Studies on Marrow Histogenesis
II. Growth Characteristics of Extramedullary Marrow Autotransplants[1] (36008)

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Autotransplantation of bone marrow to extramedullary sites results in regeneration of a new marrow organ and establishment of a marrow nodule surrounded by a shell of bone. Previous reports have dealt with the description of this developmental model (1), comparison of red and fatty marrow (2) and the supportive qualities of various tissues for extramedullary marrow implants (3). The present report deals with the growth characteristics of marrow tissue autotransplanted to subcutaneous tissue of the rat.

Materials and Methods. Male albino rats weighing 200–300 g were used in these studies. They were kept under normal laboratory conditions and were fed Purina Lab Chow and water *ad libitum*. Operations were carried out under aseptic conditions. Sodium pentobarbital was used for anesthesia.

Autotransplantation of marrow was carried out by a technique previously described (3). The knee joint was exposed and an opening was drilled through the articular surface of the femur. A polyethylene tube (gauge 160) was driven gently into the marrow cavity for the whole length of the femoral shaft. The free end of the tube was then clamped and the tube was slowly removed. The tube, now containing marrow tissue, was weighed. The marrow was then implanted in a pocket incised in the subcutaneous tissue of the abdomen. The weight of implanted tissue was determined by subtracting the weights of the polyethylene tube before and after implantation of marrow. The implants were removed at various intervals and weighed again. They were then fixed and processed for light and electron microscopy. Two groups of animals were studied.

Group I. Thirty-one implants varying in weight from 4 to 129 mg at the time of implantation were removed after 5 weeks.

Group II. Seventy-one implants with initial weights varying between 40 and 55 mg were removed on days 1, 2, 4, 5, 7, 9, 12, 15, and 30. To prevent variation due to factors other than initial weight, in this latter group the animals were chosen from the same litter, were of comparable weight, and to prevent possible diurnal variation in regenerative processes, the operative procedures were carried out in the morning.

Results. Group I. The correlation between the weight of implanted and recovered tissue is represented in Fig. 1. When the weight of implanted tissue is less than 15 mg regeneration of marrow does not occur. For implants exceeding this critical mass, a linear correlation between the weights of implanted and recovered tissues exists ($r = 0.7285$). The weight of recovered tissue is always less than that of implanted tissue with an average of about 25% (range 6–46%).

Group II. Figure 2 shows the change in the weight of implants as a function of time after implantation. It is noted that for the first 4 days after implantation the weight of implants increases to nearly 400% of the initial weight. By day 5, the weight rapidly decreases and then the rate of this decrease slows down. By day 7 the weight of implants equals the initial weight. It then further decreases and by day 12 it stabilizes around

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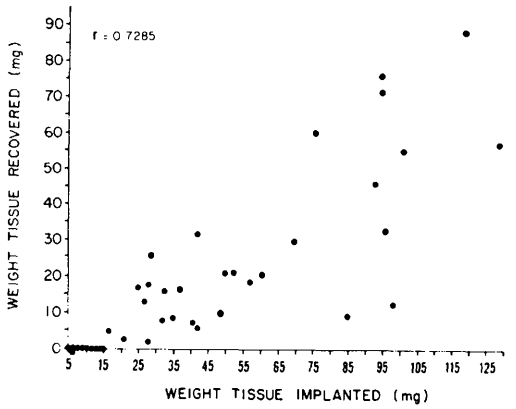

FIG. 1. Correlation between the weights of implanted marrow tissue and the bone marrow nodule recovered after 35 days. No marrow growth occurs when the weight of implanted tissue is less than 15 mg.

25% of the initial weight. These changes in the weight of implants correlate with the histological changes occurring during the growth period of the marrow. During the first 2 days the fragment of marrow becomes vascularized by invading capillary networks from the surrounding tissue. By days 3 and 4 the vascularization proceeds further and at the same time, proliferation of fibroblasts

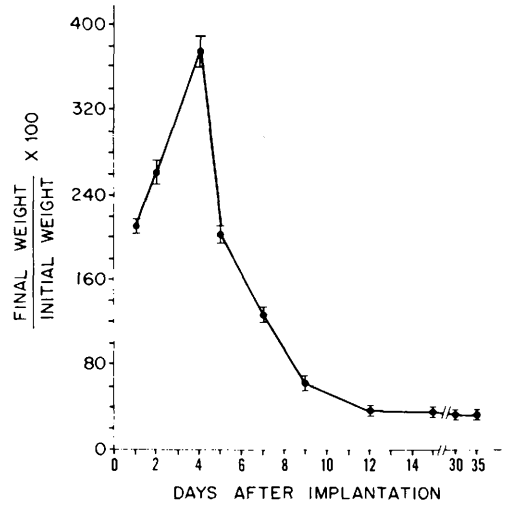

FIG. 2. Changes in the weights of implants, as a function of the time after implantation. The weight of implant is expressed as percentage of the weight of implanted tissue. Each point is the mean for three to nine experiments (± SEM).

(mesenchymal cells) richly interpersed by collagen is evident. This gives rise to a picture of granulation tissue and, at this stage, separation of the implant from surrounding connective tissue is difficult since there are no clear-cut boundaries. The collagen then concentrates in some areas to give rise to osteoid tissue. At this stage vascularization appears to be decreasing. Within the interstices of osteoid bone a loose connective tissue is left which consists of macrophages, degenerating fibroblasts, and large vascular channels which can be identified as marrow sinusoids. This space is the primordial marrow cavity. Degenerating fibroblasts in this space are numerous and they can be recognized by the presence of large autophagosomal structures (Fig. 3). Hematopoietic activity, heralded by the presence of numerous lymphoid cells, begins on day 7 and proceeds outside the marrow sinuses. Intense hematopoietic activity is associated with resorption of osteoid bone beginning on day 9. At this stage, many osteoclasts can be identified. Expansion of hematopoiesis and resorption of bone proceeds slowly and after 4–5 weeks, results in the establishment of a marrow nodule.

Discussion. The results presented here, indicate that for the development of new marrow tissue a critical mass of marrow is necessary, below which the developmental process does not proceed. This may explain the failure of most previous attempts to grow the marrow tissue in ectopic sites (4–11). This critical mass on a weight basis is roughly 15 mg. Above this critical mass a linear correlation exists between the weight of implanted tissue and that of recovered tissue. As judged from the size of scatter, this correlation is not a perfect one. It is likely that a multiplicity of factors influences the weight of the recovered nodule. These factors include the age and state of health of the animal, variation in vascularity of the supportive tissue in which the implant is made, variation in the operative procedure, particularly the amount of bleeding which invokes inflammatory response in the supporting tissue. Another factor may be the shape and spread of implanted tissue. When the implant is spread thin over a large area of subcutaneous tissue,

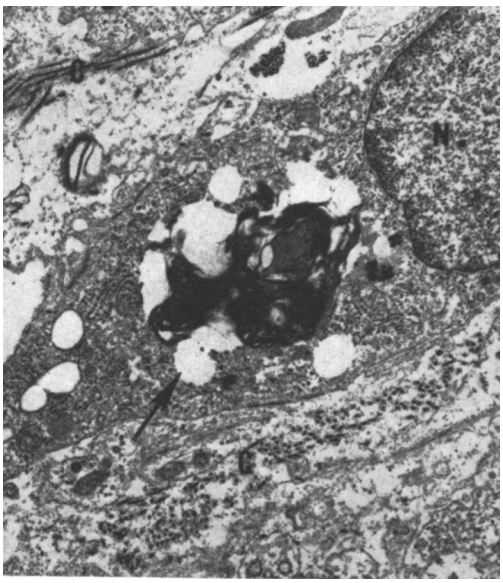

FIG. 3. Portion of a degenerating fibroblast is shown in this electron micrograph. The nucleus (N) is on the right. Note the large autophagosome (arrow). Few strands of collagen are also seen. (C) Subcutaneous implant on day 7. (× 7,500)

penetration of supporting vessels reaches most parts of the implant in time to prevent necrosis due to lack of vascular blood supply, otherwise, the central part may become necrotic before the vessels can reach it. In our second group we attempted to avoid variations due to these factors and this reflects in the smaller range of weights of the recovered implants.

The increase in weight during the first 4 days can be explained by vascular invasion carrying an intense circulation throughout the implant. Proliferation of fibroblasts and production of collagen may contribute to this weight increase during the last 2 days. After day 4, a decrease in vascularity is associated with a rapid fall in the weight of tissue and by the end of the first week and the beginning of the second week, during degeneration of fibroblasts, a further decrease in weight occurs. Cell degeneration and death, known to exist in many developmental systems (12), is intense enough to explain some of the reduction in the weight of the implant. After this period, proliferation of hematopoiesis is associated with resorption of bone, and the two processes may cancel one another, and, as a result, there is no net weight change in the implant after the first 10 days. All of this weight change must be associated with a reduction of volume. After the bone frame is set, the volume cannot diminish.

Summary and Conclusions. Based on these data, we conclude that in the rat:

1. A critical mass of marrow is necessary for extramedullary marrow autotransplants to grow. This mass is roughly 15 mg of tissue.

2. Above this critical mass, a linear correlation exists between the weight of implanted and recovered tissues.

3. During the first 4 days of regeneration, the weight of the implant increases to 400% of initial weight and this may be explained by intense circulation through the implant and, in part, by intense proliferative activity in fibroblasts. After day 4, a decrease in vascularity of the implant and degeneration of many fibroblasts results in decrease in the weight of implant which begins to stabilize around day 8.

4. The final weight of implant is always less than the initial weight (average 25%), indicating a loss of tissue during the regenerative processes.

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