In summary, the data suggest that two factors influenced the degree of change in behavior after amygdalectomy—the amount of tissue removed and the age of the operated animal. Total removal of the uncus including amygdala and the cortex of uncus produced the most severe result (in 5AU and 6AU) while removal of amygdala alone along with a small portion of cortex of uncus anteriorly produced a lesser change (in 9A and 4A). The latter type of lesion appeared to produce lesser effects in younger animals (2A and 3A) as seen from Fig. 1.

It is more difficult to specify concisely the change in behavior. The change is toward social indifference. Although operated animals react appropriately when interaction is forced upon them, they appear retarded in their ability to foresee and avoid dangerous confrontations. They do not initiate social activity. They fail to seek out and reestablish their membership in the group. When juxtaposed to their group, these operated animals tend to be attacked and banished as would-be strangers from other groups, perhaps because of inappropriate behavior and responses to approach. As solitary animals, they are vulnerable to attack and unable to compete for food.

In another study (*8*), conducted simultaneously on the effects of total amygdalectomy in free-ranging vervets (*Cercopithecus aethiops*), a somewhat similar result was obtained in that all operated subjects withdrew from social interactions, would not respond to solicitous behavior by normal group members, and none of the operated animals were observed to rejoin a social group.

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Origin of Repopulating Cells after Localized Bone Marrow Depletion

Abstract. *The restoration of marrow in a mechanically depleted segment of rabbit femur is locally determined and apparently initiated by cells normally resident in bone. This conclusion follows from results of two types of radiation experiments: local x-irradiation of the femur shaft immediately before depopulation and x-irradiation of the total body with exception of the femur diaphysis which was depopulated either just before or after irradiation. In contrast to the rapid initiation of marrow restoration in an unirradiated femur, there was little regeneration during the first 3 weeks in an irradiated femur. Recovery of the shielded depopulated femur in the rabbit that otherwise received total-body irradiation was similar to that in the unirradiated animal. Hence, it would appear that the seeding of circulating hemic stem cells is not essential to repopulation and that cells with hematopoietic potential are present in osseous tissue.*

When marrow is removed from a femur shaft either surgically (*1*) or by perfusion (*2*), trabecular bone encroaches temporarily on the evacuated space and the onset of obvious marrow restoration coincides with revascularization of the cavity and resorption of the invading trabeculae, the entire regenerative process being completed within a matter of several weeks. The stimulus for such regeneration appears to be locally determined, since the tissue that is removed represents only a small percentage of the total marrow cells and does not result in any significant perturbation of the number of peripheral blood cells. Although it is possible that repopulation is accomplished by a few cells that may have been left behind in the shaft, microscopic examination of the endosteal surface immediately after marrow expulsion by dextran perfusion has failed to disclose adherent cells (*2*). Autoradiographic analysis of marrow imprints after tritiated thymidine (^{3}HTdR) labeling also reveals that repopulation is apparently not initiated by a growing edge from the epiphyseal marrow (*2*). The studies reported here indicate that seeding of circulating stem-type cells is not essential for repopulation of an evacuated femur segment. Rather, the results suggest that the regenerative process may be initiated by cells normally resident in bone.

New Zealand white female rabbits, weighing 2 to 2.5 kg, were used as the experimental animal, and x-irradiation was employed to determine the local or systemic origin of the repopulating cells. Bone marrow was removed from a 3-cm segment of the right femur by perfusion with a dextran solution in a manner described previously (*2*). In brief, marrow removal was accomplished by perfusion and simultaneous aspiration through Turkel needles inserted into the proximal and distal ends of the femur diaphysis of the test animal under sodium pentobarbital anesthesia. Two types of radiation experiments were performed: (i) local x-irradiation of the femur shaft with 1000 r immediately before depopulation; (ii) x-irradiation (800 r) of the total body, with the exception of the femur shaft that was depopulated either just before or just after irradiation. The x-ray source was a Maxitron (General Electric) operated at a peak of 300 kv, 20 ma, half-value layer 1.65 Cu. For irradiation of the femur shaft only, the target surface distance (TSD) was 30 cm and the dose rate 360 r/min, the rest of the body being protected by a 3-mm lead shield. When the total body, except for a segment of the right femur shaft, was irradiated, the TSD was 120 cm and the dose rate 22 r/min. In this instance, the femur was shielded with 6 mm of lead; the length of shielded shaft was somewhat less (2.7 cm) than that of the depopulated area (3 cm) to minimize the possibility of sparing adjacent normal marrow. The effectiveness of lead shielding was verified by appropriate placement of lithium fluoride dosimeters.

Marrow restoration was evaluated by determining the incorporation of ^{3}HTdR into DNA and the number of cells per unit weight of marrow in conjunction with differential counts. In each treatment group two to six experimental and two control rabbits were killed at frequent intervals. One hour before the rabbits were killed they were injected intravenously with 0.5 microcurie per gram of ^{3}HTdR (specific activity, 11

curies per millimole). Right and left femurs were removed and the marrow from each was divided into two pieces and weighed. Each piece of marrow was then suspended in 4 ml of 0.9 percent saline and mixed with an electric stirrer. The nucleated cells in 0.1 ml of the suspension were enumerated with a Coulter counter, model A. Smears were prepared from another 0.1-ml portion and treated with Wright's stain for differential counts. The remainder of the suspension was used for DNA extraction; tritium activity of the extracted DNA was measured in a Packard scintillation spectrometer. Further details of the methods are given elsewhere (*2*). Since irradiated animals were included in this study, the status of marrow restoration in the depopulated femur was related to marrow indices in femurs of normal rabbits rather than to the contralateral limb as described in our earlier work (*2*).

The pertinent results are summarized in Fig. 1. There was little, if any, repopulation in the locally irradiated femur segment by 21 days after removal of marrow as revealed by either cellularity or uptake of ^{3}HTdR into DNA. In contrast, recovery of the shielded depopulated femur in the rabbit that otherwise received total-body irradiation was essentially similar to that in the unirradiated rabbit. Repopulation occurred eventually even in the femur that was irradiated with 1000 r. This began during the 4th week after removal of the marrow, and cellularity reached normal levels by 39 days. In the unirradiated femur, recovery commenced within the 1st week after depopulation and cellularity was in the normal range by 26 days. Erythroid and myeloid elements (erythroblasts and myeloblasts) appeared in the unirradiated femur by 8 days after depopulation whether or not the remainder of the body was exposed to x-rays. On the other hand, such cells were not obvious in the locally irradiated femur until the 4th week.

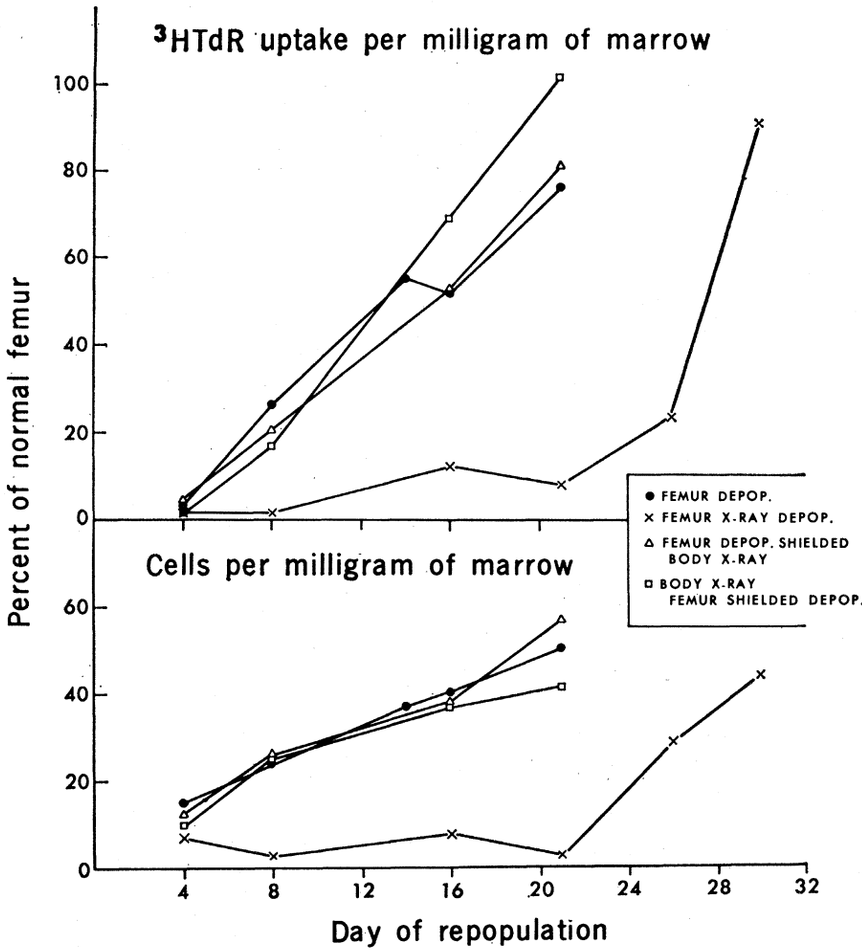

Fig. 1. Effect of local and body x-irradiation on initiation of marrow restoration in a mechanically depleted femur shaft. (The status of marrow restoration is related to control values obtained in 144 femurs. Each experimental point is based on observations in two to six rabbits. Cellularity refers to all cell types with exception of mature granulocytes and erythrocytes.)

These findings point to the local origin of the marrow repopulating cells. It will be recalled that the radiation dose to the femur was 1000 r and that to the body 800 r. The former study was performed first, and in subsequent work with body irradiation a lower dose was used to permit survival of most of the rabbits during the ensuing several weeks. However, this dose difference should have only a minor influence on the basic comparison of the significance of femur and body irradiation. Studies in progress reveal that local irradiation of the femur even with 500 r also results in a delay in marrow repopulation. If circulating hemic stem cells played a prominent role in the initiation of marrow regeneration, we would have anticipated some impairment when the body was given 800 r with the femur shielded. None was observed. Based on studies in mouse and rat, only a few percent of hemic colony-forming cells would be expected to survive after 800 r (*3*), a dose that falls within the acutely lethal range for the rabbit. It is noteworthy that there was no difference in onset of regeneration in animals irradiated just before or just after femur depopulation, the femur being shielded in each case. Hence, we may conclude that the responsible cells are not trapped in the blood clot formed immediately after marrow expulsion. From the foregoing considerations, it is unlikely that local irradiation of the femur impairs regeneration by altering the accessibility of circulating stem cells through, for example, a delay in revascularization of the depleted marrow cavity. The most plausible explanation of these results is that cells in endosteal crevices or in the bone substance itself initiate the process of marrow regeneration. Delayed repopulation of the irradiated femur could be due to the radiosensitivity of such cells, or to a radiation effect on reconstruction of the marrow microenvironment, or to both of these factors.

Several lines of evidence suggest that hemic colony-forming cells are present in circulating blood (*4*). Colony-formers also seem to be released rather promptly from a shielded normal marrow in an animal that otherwise received total body irradiation (*5*). It is generally thought that the seeding of progenitor cells promotes the recovery of irradi-

ated marrow and that this accounts for the protective effect of marrow shielding or transplants. The fact that circulating stem-type cells are not critically involved in regeneration of a small volume of depleted marrow as described here is not necessarily contradictory. For example, the probability of seeding in a small area may be quite low because of the relatively small number of circulating progenitor cells. In view of the reported abundant release of such cells from a shielded marrow, it would be of interest to study the recovery of a mechanically depopulated segment of irradiated femur when the remainder of the body has also been irradiated with exception of the contralateral femur.

Although the bone cavity appears to be devoid of marrow cells immediately after perfusion, it is, of course, possible that a few cells may be left behind. However, since stem cells represent only a small percentage of the total marrow population (*6*), the probability that any retained cells are stem cells should be correspondingly low. Mesenchymal cells are scattered throughout the Haversian system and, conceivably, some of these could serve as progenitors of the hemic colony-forming cells normally present in the marrow proper. However, the fact that donor marrow cells can more or less permanently recolonize part of a recipient's bone marrow (*7*) indicates that any influx of cells from bone is ordinarily very small, or limited to certain conditions of marrow regeneration. That cells with hematopoietic potential may be present in osseous tissue is perhaps not surprising since there are other examples of functional relations between marrow elements and bone (*8, 9*). When marrow is transplanted to an extramedullary site, the hemic cells disappear and the reticular cells that remain first give rise to bone and then to a microcirculation before hematopoietic repopulation of the implant (*8*). The picture described here is in some respects the converse since cells in bone are presumed to initiate the process of marrow repopulation. Present studies of marrow regeneration in a prelabeled femur should provide clues to the identity and location of the repopulating cells.

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Protein Content of Seed: Increase Improves Growth and Yield

Abstract. *Oat seeds with a higher protein content as a result of chemical applications in 1967 yielded 21 to 42 percent more grain in 1968. Wheat seed, whether from Michigan, Illinois, or Mexico, that contained more protein as a result of field applications of chemicals or nitrogen developed into larger seedlings. The content of protein in the seed correlated with subsequent growth and yield, indicating that the amount of endogenous protein or of a proteinaceous moiety, which can be controlled, may be an important factor in subsequent yield of major agronomic crops.*

Seed size or weight has been correlated with growth and yield; however, the explicit relation between seed protein content and subsequent growth is not known (*1*). Treatment of seeds with trace elements and fertilization of seedlings with nitrogenous compounds have increased yield, and carbohydrate and protein content of grain. Postemergence, subherbicidal rates of atrazine, simazine, and terbacil have also increased protein content of forage and grain crops (*2–4*). Wheat *Triticum aestivum* L. and oat *Avena sativa* L. seeds with higher protein content resulting from chemical treatments and nitrogen fertilization during the first generation were used to evaluate the relation of seed protein content to growth and yield in the next generation.

Total protein was determined by micro-Kjeldahl procedures (*5*), amino acids were analyzed on an automatic amino acid analyzer (*6*), and nitrate was determined (*7*). In growth chamber and greenhouse experiments, 20 uniform seeds were planted in each pot, and randomly thinned to 15 seedlings upon emergence. At harvest, plants were weighed and oven-dried for analysis (*8*).

Oat seeds from a 1967 field experiment were planted in the field on 18 April 1968, and the grain was harvested 30 July. Equal weights of seed were planted in each plot. Similar seeds were also sown in clay pots containing sandy clay loam, grown in a greenhouse at 20° to 27°C, and harvested after 19 days. Growth and yield data are the means of three replicates in randomized block designs.

The increase in seed protein content (based on Kjeldahl nitrogen) due to chemical treatment (Table 1) has been reported (*4*). However, these data show that the protein-enriched seeds as measured by amino acid analysis developed

Table 1. Relationship between seed protein (amino acids) content of oats (cv. Rodney) with growth and yield the following generation.

Chemical	Rate (kg/ha)	First generation		Second generation	
		Seed weight (mg)	Total amino acids (mg/g; dry weight)	Seedling growth (mg/plant; fresh weight)	Seed yield (kg/ha)
Control	0	29	103	187*	3830*
Simazine	.07	29	132	267	5443
Simazine	.28	30	131	282	4905
Terbacil	.14	31	127	229	4636

* F value for control compared to treatments is significant at the .05 level. The correlation of total amino acid content with seedling growth is significant at the .01 level ($r = 0.7106$) and with yield at the .05 level ($r = 0.7033$).