Studies on Marrow Histogenesis
I. The Site of Choice for Extramedullary Marrow Implants[1] (34585)

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Successful implantation of marrow fragments in extramedullary sites has already been reported (1–6). After such implantation, a sequence of histogenetic events takes place resulting in reconstruction of marrow microcirculation and a nodule of hematopoietically active marrow encapsulated by a shell of bone.

Since this process may serve as a useful model for studying the histophysiology of marrow, a further investigation of the requirements of the implants was done. The present report deals with the choice of site for extramedullary implantation of the marrow.

Materials and Methods. White Wistar rats of both sexes were used in these experiments. Animals were maintained under standard laboratory conditions and were fed Purina Rat Chow and water *ad libitum*. Anesthesia was given by intraperitoneal injection of sodium pentobarbital, 5 mg/100 g body weight. All operations were carried out under sterile conditions.

The method for obtaining fragments of marrow has been described in previous communications (1, 7); in short, a window is made in the tibia or femur using a low-speed dental drill. Marrow for implantation is obtained by polyethylene tube or lifted out on the tip of a spatula.

For implantation in the subcutaneous tissue, a pocket is made using blunt-tipped scissors, and the marrow fragment is placed within the pocket.

Implants were made in the spleen, either deep in the splenic tissue or in the subcapsular area; in the first case, a deep incision was made in the spleen, a small fragment of splenic tissue was removed and was replaced by the fragment of marrow. The two edges of the incision were then brought together and held for a few minutes until a clot formed. Generally, the bleeding was not heavy, and the incision did not open again. In a few cases oxidized cellulose (Oxycel gauze-type, Parke-Davis) was used to hold the incision closed, but this did not appear to be of absolute necessity. For implantation in the subcapsular area of the spleen, a small abrasion was made on the external surface of the organ, and the marrow fragment was placed on it. The fragment adheres to the supportive organ after the clot is formed and does not become loose or disconnected. Later, the splenic capsule extends to cover the implant.

A similar method was used for implantation in hepatic and renal sites. For implantation into the omentum, the two layers of the omentum were separated from its gastric edge and dissected carefully for 1–2 cm. The fragment of marrow was then implanted between the two layers. For implantation in adipose tissue, the perirenal fat pad was chosen; a small incision was made using the tip of the scalpel, and the marrow fragment was inserted deep into the adipose tissue.

Implanted tissue was removed after 5 weeks for examination and light microscopic study. This appears to be the earliest time that the histogenetic process is complete in all cases.

Results and Discussion. Table I summarizes the results of 267 implants in different sites. Implantation was invariably successful when it was made in the subcutaneous tissue

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TABLE I. The Results of Marrow Implantation in Different Extramedullary Sites.

Site	No. of experiments	No. of takes	Per cent of takes
Subcutaneous tissue (abdomen)	122	122	100
Spleen	38	37	97
Kidney	26	25	96
Liver	20	18	90
Omentum	40	19	47.5
Fat pad	8	2	25
Subcutaneous tissue (foot)	9	0	0
Subcutaneous tissue (tail)	4	0	0

of the abdomen; in the subcutaneous tissue of the dorsal aspect of the foot and midtail, the implants did not survive. The latter sites were chosen in order to investigate the effect of temperature on the activity of marrow nodules. Petrakis (8) has shown that a temperature gradient exists between the tail and central parts of the body, and classical experiments by Huggins and Blocksom (9)have suggested that this temperature gradient may account for the difference in intensity of hematopoiesis in central and peripheral parts of the body. The failure of marrow fragments to survive in the midtail and the dorsum of the foot could be due to lower temperature in these areas. On the other hand, greater tissue tension in these sites may have interfered with the vascularization and growth of the implants; vascularization has been shown to be a crucial step in the initiation of the histogenetic process (10).

Of 38 implants made in splenic tissue, 26 were in the subcapsular area and the results were always positive. The 12 others were made within the splenic tissue, and there was only one negative result.

Of 40 implants which were made in omentum, 36 were randomly distributed, and implantation was successful in 16 cases, while when implantation was made in a visibly vascular section of omentum, three cases out of four gave a positive result. This may reflect the requirement of a vascular bed for growth and regeneration of the marrow; omentum, being a poorly vascularized tissue, does not support the growth of marrow optimally.

In accordance with this are the results of implantation in perirenal fat pad which is also a poorly vascularized tissue, and consequently the implantation was successful only in two cases out of eight. Liver and kidney both appeared to be suitable for implantation; occasional negative results could be due to the fact that the implants became disconnected from the supportive bed.

The weight of the recovered nodules, when compared to that of the initial implant, was always less. Figure 1 shows such a relation for 20 experiments when implantation was made in the subcutaneous tissue of the abdomen. Although there is a wide variation in the weight ratio of recovered and implanted tissues, the average ratio is 24%. This average also holds true for implants made in the kidney and the subcapsular area of the spleen. However, the average ratio for im-

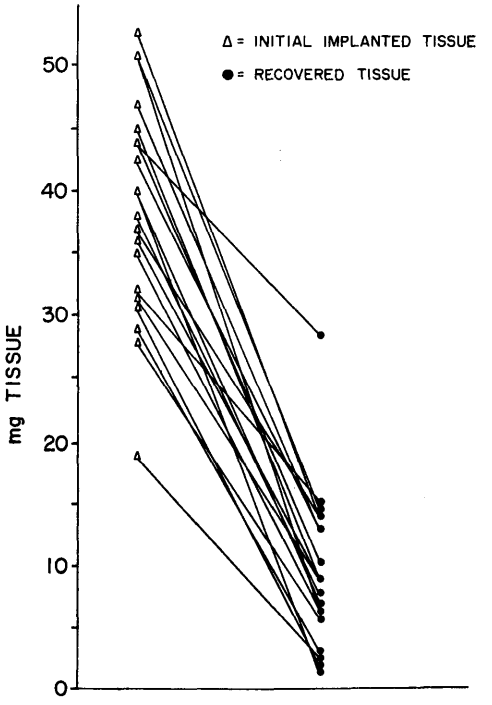

FIG. 1. Initial weight of marrow implants as compared to that of the nodule recovered after 5 weeks in 20 experiments. The implants were made in the subcutaneous tissue.

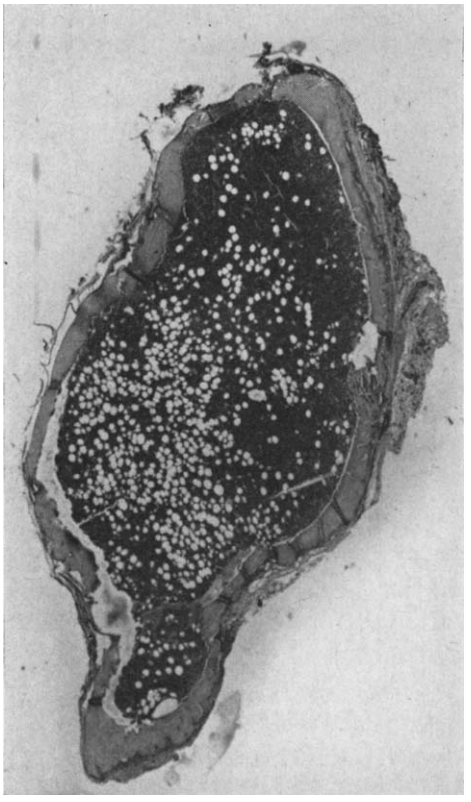

FIG. 2. Histologic section of bone marrow nodule recovered 5 weeks after implantation in the subcutaneous tissue; note the thin capsule of bone and the dense hematopoietic tissue. ×20.

plants made in poorly vascularized tissues such as the omentum and the perirenal fat pad is 3% and never exceeds 10%. This implies poor growth and possibly necrosis of the implant due to poor vascular support.

Microscopic study of the recovered nodules showed that whereas the nodules obtained from subcutaneous renal or splenic sites where hematopoietically very active and encapsulated only by a thin shell of bone (Fig. 2) nodules obtained from the perirenal fat pad and the omentum consisted only of bony tissue with very few hematopoietic elements (Fig. 3). Nodules obtained from hepatic sites were generally less active than those recovered from splenic, renal, or subcutaneous sites but decidedly more active than what was obtained from the omentum or the perirenal fat pad.

Previous work (2) has suggested that the site of choice for marrow transplantation is the subcapsular area of the kidney. Based on the present data, however, we conclude that the site of choice for extramedullary marrow transplantation is the loose, lean subcutaneous tissue, such as that of the abdomen, for the following reasons:

1. Implantation of marrow in this site is invariably successful. So far, we have used this site in more than 1000 experiments which are not included in the present data because the design of experiments was somewhat different. We have met with no failure which can be attributed to the site of implantation.

2. Hematopoiesis is most intense in nodules recovered from subcutaneous sites (as well as spleen), and on a weight basis the ratio of recovered and implanted nodules is highest.

3. The subcutaneous site is the least trau-

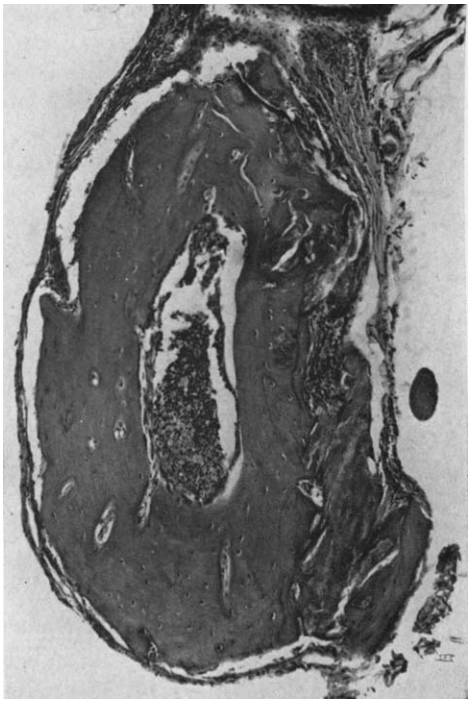

FIG. 3. Histologic section of a bone marrow nodule recovered 5 weeks after implantation in the omentum; note the thick bony capsule and scanty hematopoietic tissue. ×90.

matic, most accessible, and, therefore, most practical site for implantation.

Summary. Extramedullary marrow transplantation results in the formation of a nodule of hematopoietically active marrow encapsulated by a shell of bone. The site of choice for such implants is the loose subcutaneous tissue such as that of the abdominal wall; the high rate of success and the easy accessibility makes these implants a useful model for the study of the histophysiology of the marrow.

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