Bone and Marrow Transplants in the Rat

By Andrew D. Dorr, William C. Moloney, Geraldine Dowd and Antonio E. Boscetti

During the past several years the growth of bone marrow has been studied in transplants of autologous rib sections to the spleen and other sites in the dog. To extend these observations and utilize not only autologous experiments but also grafts between inbred animals, a method for transplanting tibial bone and marrow to the rat kidney was developed in this laboratory. Details of the technic and results of studies on this ectopic transplant of bone and marrow are described in this paper.

Methods and Materials

Three hundred adult rats weighing between 200 and 300 Gm., were etherized, a section of tibial bone proximal to the knee joint, 1.0 to 1.5 cm. in length, was removed and a longitudinal hemisection containing bone and marrow was implanted into the animal's left kidney via a retroperitoneal approach. The capsule and cortex were incised and the tibial specimen inserted into the parenchyma along the lateral border of the kidney (figure 1). The leg and flank wounds were sutured, 20,000 units of penicillin administered, and the animal was maintained on a regular diet until sacrificed. Observations on these autografts were carried out for periods varying from 6 hours to 18 months. Bone marrow either as such or mixed with cortical bone shavings was injected into the kidney with a #16 bone-marrow needle and stylet. The marrow was obtained by scraping the exposed cavity free of marrow, filling the needle with marrow and injecting the contents into the kidney by pushing the stylet through the needle. Small cortical bone shavings were obtained by whittling the cortex with a knife blade.

As controls, in a group of 48 rats, hemisections of boiled autologous bone and glass tubing without marrow were placed in the kidney and followed for 30 to 50 days. The boiled autologous bone was prepared by scraping the hemisections of bone free of as much marrow as possible and placing it in boiling water for 10 minutes. Pieces of glass tubing were prepared in size and shape resembling the hemisections of bone.

In early experiments marrow was obtained with a scalpel blade and smears prepared on cover slips. Later the paint-brush technic of Burke et al. was utilized with much more satisfactory results. The smears were stained with Wright-Giemsa and to further identify cellular elements histochemical stains for peroxidase, esterase and alkaline phosphatase activity were carried out on some preparations. For histologic sections, tissues were fixed in Zenker's solution and stained with phloxine methylene blue and Giemsa.

The mechanism of marrow growth was further studied by the use of H Thymidine. Bone and marrow cells were labeled in vivo by intracardiac injection of two µc. of H Thymidine per Gm. of body weight one hour before transplant. In vitro labeling...
Fig. 1.—A cross section of rat kidney showing recently implanted autologous bone and marrow in situ. This specimen has a large section of tibia for purposes of demonstration.

was accomplished by incubation of the transplant for one hour in rat serum containing two μc per ml. of tritiated thymidine. The incorporation of the H₃ thymidine into the transplanted cells was studied by serial autoradiographs carried out at various intervals on methyl alcohol-fixed smears of the transplanted bone and marrow.

RESULTS

Successful growth of autologous bone and marrow was obtained in 90 per cent of transplants to the rat kidney. As shown in figure 2, new bone and marrow with all cellular elements proliferated in a normal fashion. Early in these experiments HN₂ was used prior to transplant in order to enhance bone and marrow survival in ectopic sites. When it became evident that this preliminary treatment was unnecessary, the use of the alkylating agent was discontinued. In addition to the kidney, spleen, liver, round ligaments, subcutaneous tissue and omentum were used as transplant sites. Because of the ease of technic, freedom from postoperative complications, and isolation in a single organ, the kidney afforded many advantages over other locations in the rat. An important factor which greatly influenced transplant survival was the size of the bone and marrow specimens. Large bone sections caused mechanical injury and predisposed to necrosis and infection. In these experiments bone length was limited to 1 to 1.5 cm.

The fate of the transplant was studied by serial smears and histologic sections at the transplant site. Periods of observation ranged from 6 hours to 18 months post-transplantation. Sequential studies revealed that marrow elements rapidly disappeared and only a scattered few cells were found on smears taken up to the third day. As early as two days following transplant, large mononuclear cells were noted and these cells persisted in some preparations up to twenty-one days (fig. 3). In the smears, few marrow elements were present for seven to fourteen days, then cells showing morphologic characteristics similar to normal marrow elements appeared in increasing numbers. Histochemical stains were of assistance in identifying the immature and large mononuclear cells. Rat myeloid cells, including promyelocytes but not myeloblasts, showed positive reactions to alkaline phosphatase, esterase and peroxy-
dase stains used in these studies. Osteoblasts on the other hand were positive for alkaline phosphatase but negative for peroxidase and esterase activity. Red cell precursors and the large mononuclear cells were negative for all three histochemical stains.

Examination of the histologic sections provided important evidence on the manner of marrow proliferation in autologous transplants which was not demonstrable by smear preparations. In sections taken on days 1, 6 and 8, small residual hematopoietic foci were disclosed between the surviving old bone spicules and it was apparent that these foci extended with new bone growth into the adjacent marrow cavity (fig. 4). By the 15th to 21st post-transplant days, marrow filled the intertrabecular spaces of both old and new bone and had the appearance of normal marrow in all respects. After 18 months, bone marrow transplanted to the rat kidney was identical morphologically with marrow taken from normal sites in the same animal. Bone and marrow transplants from inbred litter mates (Furth-Fischer) behaved in a fashion similar to autologous grafts.

Autologous marrow alone, homologous bone and marrow, boiled autologous and homologous transplants, autologous bone chips with marrow and sections of glass tubing implanted in the rat kidney failed to demonstrate growth of bone or marrow cells. However, large mononuclear cells were present in smear preparations taken at the transplant sites of homologous bone and marrow, boiled autologous bone, boiled homologous bone and glass (fig. 5).

The experiments in vivo and in vitro with tritiated thymidine were carried out to further identify the cells in the bone marrow transplant. When $H_t$ thymidine was administered I.V. to rats with autologous transplants in the kidney,
marked activity occurring in the nuclei of immature myeloid and erythroid cells was noted in the autoradiographs. In other experiments tritiated thymidine was given to animals I.V. one hour before bone and marrow were transplanted to inbred litter mates. Marrow smears taken on the 4th, 8th, 21st and 26th days following transplant produced autoradiographs indicating labeling of nuclear remnants on days 4 and 8; no labeling was noted in later preparations, nor did normal erythropoietic precursors show any activity on day 8.

When specimens of bone and marrow were labeled in vitro with tritiated thymidine and then transplanted to inbred litter mates one hour later, no marrow growth was observed up to 15 days post-transplant in these preparations. It was concluded that at this dose level radioactivity was sufficient to inhibit growth of the transplant. In earlier transplants with abundant large mononuclear cells present, only a rare cell showed labeling and this may have been due to phagocytosis of labeled cellular particles.

Discussion

The perplexing problems of marrow growth have been investigated in many ways. Since the early work of Baikow and Chiari, many studies have been reported on the transplantation of autologous bone and marrow to extramedullary sites. Experiments in various animal species have demonstrated that transplants of live and devitalized bone, marrow, cartilage, muscle, and uroepithelium may bring about growth and proliferation of bone and marrow, usually after prolonged delay. This phenomenon is considered to be a form of "induction" or metaplasia. A second mechanism of ectopic marrow growth is
Fig. 4.—A section of autologous bone and marrow taken on the 6th day at the transplant site in the kidney; this specimen shows islands of active marrow between spicules of old bone along with new bone formation (objective 40x). To the left of the broken line, the arrows are directed at spicules of old bone enclosing residual active marrow further evidenced by the admixture of fat cells. To the right of the broken line, arrows are directed at a few of the anastomosing trabecula of new bone enclosing a fibrocellular marrow cavity.

Based on the histologic demonstration that islands of active hematopoietic cells survive at the transplant site and subsequently proliferate. Evidence for the induction theory arose from the experiments of Urist and McLean. These investigators transplanted fracture callus to the anterior chamber of the rat's eye and observed bone and marrow formation within 30 to 36 days. Bridges and Pritchard implanted bone and devitalized callus, muscle and urinary bladder epithelium in the renal capsule of the rabbit and observed the formation of bone and later marrow after a latent period of 35 to 36 days. They could not repeat these results in the rat and guinea pig. Similar observations were recorded over 30 years ago by Huggins and more recently by Spellman et al., following transplantation of uroepithelium. Unlike marrow growth due to cellular survival, bone and cartilage formation usually precedes marrow growth in the induction process. Proliferation under these circumstances is attributed to a metaplastic effect by the transplant on the fibroblast or other connective tissue cells of the host. The recent work of Bell and Knudston supports this concept. They found that in experiments on induction of bone by implantation of gall bladder and urinary bladder epithelium, bone formation did not take place in smooth muscle or in organ sites. These authors postulate than an inductor substance, present in gall bladder and urinary bladder epithelium, promotes metaplasia of connective tissue fibroblasts to osteoblasts.

Levander, DeBruyn and others, in the course of serial studies, followed ectopic marrow transplants and noted the disappearance of marrow cells, con-
Fig. 5.—Smear taken on the third day following transplantation of glass to rat kidney. Note presence of lymphocytes and large primitive mesenchymal cells similar to those found in fig. 3 (objective 40x).

nective tissue proliferation and much later marrow growth which they inferred was due to an induction phenomenon. In their experiments in dogs Owen et al.\textsuperscript{1} described rapid disappearance of marrow cells followed by the appearance of large mononuclear cells and later by regrowth of bone and marrow. The mechanism of regrowth was at first attributed to these large cells which were considered to be pleuripotential-stem cells. After further studies it was concluded that these cells were histiocytes and that the source of the marrow cell reproliferation was the transplant itself.\textsuperscript{4} In our studies a similar sequence of events was noted in the rat kidney. Large mononuclear cells were present but since they were also found following implantation of boiled bone, homologous bone, glass and other materials, it seems reasonable to assume that these cells were histiocytes reacting to a foreign body. The experiments with tritiated thymidine were not conclusive but there was no indication of a rapid uptake of thymidine which might have been expected in primitive marrow cell precursors.

Our observations on the survival and proliferation of bone and marrow transplants were in close accord with the findings of LaCroix\textsuperscript{21} in the rabbit renal capsule and of Berman and Kaplan\textsuperscript{22} in the mouse peritoneum. However, LaCroix found that marrow alone grew in the renal capsule of the rabbit while Urist and McLean noted similar results using the anterior chamber of the rat's eye. Danis\textsuperscript{23} has also grown marrow in the anterior chamber of the rat eye and he states that marrow implanted alone in other rat tissue proliferated successfully. On the other hand, Jacob et al.\textsuperscript{1} were unable to grow marrow alone in the spleen and other sites in the dog, and this has been our ex-
pericence in the rat. It is difficult to reconcile these contradictory results; however, differences in species, transplant sites and technics probably all play some role.

Ectopic autologous bone and marrow implants serve as an in vivo marrow culture preparation. Functioning in an isolated organ, such as the spleen or kidney, repeated observations may be carried out on the proliferation and maturation of all marrow elements. Since the blood supply to the transplant site can be isolated and controlled, a variety of experiments can be devised to note the effects of anoxia, drugs, chemicals, ionizing radiation and other agents on the growth and development of bone and marrow cells.

**Summary**

A simple method for transplantation of autologous bone and marrow to the rat kidney is described and the usefulness of this preparation for research on bone and marrow growth is pointed out.

While an induction mechanism cannot be conclusively ruled out by these studies, the histologic evidence strongly supports the concept that growth of implanted bone and marrow is due to survival of small islands of active bone and marrow cells and from these foci, repopulation takes place.

**Summario in Interlingua**

Es describite un simple methodo pro le transplantation de autologe osso e medulla ad in le ren del ratto. Es signalate le utilitate de iste preparato pro le recerca in re le crescentia de osso e medulla.

Ben que le hic-reportate studios non exclude categoricamente le possibilitate de un mechanismo de induction, le constatationes histologic supporta forte-mente le conception que le crescentia del transplantate osso e medulla resulta del superviventia de micre insulas de active cellulas de osso e medulla e que il es ab iste foci que le repopulation occurre.

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**References**

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