pected in 1972. Sustained research programs are essential in protecting our food supplies from potential losses of catastrophe magnitude.

Several professional groups, including the American Phytopathological Society and the Entomological Society of America, have urged that a program and facilities be established for the study of exotic pests that threaten our agriculture so that controls may be found before the pests are here. Such a program would be desirable but covers only one aspect of the problem. What is really needed is an overall strengthening of research on crop pests.

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Bone Marrow Transplantation

Research in marrow grafting has generated extensive new information for the hematologist.

C. C Congdon

The diseases acquired by man and animals during postnatal life are frequently irreversible and sometimes lead to total destruction of organs and organ systems. Because a size-function relationship exists for biological structures, an essential organ (for example, the bone marrow) can be reduced by disease to such a point that its level of function will no longer support life, and death occurs. Inadequate development of an organ, brought about by congenital disease, can have a similar effect, with death occurring when a critical level in the size-function relationship is reached. An example of this is the category of birth defects known as immune deficiency disorders. These are characterized by a failure to develop adequate amounts of lymphatic tissue, the structure that is essential for the immune response that helps prevent viruses and other microorganisms from invading and destroying vertebrate organisms.

It is primarily because of these organismal disasters that the transplantation of bone marrow represents an exciting chapter in the history of biomedical investigations on organ grafting for the treatment of pathological processes. However, the seemingly miraculous things done with organ grafting in very healthy, expendable laboratory animals lead us to imagine that such techniques will soon be applicable in the relief of some human disease. The history of progress in clinical organ transplantation shows that this mode of therapy is slow and difficult, with occasional brillant successes against a background of many futile attempts to do something for desperately ill patients (*1*).

Technique of Bone Marrow Transplantation

Bone marrow and its ancillary lymphatic tissues are usually procured and transplanted in ways quite different from those used in other organ transplants. Needles and syringes are used to aspirate the normal red bone marrow from the cavities inside bones. Suspensions of the living cells are then made in an appropriate vehicle, so that the cells can be injected directly into the bloodstream of the recipient. Once inside the blood, the stem cells of the marrow lodge in the recipient's bones in the spaces that normally contain red marrow; there they grow and replace the destroyed organ.

This procedure is not as artificial as it seems, because the stem cells of bone marrow normally circulate in small numbers in the blood of mammals. They are present along with the other blood cells that were originally produced by division and differentiation of stem cells of the same type as those residing in the marrow (*2*). A schematic representation of these patterns is given in Fig. 1. Small numbers of bone marrow stem cells are also found among the free cells in the peritoneal cavity (*3*), and larger numbers occur in the spleen red pulp and the fetal liver in mice.

A major achievement in bone marrow transplantation and related areas of research has been a new understanding of the complicated patterns of cell migration through the bone marrow, peripheral blood, lymphatic tissues, and thymus. This field of investigation, which some of us call experimental hematology, is now a major activity of many laboratories throughout the world.

Recent advances in experimental hematology which exploit knowledge gained in marrow and lymphatic tissue transplantation in laboratory animals are reported in *Experimental Hematology* and in many other publications (*4*). Much of the material in *Experimental Hematology* deals with new directions in hematology that were not anticipated when marrow grafting was first used as therapy for radiation injury. The review by Petrov and Zaretskaya (*5*) is an extensive account of experimental hematology.

The author is on the staff of the Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

How Bone Marrow Is Destroyed

In addition to hereditary defects in function, bone marrow is easily destroyed, by whole-body ionizing radiation, by many drugs and chemicals, and by neoplasms or cancers such as leukemia. The stem cell compartment of the red bone marrow (Fig. 1) is the major sensitive site. Its final products, the mature peripheral blood cells, are in most instances not very sensitive to injury by irradiation or chemical substances, but these cells have relatively short life-spans (from a few days to a few months) in the normal steady state. Peripheral blood cells are essential for life of the organism, and they will disappear unless there is a regular supply of new cells coming into the blood from the marrow. In leukemia the bone marrow is crowded out by tumor cells, a situation effectively the same as that existing when marrow is destroyed by other means.

These findings are illustrated in Figs. 2 through 5, which show destruction of marrow by x-irradiation. In the study of Fig. 2, bone marrow cells were counted at different time intervals after exposure of mice to 900 roentgens of x-radiation. If there is no bone marrow transplant these cells disappear and the mice die from bacterial infections and hemorrhage in the second week after irradiation. When genetically identical (syngeneic) bone marrow is transplanted directly into the bloodstream, the organ returns to its normal size in time to prevent death from infection and hemorrhage. Note that the speed of recovery depends on the amount of bone marrow injected.

Figure 3 illustrates the loss of white blood cells in the peripheral blood of a guinea pig given a lethal dose of x-rays. The cells disappear primarily because their normal life span is only a few days and the injured bone marrow cannot replenish the loss that occurs through aging. Bacteria gain entrance to the blood and tissue when the level of white blood cells is too low, and death from septicemia frequently results. Figure 3 also depicts return of the number of white blood cells to the normal level when bone marrow has been restored by grafting and septicemia is prevented.

Similar curves are seen for platelets in the peripheral blood. Too low a level of these elements brings about fatal spontaneous hemorrhage. The red blood cells have a considerably longer life span, and death from bacterial infections and hemorrhage occurs long before the natural aging of red blood cells causes a significant anemia.

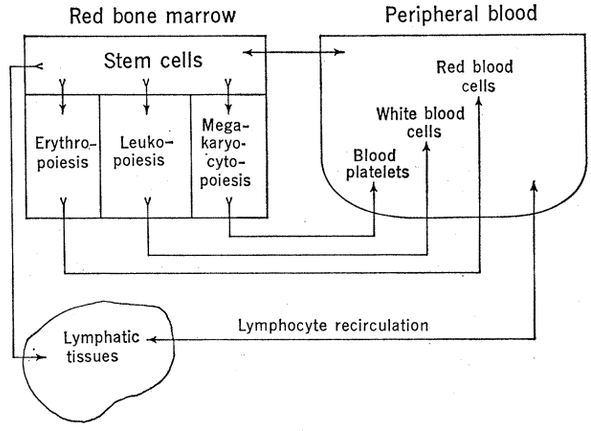


Fig. 1. Renewal system for bone marrow cells, showing major compartments. The short life span of blood platelets, white blood cells, and red blood cells requires a continuous generation of these elements by the red bone marrow. Lymphocytes enter lymphatic tissues from the blood and reenter the blood by way of the thoracic duct, according to a system worked out by Gowans and Knight (*43*).

Figure 4 carries the findings one step further, showing how differing amounts of a bone marrow transplant to different groups of mice prevent death during the 30-day period after exposure to 900 roentgens of x-radiation. Finally, in Fig. 5, the true therapeutic value of marrow transplantation, as measured by studies on laboratory mice, is shown. This work, by Doherty (*6*) and by Doherty and Smith (*7*), has as its point of departure the dose of radiation calculated to kill 50 percent of the mice in 30 days. These investigators obtained, for this dose, a value of 778 roentgens for mice that received whole-body x-irradiation without a transplant or other therapy. The dose could be increased to 1450 roentgens when the largest amount of marrow was transplanted—2×10^8 cells per mouse. It should be noted that a dose of 900 roentgens usually kills 100 percent of this type of mouse, a finding that reflects the extremely steep dose-response mortality curve for whole-body irradiation.

Autologous bone marrow transplantation in dogs and monkeys supports the findings on recovery from lethal irradiation in small laboratory animals such as the mouse. The procedure in dogs and monkeys is to remove some red bone marrow just before irradiation, store it in a viable state until the exposure has been completed, and then transfuse it back into the animal from which it was taken (*8*). In humans, for a bone marrow graft in which the donor is the identical twin of the host, who has suffered radiation damage, the recovery picture is similar (*9, 10*).

Injury to bone marrow by drugs and chemicals is a major problem in human medicine. The possibility of adverse reactions in which bone marrow is destroyed as a side effect is a frequent threat to the patient taking certain drugs. In some human cases, marrow transplants have been used successfully to treat chemical damage to the red bone marrow. Successful transplants in mice, rats, and dogs also show the general feasibility of this therapeutic method.

One of the earliest investigations, by J. K. Weston and his colleagues (*11*), demonstrated that rats exposed to the drug busulfan at ordinarily lethal doses could be treated, and death avoided, by bone marrow transfusion. Numerous early studies on the so-called radiomimetic drugs and modification of their bone marrow toxicity by marrow transfusion are summarized by Van Bekkum and De Vries (*12*).

Many other studies on treatment with marrow grafts after injury by drugs have now been made; the work is less extensive than that done on radiation damage to marrow, but the findings are generally parallel. Cyclophosphamide has been the compound of special interest because it has immunosuppressive actions as well as marrow-destroying action (*13, 14*).

Some cases in which bone marrow transplant therapy has been used to treat human patients some of whose blood-forming tissues have been destroyed by radiation or chemicals are mentioned in the next section.

Human Bone Marrow Transplantation

Organ transplants between identical human twins, like those within a highly inbred strain of mice or rats, illustrate well the basic therapeutic value of the graft, for they eliminate the complications introduced by immune reactions between genetically nonidentical donor

and recipient. The successful transplanting of kidneys between identical twins was, for example, a vital step in showing the surgical feasibility and therapeutic usefulness of this kind of organ replacement. Table 1 gives the terminology for various donor-host relationships in man and mouse.

Bone marrow transplants between identical twins have been successfully carried out on at least 13 occasions; one of the recipients was a worker who, in an accident, had been exposed to an apparently lethal dose of radiation from an accelerator (*15*). He survived with the aid of the transplant (*10*). A summary, with literature citations, of most of these transplants between identical twins appears in Bortin's compendium of 203 reported cases of human marrow transplants (*16*). Bortin cites recovery in five of seven individuals who received marrow from an identical twin as treatment for aplastic anemia. (The original marrow failure in the seven was either of unknown cause or attributed to drug reaction.) With identical twins, proving that transplantation of marrow has been accomplished is a problem because there is no way of recognizing donor cells in the patient. (Marker techniques for identifying transplanted bone marrow cells are discussed below.)

One other situation in which marrow transplant is beneficial or potentially so in experimental animals is seen in hereditary anemia in mice (*17*). If the genetic relationship of donor and recipient is very close, the anemia is curable. Hereditary anemia (for example, sickle cell anemia, or thalassemia) is a major disorder in some human populations, but attempts to transplant normal bone marrow have not yet succeeded in altering the course of the disease (*14*).

Markers for Identifying Bone Marrow Transplants

Demonstration that a transplant has survived the technical difficulties associated with wound healing and is functioning is not always easy. This has not been a major problem in skin grafting and organ transplantation but has seriously hindered efforts in bone marrow grafting.

When bone marrow cells are placed in the bloodstream, the wound-healing problem encountered with tissue grafts is absent. We expect the injected cells to leave the blood and become colonies that grow and replace the destroyed marrow, or genetically defective cells. Proving that the new cells are descendants of the transfused ones is not always a simple task, and it took several years of research to work out the techniques (*12*).

The process of showing that transplantation and growth of marrow grafts had taken place generated an entirely new area of experimental hematology, whose impact is still being felt. Perhaps the biggest achievement in this area was the demonstration that complete replacement of bone marrow, lymphatic tissue, and thymus after destruction can be brought about by transplantation of relatively small numbers of bone marrow cells.

Direct demonstration of the presence of donor cells in marrow is usually accomplished by examining the chromosome number or chromosome structure when the donor and recipient have different chromosomal karyotypes. Another technique is to react the cells with cytotoxic antibody directed against the antigenic differences between the donor and the host. Other markers for identifying the progeny of bone marrow cells that circulate in the peripheral blood are used, in addition to karyotype and cytotoxic antibody. These depend on red blood cell types, on histochemical differences, and on differences in cell products between donor and host.

Figure 6 illustrates the disappearance

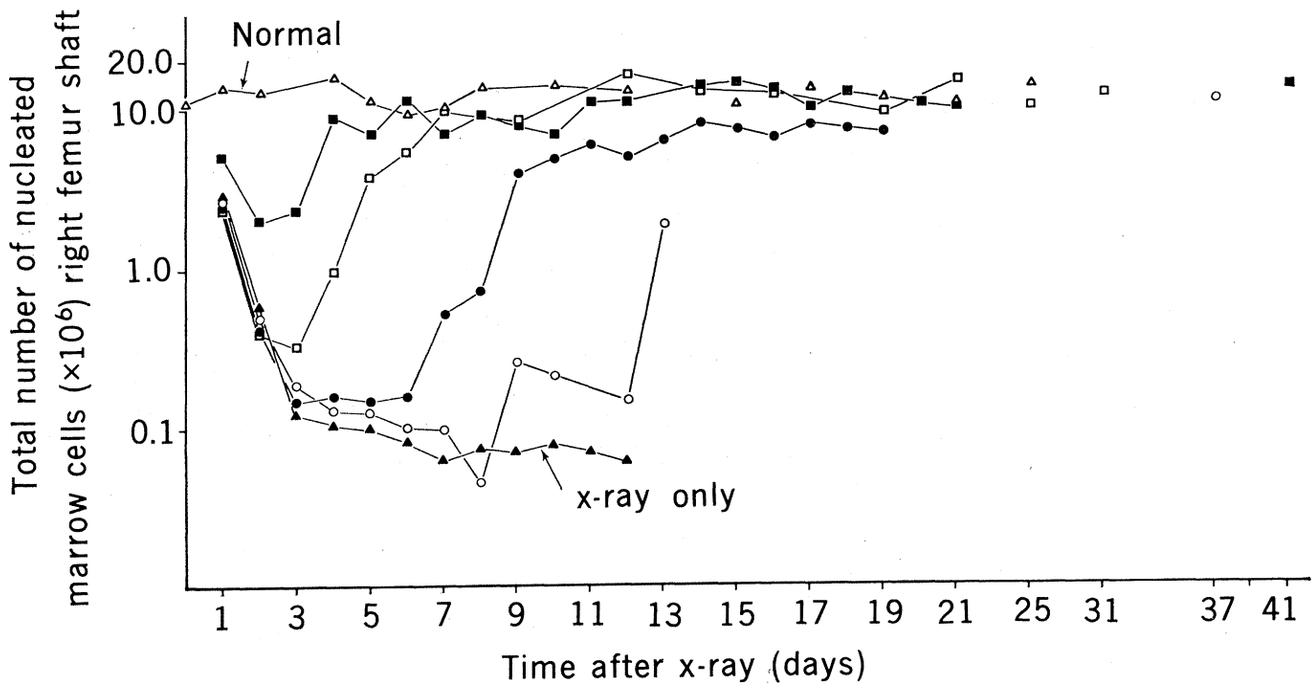


Fig. 2. Destruction of bone marrow by whole-body irradiation (900 roentgens) of mice, and repopulation of marrow after intravenous transfusion of increasing amounts of normal marrow in different groups of mice. (Open triangles) Normal mice; (solid triangles) mice that received x-ray only. (Circles and squares) Mice given a transfusion of normal marrow after irradiation: (open circles) 0.16×10^6 cells; (solid circles) 0.97×10^6 cells; (open squares) 12.87×10^6 cells; (solid squares) 237.9×10^6 cells. [From Urso and Congdon (*44*)]

of host-type red blood cells and their replacement by rat red blood cells in a mouse given a lethal dose of irradiation and then a transplant of rat bone marrow. Detection of rat and mouse red blood cells depends on the use of antiserums that selectively recognize the antigens of the two species.

In discussing the technique of bone marrow transplantation, I should also mention the route of administration. By analogy with blood transfusion, marrow transplants are sometimes called "bone marrow transfusions." When the transplant of marrow cells is placed in the abdominal cavity, migration of cells into the bloodstream and then to the bone marrow occurs, but the process is slower and less efficient than direct grafting into the bloodstream. Injection of bone marrow cells into muscle, into other organs, or beneath the skin usually gives only a small local transplant that is of little value to the host if its bone marrow has been destroyed.

Table 1. Donor-host relationships.

Terminology*	Examples	
	Human	Mouse
Autologous (same individual)	Same person	Same animal
Syngeneic, isogeneic, isologous (same genetic type)	Identical twins	Inbred strain of mice
Allogeneic, homologous (same species)	Siblings, parents, or unrelated persons	Different strains of mice
Xenogeneic, heterologous (different species)	Ape to man	Rat to mouse

* Autograft, isograft, allograft, homograft, xenograft, and heterograft are contractions often used to refer to specific examples of transplants. Similarly, autotransplant, isotransplant, and so on may also be used.

Host-versus-Graft and Graft-versus-Host Reactions

Host-versus-graft reaction. Before discussing the key problem in tissue transplantation—rejection—I must say more about compatibility between donor and host in mammalian species. If bone marrow is taken out of a mouse or a man and then put back, this autologous transplant is accepted and lives, provided the cells get adequate oxygen and nutrients for survival. The same thing is true for skin and organ grafts. We say that a genetic identity exists between the autologous donor cells and the host, because they all arose from the same fertilized ovum (*18*). When the autologous donor cells or cell products come into contact with lymphatic tissues (lymph nodes, spleen, and so on) of the host, nothing happens, because of the genetic identity. An immune response is not induced if the lymphatic tissues treat the donor material as "self," and there is no host-versus-graft reaction. Cell transplants between identical twins and within a highly inbred strain of animals are treated as if they were autologous. *Syngeneic* and *isologous* are the terms used to designate this type of graft.

With all other types of genetic relationships between donor and host, the outcome is different. In cases where the donor cells and the host tissues are not genetically identical, when the grafted cells and cell substances come into contact with lymphatic tissues of the host they activate these tissues, because they are recognized by the tissues as "nonself," not having been there when the tissues first developed. The lymphatic tissue system proliferates to produce cells and substances that attack the graft and destroy it. This is the host-versus-graft reaction that causes the failure of allogeneic or homologous grafts and of xenogeneic or heterologous grafts (see Table 1).

Bone marrow from one strain of mouse placed in a normal adult mouse of a second strain is rejected; this is the host-versus-graft reaction in an allogeneic graft. Similarly, rat bone marrow given to a normal adult mouse undergoes the same fate as a skin transplant or organ graft.

If the lymphatic tissue system is severely injured or modified in any of a variety of ways, it loses its ability to recognize "nonself" substances and it may not reject the allograft. A common means of producing this effect (a technique called immunosuppression) is to treat the animal or patient with azathioprene, cyclophosphamide, antilymphocyte serum, or whole-body ionizing radiation (*19, 20*).

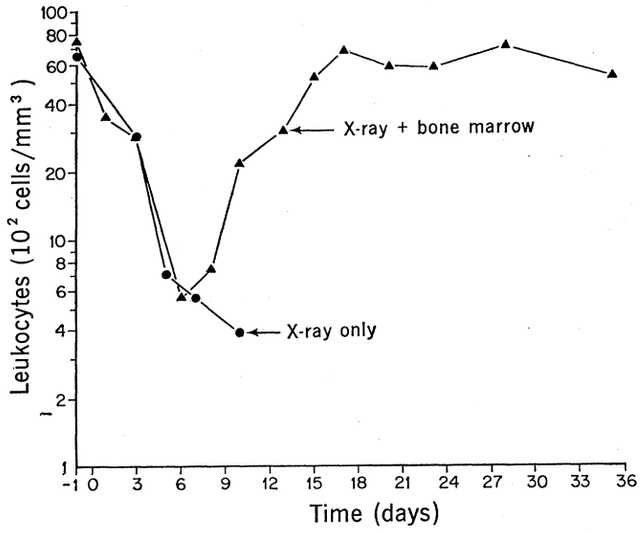
When ionizing radiation is used as an immunosuppressive agent, the bone marrow is also destroyed, so, for example, if we transplant rat bone marrow cells into a mouse previously given a lethal dose of radiation, the animal will survive because the bone marrow cells of the rat replace the destroyed ones of the mouse; they grow and function like normal bone marrow, making rat red blood cells, white blood cells, and platelets. All of this is possible only because the lymphatic tissues were also injured by the exposure. A normal mouse would have eliminated the rat cells very quickly by a host-versus-graft reaction.

The use of immunosuppressive agents poses a major problem—that of providing enough suppression to permit allografting without causing additional undesired injury from the suppressive agent itself. Continued and prolonged administration of the drugs and biologicals used is detrimental to the organism because it needs a normal immune response to bacteria, yeasts, fungi, and viruses for survival. Not only is a host-versus-graft reaction suppressed but immunologic defenses against invading microorganisms are also altered.

Histocompatibility typing is a way of measuring the closeness of the genetic relationships between donor and host in the allograft experiment. In ordinary mongrel populations, such as man or domestic animals, outbreeding, not inbreeding, is the practice, hence rejection of an allograft is always anticipated. There are, however, degrees of allograft relationships, somewhat like the range of relationships seen in blood grouping, and if matching (determination of histocompatibility) of donor and host is undertaken, it may make the job of immunosuppression less difficult and the success of the grafting more certain.

Tissue typing for determining the degree of compatibility is now an important area in transplantation research and practice, even when powerful immunosuppressive agents are available. It shows why, whenever practical, family donors are used after the degree of histocompatibility has been determined (*21*).

The major goal in replacement therapy of the transplantation type is to somehow stop the host-versus-graft reaction long enough to permit take of the graft and then to achieve tolerance of the foreign cells by the host's normal immune mechanism. This goal has not been reached for most transplant patients, and its feasibility has been demonstrated experimentally in laboratory animals. The promise of bone marrow transplantation includes realization of this goal, because if a bone marrow graft is established after the host has received a single dose of immunosuppressive agent, a new lymphatic tissue



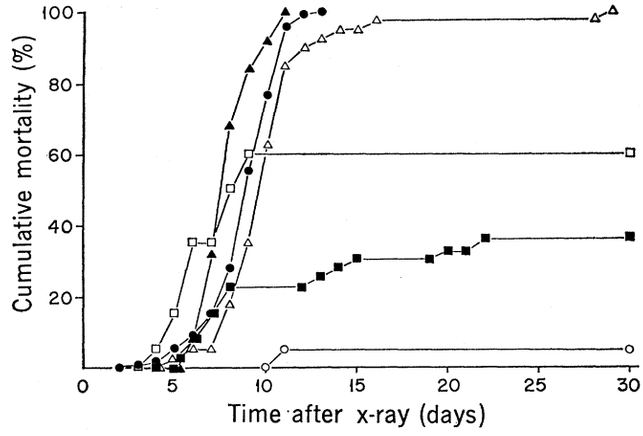


Fig. 3 (left). Loss of white blood cells in guinea pigs (strain 13) exposed to 750 roentgens of x-radiation. White blood cells were recovered after a graft of marrow into the bloodstream. [Lorenz and Congdon (*38*)] Fig. 4 (right). Reduction in radiation mortality after x-irradiation (900 roentgens) and intravenous bone marrow transplantation, in mice. (Solid circles) Mice that received x-rays only. (Triangles, squares, open circles) Mice given normal bone marrow intravenously after irradiation: (open triangles) 0.007×10^6 cells; (solid triangles) 0.016×10^6 cells; (open squares) 0.61×10^6 cells; (solid squares) 0.97×10^6 cells; (open circles) 12.87×10^6 cells. [Urso and Congdon (*44*)]

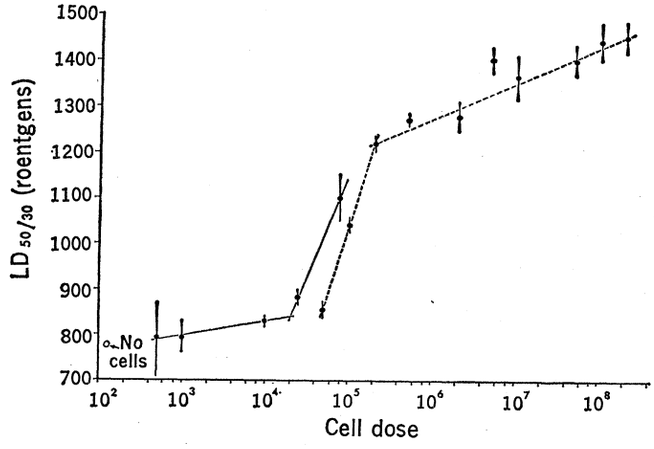
can be derived from it that will accept an organ graft of the same genetic origin as the new tissue without further treatment with drugs.

The trouble is that bone marrow grafts and cells growing out of them have the potential for attacking the host, and the graft-versus-host reaction dominates work in the marrow transplant field (*20*).

Graft-versus-host reaction. The idea that a graft of foreign cells can attack the host came to the attention of transplantation investigators in the early 1950's. This phenomenon is not seen with skin, kidney, heart, liver, and many other organ allografts, but it is a major, still unsolved, problem in the transplantation of foreign bone marrow, white blood cells, and lymphatic tissues. This special group of tissues is frequently referred to as "blood-forming tissues."

Not long after the first bone marrow and spleen transplants had been performed in mice exposed to lethal doses of radiation, it was noticed that prompt recovery from bone marrow failure took place, but that during the next few weeks death occurred from a secondary wasting disease. Figure 7 illustrates the cumulative mortality. The secondary disease did not occur when blood-forming tissue genetically identical to the host material was grafted. Theory predicts and practice shows that secondary disease does not occur in bone marrow transplants between identical twins in man, and in marrow grafts within an inbred strain of mice, rats, or guinea pigs (that is, when the relationship between donor and host is syngeneic, or isologous). An important additional point is the finding of reduced severity of the graft-versus-host reaction when the donor and host, even though not genetically identical, have been closely matched by histocompatibility testing.

In the case of identical-twin transplants, or of other syngeneic transplants, of blood-forming tissues, immunologically competent cells capable of a graft-versus-host attack grow out of the graft, but they see only "self" in their environment and are not activated. In the case of an allograft or



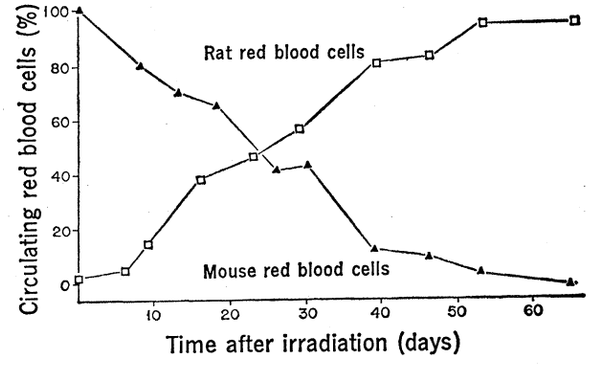


Fig. 5 (left). Effect of the dose of syngeneic bone marrow cells on the amount of radiation exposure that kills 50 percent of the mice in 30 days. [Doherty and Smith (*7*)] Fig. 6 (right). Loss of mouse red blood cells through natural aging and their replacement by rat blood cells in an irradiated mouse given a transplant of rat bone marrow. [Makinodan (*45*)]

heterograft of blood-forming tissue, the cells growing out of the graft see "nonself" in the foreign environment and are activated to produce cells, and presumably humoral substances, that injure the host.

The major sites of injury in graft-versus-host reactions are the lymphatic tissues, the skin, the intestine, and possibly the liver of the host (*12*). The failure of lymphatic tissues to regenerate as they do in syngeneic grafts means that the recipient animal or man cannot adequately control the microorganisms present in the flora of the skin, intestine, and other tissue (*22*). For example, in one human marrow transplant, where an attempt was being made to eradicate leukemia, the patient died apparently from influenza after a successful take of the transplant. The death of another transplant recipient may have been the result of gonorrheal septicemia from an old, inactive pelvic venereal disorder (see *14*). Dermatitis of an extremely serious kind, diarrhea, loss of weight, and sometimes poor liver function are additional manifestations of a graft-versus-host reaction.

In addition to the belief that poor immune function and the cytotoxic action of graft versus host in several organ systems are a basis for mortality from secondary disease, the idea that a metabolic disorder either precedes, coexists with, or results from the graft-versus-host reaction has been proposed. Failure of hair growth and excessive loss of body weight were the primary observations that led to postulation of a biochemical or metabolic lesion. Kretchmar and Price (*23*) point to the metabolic pathways involved in serine metabolism as a central area of interest for this approach.

The graft-versus-host reactions in bone marrow transplantation in humans have been of overwhelming importance and are the major problem in these experiments (*24*). Such reactions are also of great importance in monkey and dog marrow grafts between unmatched donor and recipient. In Table 2 are listed some of the approaches that are being used in attempts to control graft-versus-host reactions in various laboratories.

A technique of potential significance is the experimental design used in mouse marrow grafting. It is based on observations in this species that spontaneous cure and sometimes the avoidance of secondary disease occur even when the genetic differences between donor and host are very great. The attempt is to discover the unknown variables associated with long-lived chimeras (*25*) having foreign bone marrow, on the assumption that this information would be of value in dog, monkey, and human studies (*26, 27*). In mice, age of animal supplying the donor marrow, time of marrow injection after irradiation, number of cells transplanted and other variables were systematically studied in a series of mathematically designed experiments. They revealed significant reductions in mortality from graft-versus-host reaction when the known variables were carefully controlled; but they also revealed the presence of unknown variables probably associated with the environment and past history of experimental mice used in the studies. These unknown or poorly controlled variables are important and need to be investigated for their relevance to larger animal and human bone marrow transplants.

A new contribution, by Hellström *et al.* (*28*), related to unusually successful bone marrow transplants in dogs, suggests that the so-called immunologic tolerance of the long-surviving radiation chimeras in experiments in which irradiation was followed by allogeneic transplants is a result of blocking serum factors produced by lymphocytes growing out of the original bone marrow transplant. The potential importance of this piece of research is very great. If the results are confirmed and the technique is found to be applicable to species other than the dog, the discovery ranks with earlier original observations of recovery from radiation damage through marrow transfusion, the establishment of the transplantation mechanism that led to recovery, and the explanation of secondary disease as a graft-versus-host process.

A long-range goal in marrow transplantation is to use marrow grafts as a means of promoting acceptance of other organs, such as liver, heart, and kidney. Investigations in the mouse have shown that, once the foreign marrow is established, skin taken from the same donor as the marrow can be successfully transplanted without further immunosuppression. The transplantation of foreign bone marrow in lab-

Table 2. Some approaches to the control of secondary disease.

1. Immunological compatibility: histocompatibility typing and matching of donor and recipient
2. Immunological suppression
   a. Treatment of marrow recipient
      a-1. Methotrexate
      a-2. Cyclophosphamide
      a-3. Antilymphocyte serum
   b. Treatment of marrow donor: antilymphocyte serum
3. Removal of immunologically active cells
   a. Manipulation of the marrow in vitro
   b. Cell separation
4. Innate absence of immunocompetent cells: use of fetal and newborn blood-forming tissue from the donor

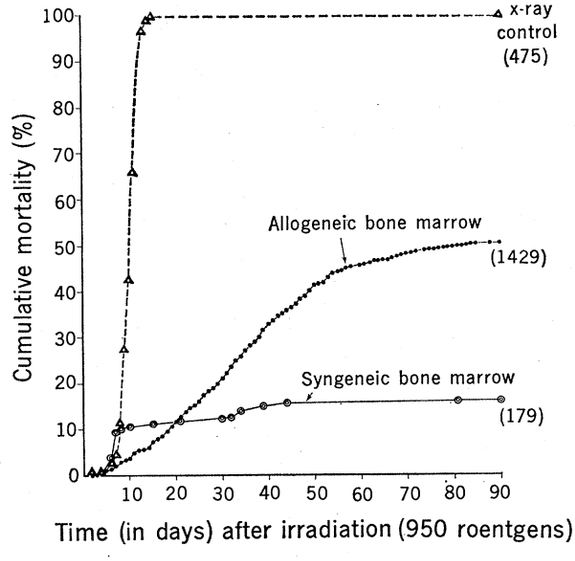


Fig. 7. (Triangles) Cumulative mortality in lethally irradiated mice exposed to x-rays alone (primary disease). (Solid circles) Secondary disease (graft-versus-host reaction) accounts for the mortality after grafting of allogeneic bone marrow. (Open circles) The few deaths after grafting of syngeneic marrow were from primary radiation disease. Numbers in parentheses are the numbers of experimental animals. [Data from Congdon *et al.* (*26*)]

oratory animals constitutes the important feasibility experiment for many of the goals in organ transplantation, in particular that of achieving immunosuppression with only a single-pulsed injury to the host.

Graft-versus-cancer reaction. The potential of immunotherapy in cancer is a very old idea, going back many decades. Probably the earliest observations of importance are the clinical and histologic evidence for natural host resistance to spontaneous human tumors. The histologic evidence is based on unusual cellular reactions in regional lymph nodes near some cancers and on the presence of a lymphocyte infiltration in certain primary tumors. Clinical evidence of curability and of slow tumor growth correlates to some extent with these histologic findings.

In the past decade there has been a veritable deluge of experimental studies demonstrating antigens in induced and spontaneous tumor cells; these antigens are not present in normal cells of the tumor-bearing animal host. The results provide the major justification for use of foreign bone marrow therapy in leukemia and related neoplasms. Graft-versus-cancer therapy is also used when there is an overt oncogenic virus that maintains the leukemia.

Early attempts to use bone marrow grafts in the tumor-bearing animal as adjuncts to permit supralethal exposure to radiation or drugs failed to demonstrate any significant benefits of the marrow other than its ability to promote recovery from the supralethal exposure. Results of current studies, however, seem to warrant continued efforts with graft-versus-cancer and other immunotherapeutic techniques as possible ways of adding to the benefits of standardized treatment regimens (*29*).

Immune Deficiency and Other Genetic Disorders

Bone marrow transplantation experiments indicate the possibility of cure for at least two types of immune deficiency disorders in man (*30, 31*). In the Swiss type of hypogammaglobulinemia the patient is not able to reject foreign cells, thus showing an absence of the host-versus-graft reaction. In this disorder, numerous other measures of normal immune function also reflect the absence of effective lymphatic tissue and its ability to respond to antigenic stimuli.

Bone marrow transplant cures this situation, provided there is histocompatibility between the marrow donor and the recipient, so that a lethal graft-versus-host reaction does not develop. The longest observation, to date, of a child treated with marrow transplant for this rare disease is about 2 years (see *14*). Not every patient so treated has survived the graft-versus-host reaction, but those who have appeared to be cured. The Wiskott-Aldrich syndrome has also been partially reversed by marrow graft (*30*). The ability of marrow stem cells, when grafted, to generate a new lymphatic tissue system of donor-cell origin as well as a new thymus is one of the remarkable features of these cells.

The cure of thalassemia and of sickle-cell anemia in man has been another goal for marrow grafting, but successful results have not yet been attained. In the genetic anemias the host-versus-graft reaction must be avoided through histocompatibility matching of donor and host and through treatment of the host with immunosuppressants. The graft-versus-host reaction is still a major hazard, even when the take of the marrow graft is assured.

Cell Separation, Bone Marrow Banking, and Tissue Culture

Cell separation. All organs and tissues are made up of mixtures of different cell types. The predominant cell type that gives the organ or tissue its unique functional and anatomical character is the parenchyma; parenchymal elements are held together by cells known as stromata. This distinction between a parenchyma and a stroma is a useful but not a perfect concept. In analytical biology and biochemistry, refinement of the analysis by study of a single cell type in any given organ or tissue is a long-range goal. The situation is somewhat similar to that of an earlier era of microbiology when investigators had to work with mixtures of organisms because the technology of achieving pure culture had not been developed.

For bone marrow, lymphatic tissues, and blood forming cells in general, cell separation by physical methods or by a shifting of cell populations (through any of a wide variety of approaches) is an important contemporary goal. The experimental hematologist needs relatively pure cell populations for his analytical studies; the experimental therapist prefers to transplant or transfuse individual cell types according to their functional characteristics.

Cell separation of the peripheral blood by centrifugation is widely used to obtain packed red blood cells, platelets, and white blood cells for therapeutic purposes. Although blood has no stromata to be removed, resolution of the different types of white blood cells into useful functional types has been partially successful (*32*). Separation of thoracic duct lymphocytes into functional classes is a somewhat similar problem.

When bone marrow, thymus, and lymphatic tissue are to be separated, not only do the stromata have to be removed after tissue homogenization but separation of parenchymal elements from their mixtures in the cell suspension (which is now like peripheral blood) has to be undertaken (*33*). The procedures involve great technical difficulties, and this is why the simpler approach of shifting cell populations in vivo in the donor has often been used by the experimentalist, even though, with this approach, only relatively enriched cell populations, rather than pure cell types, are transplanted.

For bone marrow transplantation the immediate goal has been to remove fast-growing, immunologically competent cells that cause lethal graft-versus-host reactions from the cells that give rise to erythropoiesis, myelopoiesis, and megakaryocytopoiesis. Only limited success has been achieved to date.

Bone marrow banking. Maintenance of functional cells and organs for transplantation purposes at 37°C is possible for only very short periods; soon there is significant cell death. For example, at this temperature suspensions of bone marrow cells in tissue culture show 25 percent loss of function after 6 hours and 80 percent loss after 24 hours (*34*). At temperatures ranging between ice-bath temperature and 20°C, the period before major loss of function is longer; some cells survive for several days.

Preservation of bone marrow for use weeks, months, or years after removal from the donor is entirely feasible, and demonstration experiments have been carried out (*35*). A rule-of-thumb approach that makes banking of human bone marrow now entirely feasible is based on long-established animal-husbandry techniques for preserving semen. The empirically derived rates for cooling the cell suspension are 1

degree per minute from about 0° to −15°C, then fast cooling to −76° or −196°C for storage. Rapid warming to room temperature is used to thaw out the cell suspension. Chemical additives that prevent cryogenic injury are glycerol and dimethylsulfoxide.

For further development of long-term banking procedures, studies are needed on (i) detailed control of cooling rates, (ii) the optimum storage temperature, (iii) optimum warming rates, and (iv) the use of nontoxic chemical additives that prevent death of cells during the freezing and thawing process (*36*). The techniques work only for cells in suspension, and attempts to use the same procedures for long-term preservation of solid organs, such as kidney, heart, or liver, or for preservation of the whole animal are still unsuccessful in terms of preserving functionally active tissues.

Tissue culture. A goal of great practical value for human medicine would be to obtain an inoculum of human marrow, explant it to a tissue culture environment, grow large quantities of cells with functional capacity, and use these for transplantation, to restore destroyed or defective marrow (*12*). This goal has never been achieved in any mammalian species, although several attempts have been made. In the present state of tissue culture methodology, cells that survive and proliferate in a tissue culture medium do not restore organ function when reinjected.

Historical Aspects of Bone Marrow Transplantation

Like nearly every research area in biology and medicine, marrow grafting has a multicentric origin, deriving from many related pieces of research and thus reflecting the interacting and interdependent character of progress in science. Bone marrow therapy for a great variety of human blood disorders has been proposed and attempted over many decades (*37*). We now view these seemingly primitive therapeutic experiments with chagrin, but they show the persistence of a basic idea of organ therapy that expresses itself in the availability of useful hormonal extracts from endocrine tissues and that now is extensively studied in the form of tissue transplantation. In the theory of therapeutic processes there seems to be a theme of substitution or replacement that goes back to earliest man, when it was thought that one could gain courage and other virtues by eating the proper kind of flesh.

In 1949 Leon Jacobson and his colleagues at the Argonne National Laboratory in Chicago performed prime experiments based on the blood-forming ability of the spleen in mice to protect against lethal whole-body irradiation.

During World War II the late Egon Lorenz and a group of scientists at the National Cancer Institute, in Bethesda, Maryland, undertook a large project on the effects of long-term irradiation. This project was cosponsored by the Argonne National Laboratory and represented a highly successful interagency agreement between the U.S. Atomic Energy Commission (earlier called the Manhattan Project) and the U.S. Public Health Service.

The close association of the Chicago and Bethesda groups led Lorenz to propose and organize the bone marrow transfusion experiment to prevent death from acute radiation in mice and guinea pigs. His goal was to explain the results obtained by the Argonne group on the spleen's ability to provide protection (*38*). Delta Uphoff of the Bethesda group has preserved an important letter that Lorenz wrote her on 6 August 1950, when he was traveling in Europe. In this letter, now a valuable historical document, he proposes the experiment that his group later successfully performed (*39*).

At the same time that Jacobson and Lorenz were having their remarkable success, the idea of using bone marrow transfusion to treat radiation injury was being tested in independent studies by other workers. These include Rekers and his group, who were studying irradiated dogs at the U.S. Atomic Energy Project, University of Rochester (*40*); Talbot and Gerstner (*41*), who were working with irradiated rats at the U.S. Air Force School of Aviation Medicine; and Hilfinger and Ferguson (*42*), who were using irradiated rabbits in a study at Syracuse, New York.

After Lorenz's work at Bethesda was reported, a great wealth of information developed. Various research centers took up the study of bone marrow and spleen transfusion for the treatment of bone marrow failure. San Francisco, Harwell, Rijswijk, Oak Ridge, Cooperstown, Paris, Boston, Stanford, Toronto, Houston, Prague, London, Baltimore, Villejuif, Seattle, and many other cities were sites of progress in explaining and developing marrow transplantation. Van Bekkum and De Vries have reviewed the early published work in their book *Radiation Chimaeras* (*12*).

When goals are achieved, or even partially achieved, it often pays to examine the processes involved. In my view, the first critical step was the collaboration between the Manhattan Project and the U.S. Public Health Service in interagency research at Chicago and Bethesda. The U.S. Atomic Energy Commission continued to actively promote collaboration between its Argonne National Laboratory and the National Cancer Institute until the death of Lorenz in 1954.

A second important factor in the history of bone marrow transplantation has been the extremely active interest that Alexander Hollaender, emeritus director of the Biology Division at the Oak Ridge National Laboratory, has taken in this field from 1954 to the present. He saw very early the value of genetics, immunology, cell separation, and tissue culture to marrow transfusion studies. Even more significant was his insistent initiation and promotion of international meetings that came to be known as the "Bone Marrow Conference." These ad hoc sessions allowed everyone to have his say and formed a kind of lasting coherence within an international group of investigators that is still at work. Hollaender also emphasized the need for continuing detailed communication between the research clinician and the animal investigator.

Results of the many conferences, visits, and meetings have yielded 20 issues of *Experimental Hematology* since 1957, and, through brief summaries, these publications have given scientists in all countries rapid access to new developments in the field. The attention that marrow grafting in laboratory animals has received, even though its fruitful clinical application in man has been less extensive than that of kidney grafting, has probably been a major impetus in the field of human organ transplantation.

A third factor of importance in the process of applying the work done between 1949 and 1969 to disease problems in man has been the Cooperative Group on Bone Marrow Transplantation in Man, organized by the Transplantation Society at the suggestion of George Mathé of Villejuif, France. This group of more than ten active clinical research units, attempts to deal with the difficult problem of applying

marrow grafts to blood disorders of man.

At present the bulk of data from human studies is derived from work being done in Villejuif, Rijswijk, Leiden, Minneapolis, London, Baltimore, Bethesda, Seattle, Madison, Milwaukee, and still other sites.

The history of bone marrow transplantation is that of men, of ideas, and of facts.

Summary

The goals in bone marrow transplantation are its application to the treatment of diseases arising in the blood-forming tissues of man. Techniques for procuring and grafting marrow are of the needle-and-syringe type and are based on the normal physiological processes in which stem cells circulate through blood and other fluids of the mammalian organism. Destruction of bone marrow by irradiation, chemicals, or unknown agencies provides the immediate experimental system for demonstrating the therapeutic value of marrow transplants. Genetic diseases characterized by abnormal marrow function are also modifiable by grafts of blood-forming tissues. Studies with identical twins are critical experiments for showing the clinical value of grafts, even though the transplanted cells cannot be identified by the usual marker techniques. Among the best results seen with marrow grafting is the presumed cure of certain rare hereditary immune-deficiency disorders of children.

A major problem in bone marrow transplantation—one that delays its wider clinical application—is the immune reaction from cells growing out of the foreign transplant which attack the host (the graft-versus-host reaction). Attempts to use a graft-versus-host response to eliminate tumor cells is a part of the marrow research program.

The history of the processes that led to some of the achieved goals in marrow grafting shows the usual multicentric origin of an idea. Certain individuals play critical roles in developing the idea. Finally, a body of knowledge is accumulated that opens up or limits prospects for the future.

In bone marrow transplantation, future achievements will depend in part on the progress that is made in the areas of cell separation, bone marrow banking, and tissue culture.

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