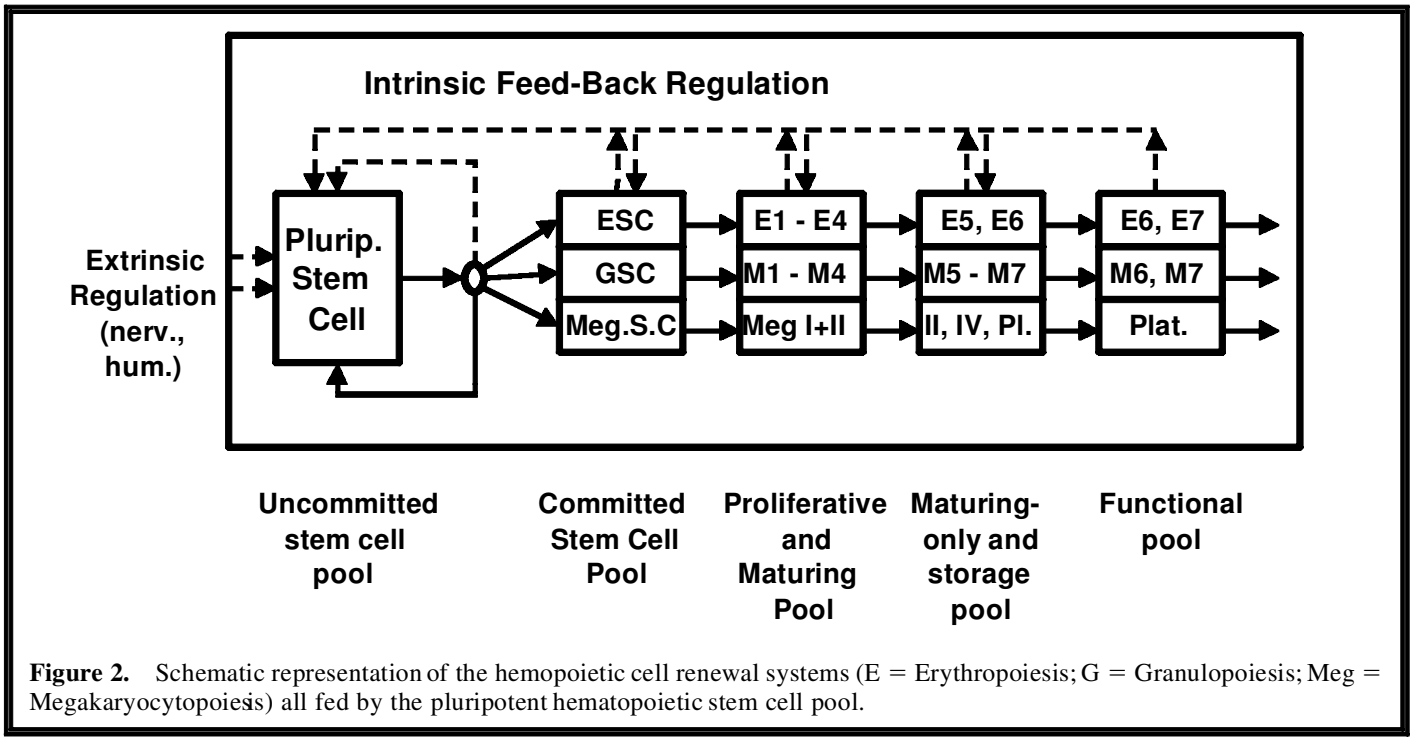

Figure 2. Schematic representation of the hemopoietic cell renewal systems (E = Erythropoiesis; G = Granulopoiesis; Meg = Megakaryocytopoiesis) all fed by the pluripotent hematopoietic stem cell pool.

niques (pioneered by Till and McCulloch (22)), and by Metcalf et al. (23). A new era of research was opened by cell surface marker technology and by the evidence that $CD34^+$ cell suspensions contained—in man—those cells capable of restoring the entire hematopoietic system, if myeloablated with appropriate methodology (for instance, whole body radiation exposure (24, 25, 26, 27)). The pool of stem- and progenitor cells is very inhomogeneous. On balance, if one of the cells divides in the pluripotent stem cell pool and leaves the compartment triggered into differentiation along a specific cellular lineage, the other cell remains in the pool in order to maintain its size. All in all, "stem cells" are still best characterized by Metcalf and Moore (23) " . . . stem cells are defined as primitive haemopoietic cells capable of extensive self-replication and endowed with a multiple differentiating capacity. A second class of ancestral cells is also recognized which is restricted in differentiating capacity to one or two lines of differentiation, has limited proliferative capacity and is sensitive to specific regulatory factors, which are not thought to act on stem cells".

It is also of importance to remind to the fact that stem cell endowed with unrestricted replicative and proliferative potentials are to a large extent at a "cytokinetic rest". They are in a G_0 state >90% (28) and can be called into replication and proliferation if the need arises, such as after a radiation induced cell loss in part of the stem cell pool (for instance, after partial or inhomogeneous irradiation).

The time to "rebuild" the granulocytic cell renewal system after hemopoietic stem cell transplantation is in the order of 10–12 days. It takes about 4 days for the granulocytes to mature after the last myelocyte division and to be released into the blood (19). It takes about 6 days for cells from the myeloblast to the myelocyte stage (19). Therefore, the replication of stem cells must be relatively rapid to produce enough cells to be triggered into the cellular proliferation and maturation process (29). The transit time for erythropoietic cells from the earliest cell type (proerythroblast) to the release of red cells into the blood is about 5–7 days (30, 31), and the equivalent time to produce blood platelets is about 10 days in the human being (4, 32).

How do the hemopoietic cell renewal systems respond to an exposure to ionizing radiation?

This topic has been discussed extensively in a number of monographs (33, 34). However, for the purpose of this presentation it is useful and sufficient to distinguish the responses to a homogeneous acute penetrating radiation exposure from a chronic radiation exposure at different dose rate levels.

Of particular importance for both types of radiation exposure (acute, chronic), it is essential to note that non-dividing maturing-only cells are relatively radioresistant. Thus, it is known that mature granulocytes, red cells or platelets would tolerate high irradiation doses, the D_0 being more than 50 Gy (33, 34). On the other hand, stem- and progenitor cells are very radiosensitive. Their D_0 was determined experimentally in vivo as well as by in vitro techniques to be between 0.6 and 1.6 Gy (22). Due to the exponential nature of the cell survival curves, this means that after an acute exposure of about 10 Gy only 1–3 out of 1,000 stem cells would remain intact from which a regeneration of the system could start. However, this exponential nature of the cell survival curve of stem cells also means that after several tenths of Gy total body acute radiation exposure most, but not all, stem cells are destroyed and therefore a late autologous recovery can be observed under appropriate experimental conditions.

As far as the dividing maturing pool of cells in the erythropoietic and granulopoietic as well as lymphopoietic series is concerned, the cells will partly be affected depending on their radiosensitivity during their cell cycle. It is of interest to note that the result of this type of early response to acute whole body radiation exposure can be found in bone marrow and blood as radiation induced abnormal cells (mitotically connected abnormalities) with binucleated cells, cells with karyomeres or giant cells due to nuclear but not cytoplasmic division (35). Thus, a very careful early examination of bone marrow and blood after suspected or proven acute radiation exposure is very useful to assess the extent of injury to the system. Of major clinical importance are two response types of the hemopoietic cell renewal systems. In Figure 3, the typical cell response pattern of blood granulocytes and thrombocytes are given for the case of an "irreversible" and a "reversible" hemopoietic failure. H4*) stands for the

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)** ***Grading code developed for assisting the physician to characterize the severity of damage to hematopoiesis (H) with severity levels of 4 (irreversible damage), 3 (reversible, but supportive therapy required), 2 (reversible, some therapy needed), 1 (reversible, no specific therapy required) (for detailed explanations see reference 36)

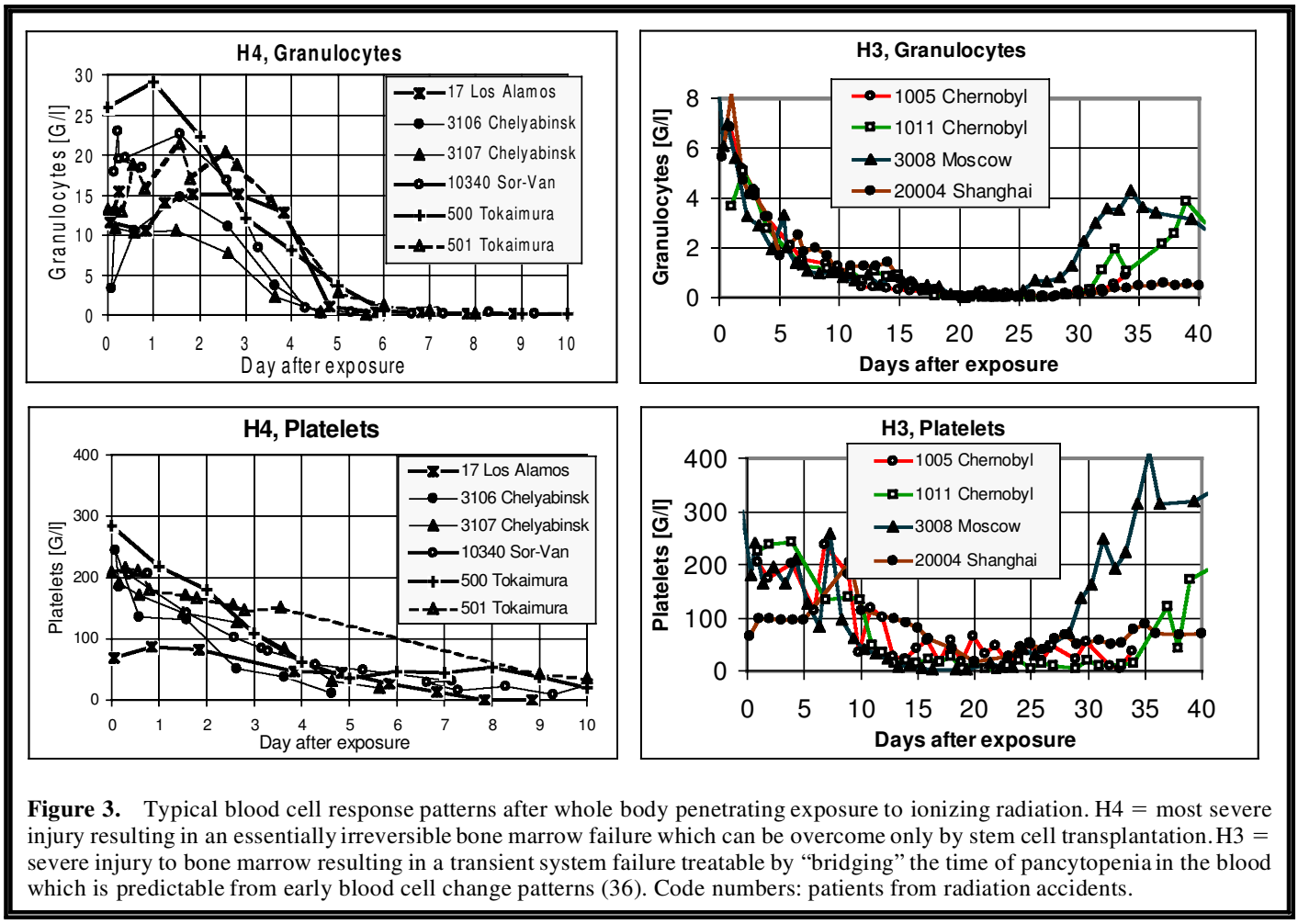


Figure 3. Typical blood cell response patterns after whole body penetrating exposure to ionizing radiation. H4 = most severe injury resulting in an essentially irreversible bone marrow failure which can be overcome only by stem cell transplantation. H3 = severe injury to bone marrow resulting in a transient system failure treatable by "bridging" the time of pancytopenia in the blood which is predictable from early blood cell change patterns (36). Code numbers: patients from radiation accidents.

pattern that can be observed after an "essentially irreversible" damage to the hemopoietic system. This pattern is characterized by an early granulocytosis during the first 1–3 days after exposure and a subsequent rapid decline with cell counts approaching zero between days 5 and 6.

As far as platelets is concerned, the response that is typical for an "essentially irreversible" damage to the hemopoietic system (H4) is that the platelet number decreases progressively and rapidly to critical low levels below 50,000 per mm^3 within 10–12 days.

This pattern is characteristic for the fact that under the circumstances of a myeloablative radiation dose the pools of proliferating and differentiating cells as well as the stem cell pool is essentially eradicated. The blood cell degenerative pattern reflects the fact that granulocytes and the maturing-only pool are initially not affected by these dose levels and mature out to replace for a period of about 4 days the blood pool of granulocytes. Once the transit time of the maturing-only pool is elapsed, no further cells appear in the maturation-only pool, because of the destruction of the proliferating/differentiating and stem cell pools. As far as the platelets is concerned, the pool of megakaryocytes is apparently damaged together with the stem cell pool in such a way that the platelets that are still available are released into the blood but are not replaced by newly formed megakaryocytes and platelets and therefore they disappear from the blood within 10 days (which is the maximum life-span of a platelet).

Typical for such an irreversible damage to hemopoiesis is also the sharp decline of lymphocytes to very low levels (less than 100 per mm^3) essentially within 24 hours. This is due to a direct effect on lymphocytes capable of further proliferation and differentiation and on their migratory function that allows them to recirculate between the blood and the lymphatic tissue (37, 38).

Such an essentially irreversible damage to the hemopoietic system denoted with the grading H4 can only be overcome if a transfusion of autolo-

gous or appropriately selected allogeneic stem cells are given (39). If this can be achieved, then one would expect a first sign of regeneration of granulocytes and platelets after 10–12 days at best. This time is needed for replication of cells in the stem cell pool, their recruitment into differentiation and proliferation and their maturation into blood going mature cells.

In contrast, the response pattern to acute ionizing radiation characteristic of a potentially autologous recovery, are given in Figure 2 under the term "H3". One can now recognize a different blood cell response pattern. There is a mild granulocytosis within the first 3–4 days, thereafter a decline which is, however, stopped by an "abortive recovery" around day 10, followed by a nadir around 25–30 days and a subsequent recovery of the cell counts. As far as the platelets is concerned, the pattern "H3" is characterized by a slow decline of platelets (a wide shoulder of 10–15 days), followed by a nadir between 25 and 30 days. This "shoulder" in platelets is functionally speaking equivalent to the abortive rise of granulocytes. It means that somewhere in the bone marrow in both systems there are some cells at the stem cell level remaining that are injured but still capable of performing a number of multiplicative divisions (33). It has been assumed by the "injured stem cell hypothesis" (33) that such injured stem cells can still undergo a number of divisions before the clone dies out. This is assumed to be the mechanism for the appearance of an "abortive rise" of granulocytes and a delay of decline of platelets with a nadir between 20 and 30 days. The more injured stem cells are remaining in the marrow, the broader would be the "abortive rise phase" both in granulocytes as well as in platelets. Nevertheless, it can be assumed that the final recovery in the "H3"-patients beyond day 30 and 35 is due to the recovery of essentially intact or totally repaired stem cells.

These findings are compatible with the following assumptions: After acute whole body radiation exposure the effects on blood cells are largely determined by the degree of injury to the stem cell pool. An "irreversible damage" of the stem cell pool can be assumed, if it is reduced to less than 1% of normal (40). The granulocytes, under these circumstances, show an initial granulocytosis and a disappearance from the blood stream between days 4–6. The platelets disappear from the blood within 8–10 days.

A "reversible damage" (spontaneous recovery possible) of the stem cell pool may occur if more than 5% stem- and progenitor cells remain sufficiently intact for replication and differentiation (40). Under these conditions, granulocytes show an initial granulocytosis, a decline towards days 8–10, an abortive rise between days 10 and 20 and a nadir around days 20–30 after irradiation. The platelets decline towards low values 15–25 days after a "shoulder" of 8–12 days.

These response patterns of hemopoiesis to a single acute whole body radiation exposure are entirely different from those that one would expect after chronic radiation exposure.

The most extensive experimental studies on chronic radiation exposure effects have been performed by the group of Tom Fritz and Tom Seed and associates at the Argonne National Laboratory between about 1970 and 1990 (41, 42). They have irradiated dogs for their life span. The exposure doses were between 0.3 and 54 cSv per day. In Figure 4 the blood cell counts are selected for 5 dogs out of the exposure group of 75 mSv per day. It is of interest to note that some dogs die after about 250 days. At that time, the total dose absorbed by the dog, was in the order of 1,800 cSv which, if given as an acute dose, would have killed the dogs within 10 days. Some other dogs died only after about 700 days. By that time, they had absorbed a total dose of 5,250 cSv. If that dose was given in one acute exposure, the dogs would have died within hours. The cause of death in these chronically radiation exposed dogs was always a hematopoietic failure. It can be seen (Figure 4) that—during the irradiation period—the granulocytes remain at satisfactory blood cell concentrations until they decrease progressively—as do the platelets—during days/weeks before death from hematopoietic failure.

These principally important experimental findings have been discussed in several publications and can be summarized here as follows: after experimental chronic whole body radiation exposure, such as studied systematically in the Argonne National Laboratory experiments in dogs, a response pattern of the stem cell pools appears to be of crucial importance (42, 43). At dose rates of 30–40 mSv per day, the hemopoietic cell renewal systems are able to cope with the increased cell losses from the stem- and proliferation compartments by increasing cell replication and proliferation. Under these circumstances the system is able to survive for years having absorbed more than 1,000 cSv of total body irradiation in some cases. At dose rates above 30–40 mSv per day, the hemopoietic system will fail sooner or later.

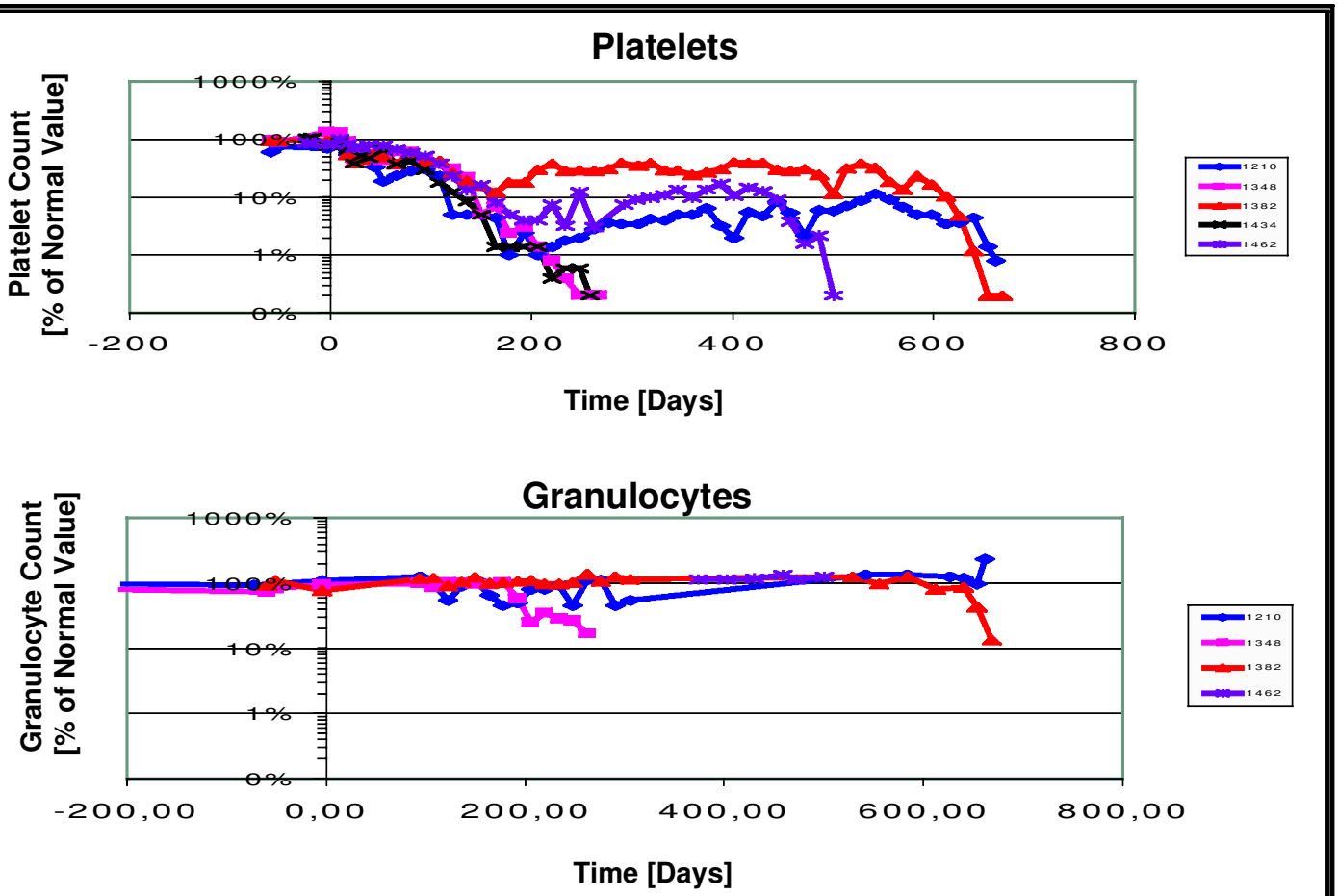


Figure 4. Blood cell responses in beagles exposed with 75 mSv/Day for lifetime to gamma-irradiation at the Argonne National Laboratory by the group of T. Fritz, T. Seed et al. (41, 42). Note that a lethal thrombocytopenia can occur after nearly 300 days, in other dogs at 500 or at 700 days. Granulocytes remain fairly constant in spite of daily radiation exposure until the hemopoietic system fails.

There is a wide spectrum of possible survival times. Some dogs may survive 75 mSv per day only for one year, some others may survive even 8.5 years with a total dose of 23 Sv.

The findings including the use of simulation models (43, 44, 45, 46) are compatible with the assumption that survival depends on a stochastic process of cell death and cell loss in the stem cell pool as first suggested by D. Graessle (45).

3. STRUCTURE AND FUNCTION OF BONE MARROW UNITS IN THEIR RELEVANCE FOR RADIATION EFFECTS

The data presented in the previous chapter of this presentation indicate the enormous cell turnover providing the organism with all essential blood cell elements that are necessary to supply the organism with oxygen (erythrocytes), with phagocytic elements that appear to clean continuously the mucous membrane by migrating through them into the inner surface of the organism (granulocytes) and to assure vascular integrity (platelets) (1, 2, 3). It also was evident that the daily turnover of bone marrow produced cells is about 500 billion. Thus, the question is legitimate as to the detailed function of the organ system which is providing lifelong the essential blood cell elements to assure the integrity of the entire organism. The bone marrow is an organ of about 2,600 g of which 1,400 g are assumed to be actively participating in blood cell formation. It is composed out of many sub-units that are distributed through the about 206 bones of the adult human organism acting as semi-autonomous organ units. These are part of the bone marrow system that acts as one organ due to the complex regulatory mechanisms that will be discussed in Chapter 4 of this review.

It appears essential to describe first the struc-

ture of one of the many bone marrow sub-units (47, 48, 49).

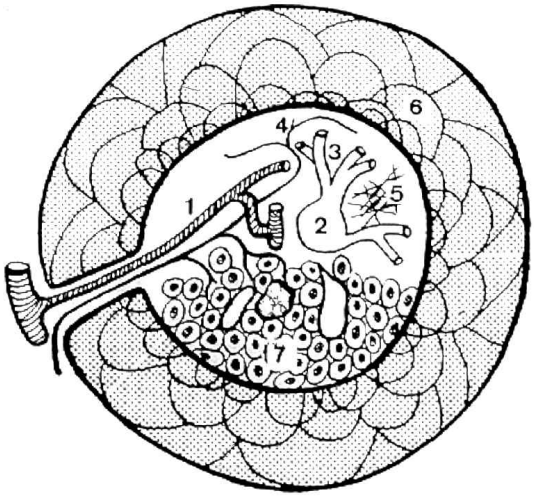
In Figure 5, a cross section of a rat femur is shown schematically. Of obvious importance is the rigid capsule of this organ unit, the bone marrow cortex (No. 6). The cavity of such a bone is filled with a cellular stroma consisting of the vascular system with nutritive vessels (No. 1) and a very complex sinusoidal system (No. 2 and No. 3) collecting newly formed cells and release them into the venous side of the peripheral blood. One has to consider the reticular network of cells (No. 5) as well as the central sinusoid (No. 2) collecting newly formed cells from the sinusoidal system. Of particular interest is the fact that the bone marrow units are innervated by both myelinated as well as unmyelinated nerve fibres (No. 4) (50, 51).

As far as the "parenchyma" (No. 7) is concerned, it obviously is composed of all the different immature and mature blood cell precursors.

A lot of knowledge is available about the inhomogeneity of "cell clone" distribution of the erythropoietic, granulocytopoietic and megakaryocytic cells within the parenchyma. An extensive discussion of the available facts can be found in several monographs (21). Of particular importance for this presentation is the experimental observation in mice and dogs about the fact that hematopoietic stem- and progenitor cells are not randomly distributed throughout the parenchyma but are localized close to the endosteum of the bone. This has been studied extensively by Lord (53) and Shackney (54) and by us (52). In dogs given lethal whole body radiation exposure followed by autologous blood stem cell transfusion, the hematopoietic foci of regeneration are closely related to the endosteum before the rest of the marrow is populated (52). A similar finding is observed in the embryonic marrow: the first hemopoietic colonies after "seeding" of the stromal matrix are seen next to the endosteum (52). In mice, there is a clear-cut gradient progenitor cell distribution between the periphery of the marrow (vicinity of the endosteum measured as CFU-S (53)). Shackney describes in his papers the "gradient" of cell development, starting in the endosteal part and progressing centripedally (54).

In Figure 6 the microvascular architecture of such a bone marrow unit of rats is shown (47, 48, 49). The arterial blood supply comes from a foramen nutritium through which arterial vessels enter the bone marrow cavity in a particular way (Figure 6a). They divide into a diaphysial and into an epiphysial part and appear to wind their way through the bone marrow cavity. Of interest is also that particularly the inner third of the cortex is filled with nutritive vessels (Figure 6b) which appear to be essentially ending loops of the bone marrow vascular architecture as will be shown later. If one wants to visualize the sinusoidal part of the bone marrow circulation, one can see a sinusoidal segment which is distended with white ink (Figure 6c and 6d). This is the collecting system for the newly formed blood elements entering the circulation.

The microvascular architecture can be visualized by injecting India ink into the arterial circulation. The filigran structure of the microvascu-



Stroma

Vascular- System

Nutritive vessels (1)
Sinosuidal system (2, 3)

Bone marrow cortex (6)
Reticular Cells (5)
Innervation (4)

Parenchyma (7)

Figure 5. Structure of a bone marrow unit: schematic cross section of the essential elements of bone marrow function.

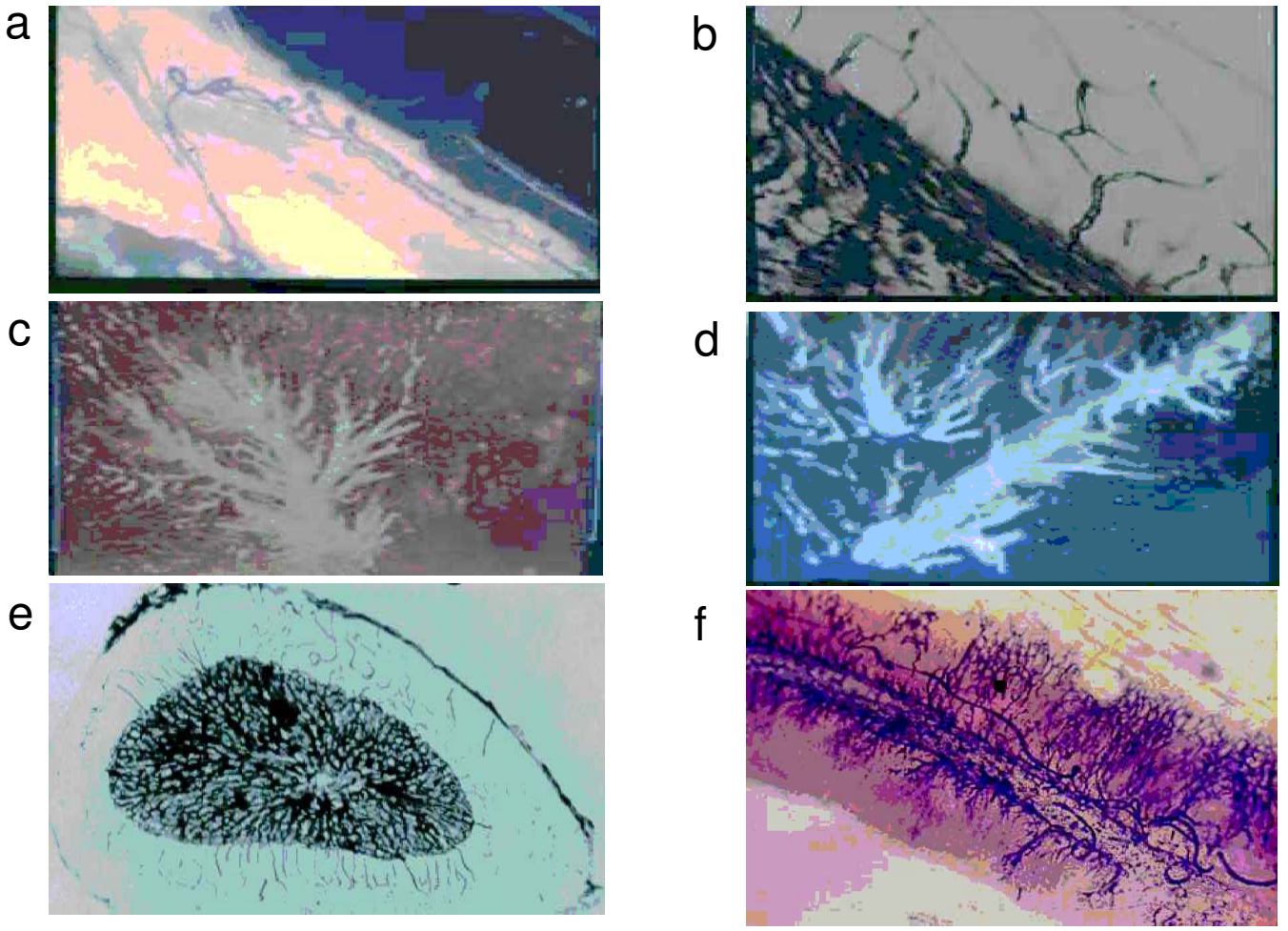


Figure 6. Visualization of the microstructure of a bone marrow unit of the rat (femur): a: Nutrient artery entering the bone cavity (plastic injection technique); b: vascular channels of the cortex as "loops" of the bone marrow vessels; c and d: Sinusoidal segments after injection of white India ink from the venous side; e and f: microvascular architecture of the bone marrow after India ink injection from the arterial side. e: cross section of a rat femur with a cross of the central sinusoid and evidence for the cortical loops of marrow vessels. f: longitudinal femur bone marrow with central sinusoid and arterial capillaries "winding" their way through the marrow. Sinusoidal segments rectangular with respect to the collecting central sinusoid.

lar architecture (Figure 6e) is clearly shown in the cross section of the femur (48). The arterial capillaries would be shown in this picture by cross section. A longitudinal section of the bone marrow is presented after India ink injection (Figure 6f). One can clearly recognize the sinusoidal segments entering rectangular into the central sinusoid while the arterial capillaries wind around and between sinusoids forming sometimes loops.

Of great importance for the regulation of the bone marrow is the innervation of the bone marrow by myelinated and unmyelinated nerve fibres both as far as the bone marrow units is concerned as well as the entire organ system. That the bone marrow is innervated, is already known since 1901 when Ottolenghi discussed the presence of nerves surrounding marrow arteries with fibres passing into the parenchyma (reviewed in 59).

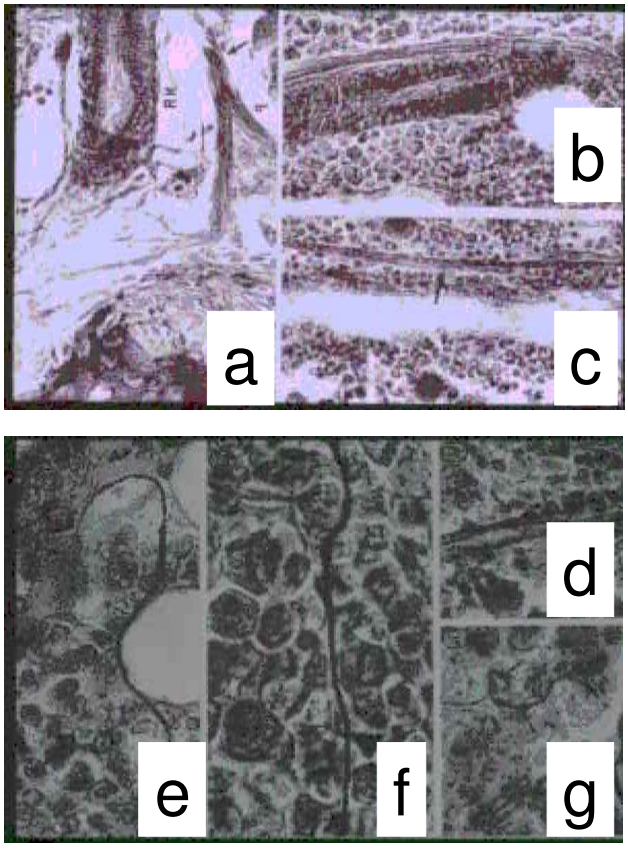
It was of great interest to note that the number of nerve fibres entering a bone marrow unit is enormous. W. Calvo in our group (52) isolated the nerve entering the tibia of a 22-week old fetus. He made cross sections and studied them by electronmicroscopical techniques forming actually a large picture like a puzzle so that he was able to identify and count all unmyelinated fibres entering the tibia. He enumerated a total of 3,951 nerve fibres. Myelinization in the embryo commences during the 6 months of pregnancy. It is known from studies in rats (55) that myelinization coincides with the time that erythropoiesis gets under the control of erythropoietin.

In the rat, the innervation was studied in great detail by W. Calvo (51, 56, 57). In Figure 7 one can see in Figure 7a the foramen nutritium where a nerve divides into a periostial and a medial branch. In Figure 7b a nutrient artery is demonstrated accompanied by a satellite nerve. In Figure 7c one can recognize a nerve next to a central sinusoid entering the parenchyma. Figures 7d through 7h demonstrate different nerve fibres myelinated and unmyelinated as indicated in the legend of the figure.

It is assumed from a number of studies contained already in the older literature (58) that the myelinated fibres transmit afferent signals to the brain. Due to the fact that such myelinated fibres are ending between hemopoietic cells and the parenchyma, one may speculate that these fibres provide signals about the local pressure situation in the bone marrow unit (rigid capsule does not allow any volume change within the bone in spite of continuous cell proliferation). One may also speculate that the non-myelinated fibres are efferent fibres giving signals to the arterial side of the bone marrow circulation regulating blood flow to the sinusoidal system.

It was P. J. Branemark (59) who was able to study the bone marrow blood flow by intravital microscopy. He used the rabbit as an experimental animal and looked at the bone marrow circulation by a microscope as indicated in Figure 8. Depending on the experiment, he was able to examine quantitatively the blood flow in distended areas of the microcirculation as well as in the restricted areas. On the basis of his studies he was able to give diameters of the different elements of the circulation. He measured in the capillary bed in a marrow of ordinary activity the arteriole with 10 micron, the capillary with 8 micron, the sinusoid with 15–16 micron and the venule with 12 micron.

From all these studies trying to analyze the microarchitecture of the bone marrow units and their functional activities, it may be justified to suggest the following way in which marrow cells



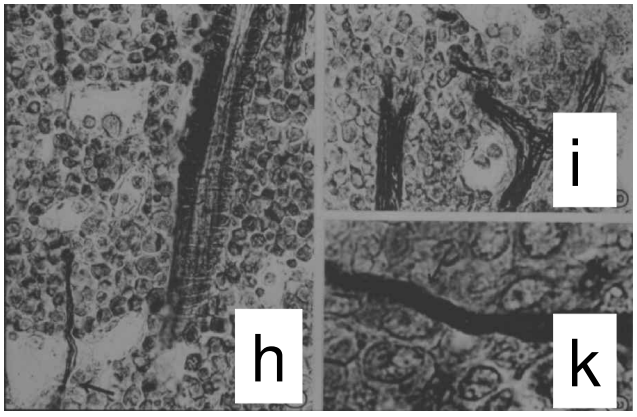


Figure 7. Innervation of the rat bone marrow (femur) with myelinated and unmyelinated nerve fibres.

a Area of the nutrient foramen. A nerve divides into 2 branches following the nutrient artery
b Branch of nutrient artery with a satellite nerve
c Along the wall of the central sinus a nerve gives off a branch (arrow) into the parenchyma
d Nerve fibre ramifies, ending among the cells of the parenchyma
e Two nerve fibres following the curvature of a fat globule.
f Myelinated fibre arching around a cell. The myelin appears as an unstained sheath around the fibre
g Non-myelinated fibre reaches the wall of a capillary
h Arteriole is followed by a nerve bundle
i Exchange of fibres between two nerve bundles producing a chiasm
k Myelinated nerve fibre traverses the parenchyma to contact various cell types

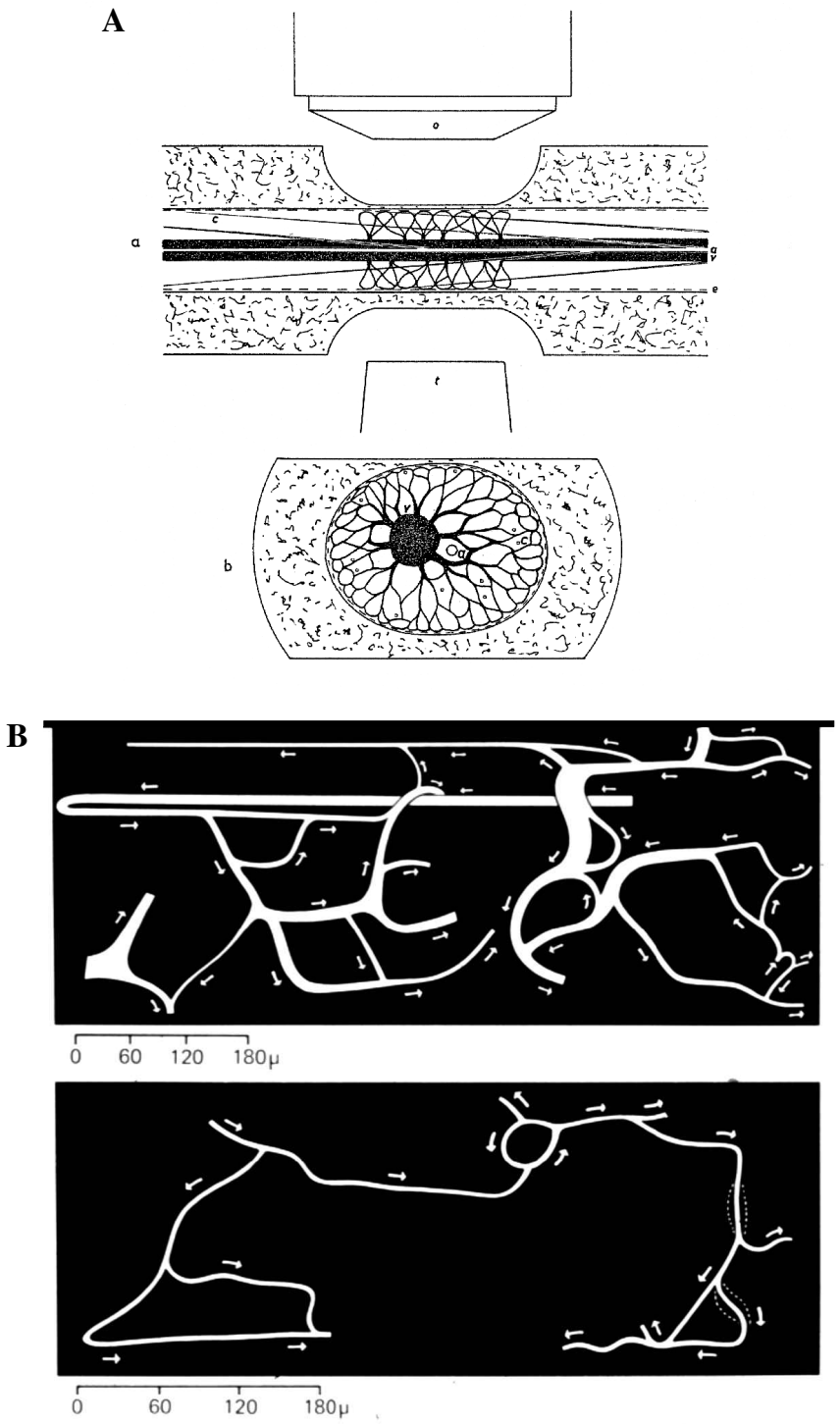


Figure 8. Vital microscopy of bone marrow blood flow and circulation (59). a: Microscopic setup to monitor blood flow patterns in the bone marrow; b: visualization of "distended" (upper panel) and "closed-down" marrow capillaries and sinusoids.

proliferate and are released continuously (Figure 9).

First of all, one may well speculate that there is a balance between the growth pressure in the parenchyma and the blood flow in the sinusoidal system (Figure 9a). This equilibrium is guaranteed by the rigid capsule, the bony cortex. While there is growth pressure outside the sinusoids exercised by continuous cell proliferation, there is within the sinusoidal system a pressure from the blood flow. The sinusoidal wall is unique in the entire organism, it has nothing in common with veins. It consists of an endothelial cell lining without any support that is usually given to the

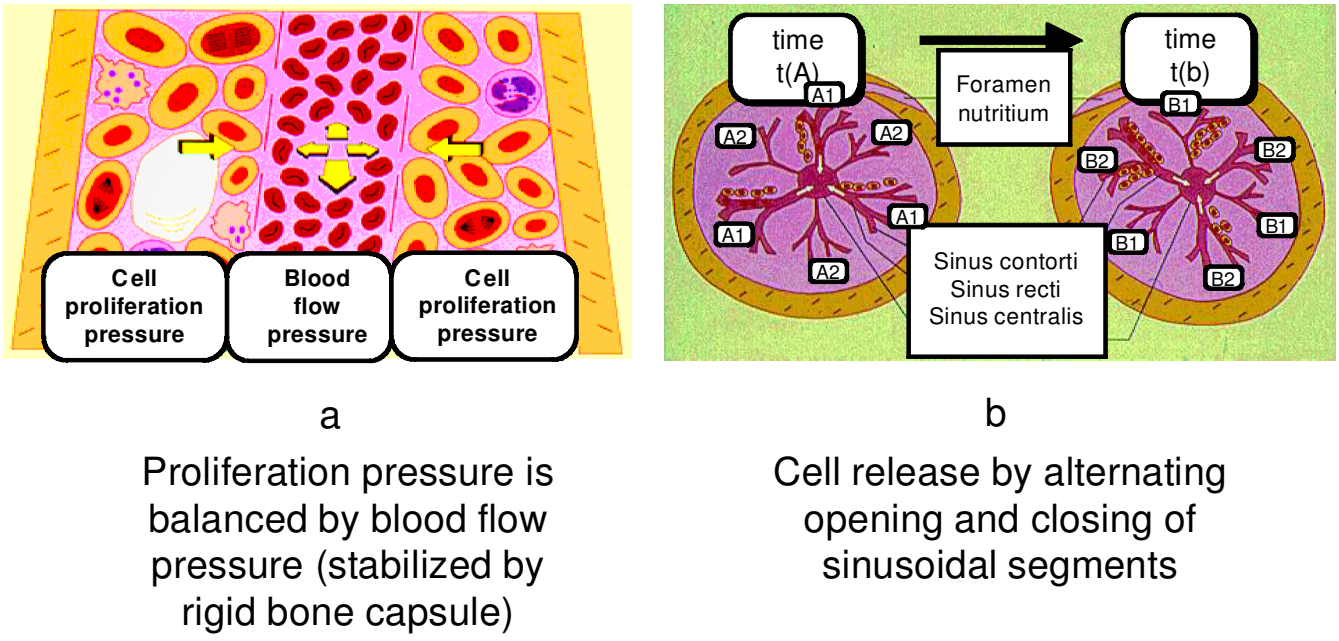


Figure 9. Hypothetical functionality of the bone marrow: for the function of the bone marrow as an unlimited and self-sustaining "ware-house" of blood cells, the rigid cortex is of central importance. This allows a very special cell proliferation and sinusoidal interplay. There is evidence for the continuous blood cell release on the basis of an alternating opening and closing of sinusoidal segments.

arterial or venous side of the capillary bed (in German: uferstaendige Retikulum-Zellen). Thus, this "shore lining endothelial cells" separate the parenchyma cells from the flux of blood. It is a specific lining since it allows the penetration of mature elements, such as erythrocytes, granulocytes of different type, lymphocytes and platelets. It does not allow under normal circumstances the emigration of cells of the proliferative/differentiative cell pools nor the maturing cell pools. However, as we will see later, it apparently allows some type of stem cells to be released into the vascular system and to migrate freely throughout the circulation and re-entering the parenchyma again (60). How can such a system function? A hypothesis is given in Figure 9b. It is assumed that the sinusoidal segment A2 is "closed" due to the fact that the cell growth pressure forced a sinusoidal segment to close down. If there is then a signal to increase the local blood flow, this sinusoidal segment opens by allowing blood to flow taking along the mature cells lining the sinusoidal segment (B2). The same sequence may occur in other marrow unit areas. Thus, one might assume an alternating opening and closing of the sinusoidal segments, providing a continuous flux of cells from the parenchyma via the sinusoidal segments and collecting sinusoid into the periphery within the particular confines of the bone marrow sub-unit structure.

Evidence for that type of regulation came from studying the radiation effects on such a system. Key elements of this are presented in Figure 10. On the left side one can see the result of local irradiation of a rat femur (61). It was a very localized exposure with x-rays (dose: 600 cGy). Three days later the irradiated part and only the irradiated part was distended with blood. If one injects India ink into such a marrow, then one can see a relatively sharp demarcation of the part of the microarchitecture distended with blood and the unperturbed structure of the intact sinusoidal system.

On the right side of Figure 10 one can see the normal marrow architecture in the histology of a rat bone marrow (62, 63, 64). Under normal circumstances, one cannot recognize many distended sinusoids. This, however, is completely different 24 hours after radiation exposure of the entire organism (dose: 650 cGy), because now there is a wide distension of sinusoids and the beginning of a breakdown of the sinusoidal system so that erythrocytes spread throughout the parenchyma. This would be in agreement with the hypothesis that the growth pressure of pro-

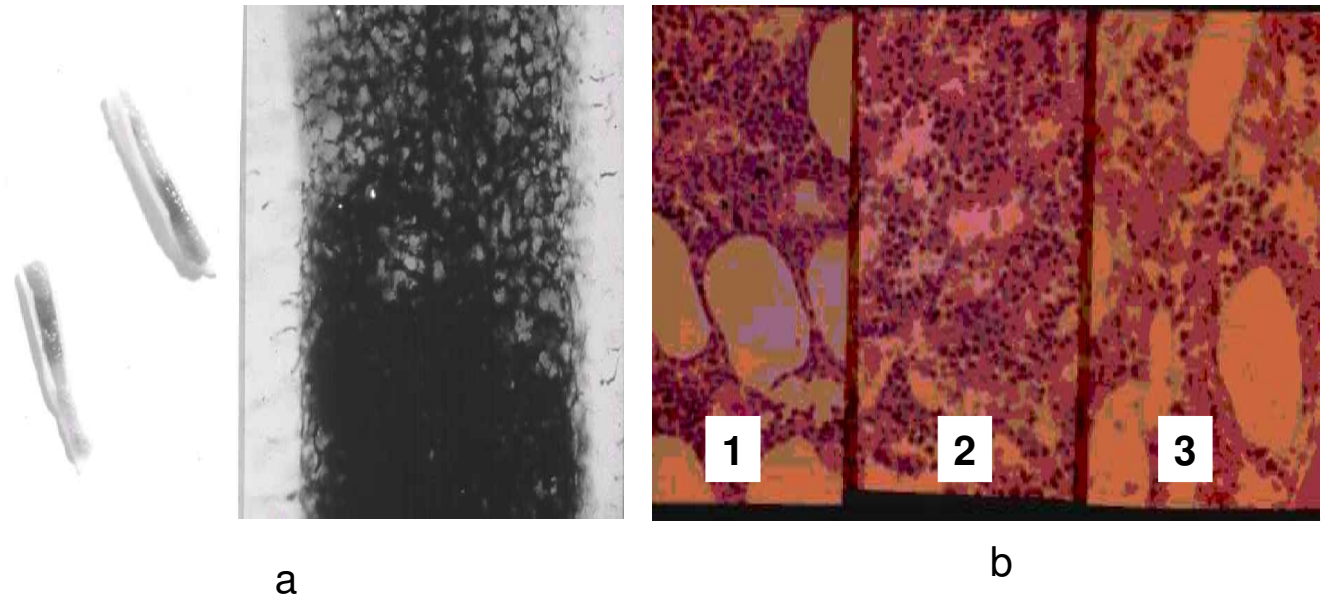


Figure 10. Bone marrow of rat after ionizing radiation exposure: a: bone marrow after segmental irradiation of a rat femur. Note the dark red marrow segment (left) and the India ink visualization of microcirculation (right) after 650 cGy as a result of local breakdown and subsequent local non-thrombopenic hemorrhage. b. Histology of a rat femur: [1] normal cellularity. Erythrocytes contained in sinusoids. [2] 24 hrs after 650 cGy distension of sinusoids at the cost of cell disintegration. [3] 3 days: a complete breakdown of the microarchitecture with a degeneration of blood forming cells and a diffuse non-thrombopenic hemorrhage.

liferating cells decreased completely allowing now the distension of sinusoidal segments until they break (3 days after exposure). One can recognize at that time a "hemorrhagic" bone marrow in spite of the fact that the platelet count is essentially normal. It is of interest that in early studies of the radiation effects on rats one could see that the initial granulocytosis shows several waves which were in agreement with the appearance and disappearance of mature cells in the marrow that were obviously released into the peripheral blood (62).

This raises the question whether the damage to the bone marrow is only due to the radiation induced death of stem- and progenitor cells as well as on their dividing progeny or whether the injury is more severe than could be expected on purely dosimetric grounds (D_0 determinations) due to the severe perturbance of the microvascular architecture.

One may summarize the significance of the structure and function of the local bone marrow unit without and after radiation exposure in the following way:

- An essential prerequisite for cell production and release in a bone marrow unit is the rigid capsule (bone). This can be assumed to grant a balance between cell production pressure and blood flow pressure to drain newly formed cells.
- Under steady state conditions only a few sinusoidal segments are distended (rinsed) by blood. Once mature cells are flushed out from one sinusoidal segment, another segment becomes distended to allow cell emigration enabling a constant cell-flux into the collecting main central sinusoid and from there into the venous side of the circulation.
- Radiation exposure in excess of about 50 cGy to a local bone marrow unit reduced cell growth pressure due to interphase and mitotic death (cell loss). This results in a corresponding stepwise increasing distension of sinusoidal segments starting within minutes to a few hours after exposure.
- If the radiation dose is sufficient (>100 cGy) the radiation induced cell destruction is followed by a flooding of the marrow unit with red cells ("non-thrombopenic bone marrow hemorrhage").
- There is evidence that regeneration of blood cell production in a bone marrow unit can be resumed only if the microvascular architecture including the sinusoidal structure is restored (62, 63, 64).

4. HEMOPOIETIC BONE MARROW AS A DISSEMINATED BUT INTEGRATED ORGAN SYSTEM

As stated in Chapter 3 of this paper the hematopoietic bone marrow system consists of many bone marrow units distributed throughout the skeleton.

One may wonder in what way this organ system spread out throughout the about 206 skeletal bones acts as one organ system. The experienced clinician knows that if he is taking a cytological or histological specimen of the bone marrow of the sternum and compares it to the bone marrow of an iliac crest he usually finds a very similar composition of the marrow tissue. In diseased states, such as bone marrow aplasia or leukemia, again all bone marrow units appear to present a very similar picture. Thus, in the clinical situation a biopsy or aspiration of sternal marrow is considered to be of equal value than the sample taken from the iliac crest or from any other site of active blood cell formation.

Therefore, the question arises as to the mechanisms by which the bone marrow units are unified to react as one organ system. Regulatory mechanisms have been studied in various ways and there is a general agreement that the hemopoietic system is regulated by neural factors but also by a variety of humoral factors. One of the first investigators to study the neuroregulation of the hemopoietic system was Ferdinand Hoff in Germany (65) and there were also extensive studies by Komiya in Japan (66). As far as humoral regulations is concerned, the recent scientific advances in the field provide a lot of evidence on both stimulating and inhibitory factors governing blood cell production. The state-of-the-arts is summarized in several textbooks and monographs (1, 2, 67, 68, 69, 70).

However, much less attention has been paid to the physiological and pathophysiological role of circulating hemopoietic stem- and progenitor cells. Their role has extensively been studied in our group between 1970 and 1985 and results of these and other international studies have been compiled in a monograph (39). Some of the crucial results can be summarized as follows:

First of all, it was shown that stem- and progenitor cells have to be considered to be normal elements of the peripheral blood. It was first shown by Kreutzmann et al. (71) and by Grilli et al. (72) that there is a steady state level of progenitor cells measured as BFU-E and CFU-C. In 3 human volunteers studied for 70 days in terms of the CFU-C content of the blood it was found that each volunteer has his particular level of progenitor cells. There is also evidence for some normal physiological oscillations in 3 normal volunteers with a period of 19, 23 and 25 days.

It is now considered to be common knowledge that $CD34^+$ cells as indicators of the presence of pluripotent stem cells are present in the blood, can be collected by means of leukocytapheresis and successfully transplanted into suitably conditioned recipients (73). In our own studies in 1980 a 4-hour leukocytapheresis was able to collect 8.7×10^5 progenitor cells, measured as CFU-C, without any mobilization (74).

In experimental animal studies, beagles were subjected to long-term leukocytapheresis to collect progenitor and stem cells and to study their dynamic properties (75, 76). If one is subjecting a beagle to a 12.5-hour leukocytapheresis, one can show that (Figure 11) the blood content of colony forming units in culture decreased with the time period of leukocytapheresis to 30% of normal. During this time, 60 times the number of CFU-C were collected from the blood than are normally present in the circulation. This must mean that some progenitor cells are ready to be released into the peripheral blood from extravascular parenchymal spaces. After the end of leukocytapheresis there is a rapid recovery of CFU-C in the peripheral blood.

Between 5 and 10 days after cessation of the procedure one can observe an overshoot in blood CFU-C before they return into the normal level. In principle, the same findings were seen in other experimental dogs subjected to a 24-hour leukapheresis procedure (76).

Of particular importance for this presentation are experiments in which a partial body irradiation was performed (77, 78, 79). Figure 12 shows the results of one of the studies. In these dogs, 70% of the bone marrow was irradiated and 30% of the bone marrow was protected. In the peripheral blood one observed initially a marked depression of granulocytes and of lymphocytes but also of GM-CFC. Then there was a rapid recovery of granulocytes beyond day 20 indicating that somewhere unirradiated bone marrow must have remained from which regeneration can occur. A rapid but transient increase of GM-CFC was seen in this experiment within 10 days (Figure 12B). Of particular importance was the rapid increase of GM-CFC in the irradiated bone marrow sites

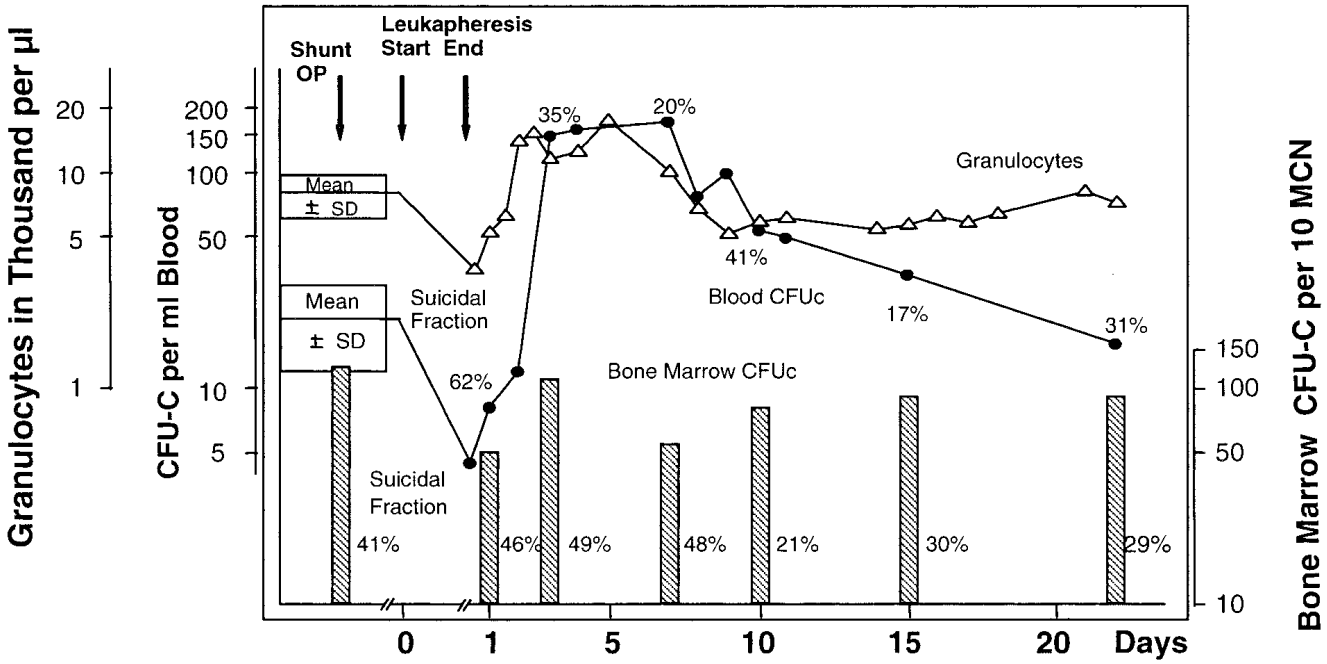


Figure 11. Blood cell changes in a dog subjected to a 12.5 hour-leukocytapheresis after establishing an arterio-venous shunt and mobilizing mononuclear cells by means of dextran sulfate. Note—in particular—the pattern of change for the blood CFU-C concentration: massive reduction at the end of CFC. Thereafter, a rapid rise and overshoot after 5–10 days and a subsequent return to normal values. Also note the changes in the suicidal fraction of progenitor cells in blood and bone marrow.

and an activation of GM-CFC in protected bone marrow (Figure 12C).

These data and results of several related studies (78, 79, 80) are compatible with an intense stem cell migration stream associated with hemopoietic responses after such a partial body irradiation restoring the entire hemopoietic system. Such migration streams of mononuclear cells have been discussed several years ago by Yoffey (81).

Finally, it appears of importance to show that the restoration of an irradiated bone marrow hemopoietic system is possible by the transfusion of stem cells that are derived from fetal liver, from bone marrow or from the peripheral blood (82, 83, 84, 85, 86).

The results of our own studies (85) in this respect are summarized in Figure 13. In these experiments the same number of GM-CFU was given in absolute terms to dogs irradiated with a total of 1,800 cSv. All of them received progenitor cell doses in the order of 1.5×10^6 CFU-GM per kg body weight for bone marrow and blood stem cell transplantation and 1.6×10^6 for fetal liver cells. Figure 13 indicates clearly that the transfusion of fetal liver derived hemopoietic progenitor cell results in a very rapid restoration in the peripheral granulocyte pool. Within 20 days the numbers are back to 100% of normal and there appears to be even a slight overshoot. The blood derived stem- and progenitor cells result in an equal rapid return of granulocyte values to safe levels, but it takes more time for the granulocyte renewal system to recover completely. The bone marrow derived stem and progenitor cells result eventually also in a complete recovery, but the process takes much longer.

These findings are interpreted to mean that the fetal liver derived hemopoietic stem cells have a much higher replicative potential than the adult stem cells. While the computer-assisted model could fit the recovery data of bone marrow—and blood derived stem cells utilizing a replication probability value of 0.63 (87), it was necessary to assume a replication probability of 0.95 for fetal stem cells. Such assumptions would be compatible with the rapid initial increase and a complete restoration on the granulocytic system very soon after transplantation of fetal tissue derived stem cells. The blood derived stem cells also show a very rapid initial recovery, but it appears to take longer for the stem cell pool to recover due to the fact that apparently blood derived adult stem and progenitor cells have a lower inherent

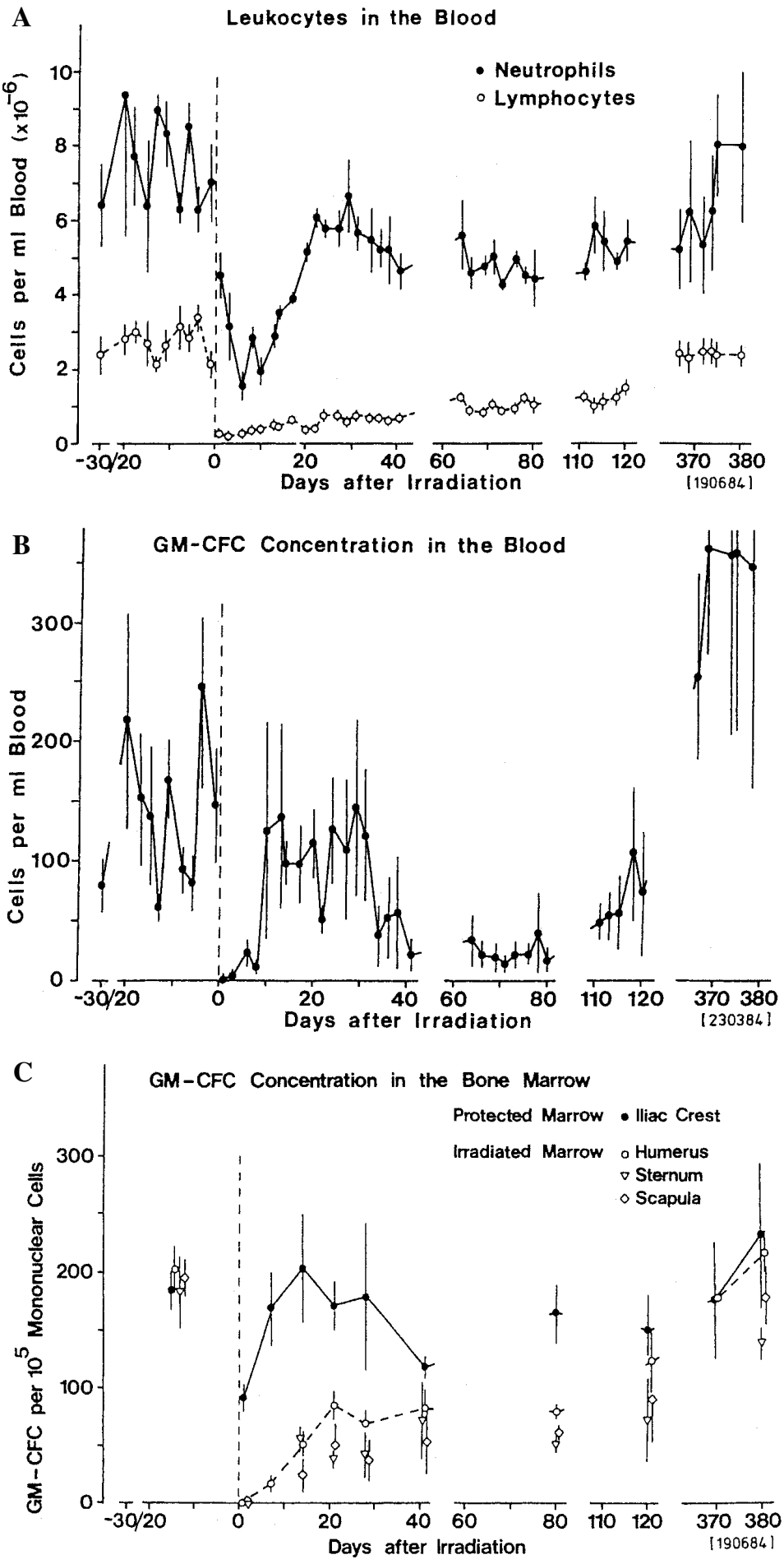


Figure 12. Blood cell changes after partial body irradiation of a dog. 70% of the bone marrow irradiated with 1170 cGy of x-rays while the lower part of the body was shielded. A: Changes of the blood neutrophils and lymphocytes. B. GM-CFC concentration in the peripheral blood. Note the initial decline and the subsequent "overshoot" phase during the time of repopulation of irradiated bone marrow (C). The "late" pattern of colony forming units GM-CFC between 370 to 380 days remains to be elucidated.

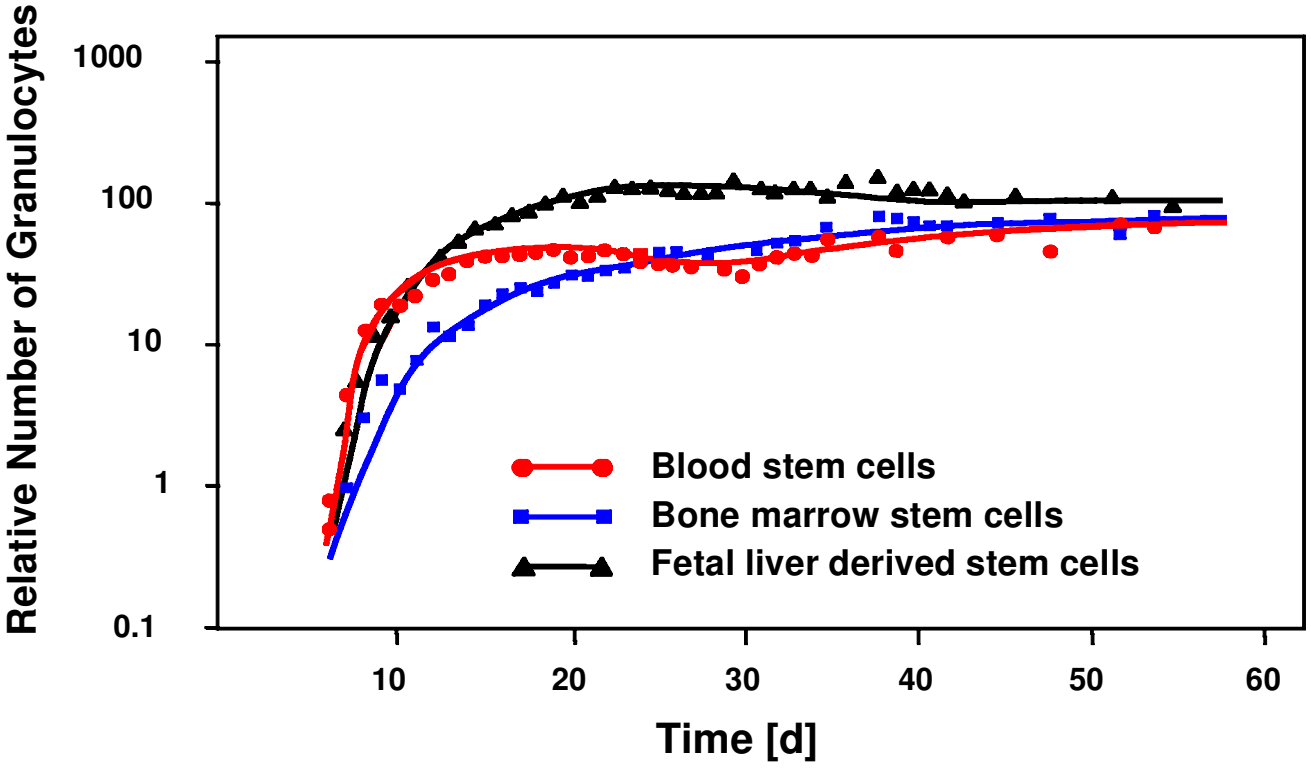


Figure 13. Pattern of granulocyte recovery in dogs exposed to 1800 cGy whole body exposure and transplanted with about equal numbers of granulocyte-macrophage colony forming units (CFU). Note the rapid granulocyte recovery within about 10–15 days after blood stem cell and fetal liver transplantation. Note also the complete and in part overshooting recovery after fetal liver derived cells while the final recovery after blood and bone marrow derived cells is comparatively delayed. The solid line represents the results of regeneration studies using a granulocyte biomathematical cell system model (86).

replicative rate (87). That the bone marrow derived hemopoietic stem- and progenitor cells are not as efficient as blood derived stem cells—at least initially—is considered to be related to the fact that the aspiration of bone marrow to harvest stem cells results not only in stem- and progenitor cells capable of entering the blood, but also in tissue residing stem- and progenitor cells. Thus, the highest replicative rate found in blood derived stem cells is not available and therefore the slower recovery might be explained (82).

These experimental data suggest that circulating stem- and progenitor cells have a function to assure a sufficient concentration of replicating and proliferating stem- and progenitor cells in the local bone marrow units. The stem cell migration streams appear to be of paramount importance for the understanding of the function of the hematopoietic bone marrow organ system.

In this context one has to mention another problem area, that of the pathophysiology of extramedullary hemopoiesis. In some disorders and in some experimental conditions, hemopoiesis in the adult organism can occur in sites other than the bone marrow. Extramedullary hematopoiesis ("myeloid metaplasia") is found in spleen, liver, but also in lymphnodes under diseased conditions (1, 2). After bone marrow and blood stem cell transplantation there is in experimental dogs (88) and in man (89) a transient period of extramedullary hemopoiesis in the spleen during hematopoietic recovery.

Extramedullary hemopoiesis is also found in animals given radioactive strontium incorporated into the skeletal bone and providing continuous irradiation of the bone marrow contained in the bones. If such an irradiation continues long enough, then there is evidence of a migration of bone marrow hemopoiesis to extramedullary sites, such as the spleen, as shown in swine (90).

Thus, one has to recognize the enormous dynamics that are inherent in the regulatory mechanism governing lifelong hemopoietic cell production and removal.

5. CONSEQUENCES FOR BONE MARROW RADIATION DOSIMETRY

In the context of this workshop on "Bone marrow radiobiology and radiation dosimetry: bone marrow physiology and radiobiology" it appeared to be important to summarize some aspects of the structure, function and regulation of the bone marrow hemopoietic system in their relevance for radiation dosimetry considerations of the bone marrow. Many details could not be dealt with but can be found in the cited literature.

Any attempt to analyze and assess the impact of ionizing energy on bone marrow rooted blood cell formation has to recognize the structural and functional as well as regulatory mechanisms that govern the homeostatic equilibrium between cell production and cell removal in this organ system distributed under physiological conditions throughout the skeleton in the organism.

In bone marrow system dosimetry, it is essential to utilize specific analytical approaches to assess the impact of ionizing radiation on the different structural components of the total hematopoietic bone marrow system. In studying the dosimetry, one has to distinguish and use (separate from each other) indicators of exposure to ionizing radiation (external and internal) from indicators of effect (local and general) and of repair (different levels of biological organization).

For the understanding of radiation morbidity and lethality of the hemopoietic bone marrow system it is essential to recognize the complexity of the stem cell system and its dynamics. The measurement of external or internal exposure has to consider the skeletal-wide distribution of the stem cell system, its dynamics of maintaining sufficient stem cell number locally (at the stem cell niches beneath the endosteum) and their potential to migrate from any bone marrow site to extramedullary localizations (spleen, liver, lymphnodes) providing a temporary home for blood cell formation.

Local bone marrow responses to ionizing radiation can be assessed by indicators of effect and of repair. Aside from the effect on replicating, proliferating and differentiating stem- and precursor cells and their inhomogeneous distribution in the marrow, it is essential to recognize the radiation effects on the micro-vascular architecture due to the perturbations of the balance between cell proliferation and blood flow pressure. These alterations of the bone marrow sinusoidal structure and function result early after exposure in a non-thrombopenic hemorrhage. This has to be cleared away before regeneration can commence from remaining or immigrating intact or repaired stem cells.

Any inhomogeneity of whole body radiation exposure (external as well as internal) and any protraction of the total dose given (for instance by fractionations) results in an increased chance of spontaneous recovery of part of the stem cell system due to its migration dynamics, due to a less extensive perturbation of local microcirculation respectively, due to changes in stem cell radiation sensitivity or due to a rapid DNA repair process.

Thus, in bone marrow radiation dosimetry it is of paramount importance to not only consider physical factors but to recognize the complexity of regulatory mechanisms at the different sites and biological levels of the bone marrow organ system that are influenced by penetrating ionizing irradiation.

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