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# The Effect of *in Vitro* Irradiation on the Subsequent Growth of Ectopic Splenic Autoimplants[1] (38630)

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Splenic fragments autotransplanted to ectopic sites undergo almost complete necrosis with subsequent regeneration into splenic tissue having a microscopic structure indistinguishable from that of the original organ (1–3). A similar process occurs when splenic fragments are incidentally implanted on the peritoneal surface during gastric surgery or splenectomy. Such fragments regenerate into functional splenic tissue and may cause recurrence of the disorder for which splenectomy was originally performed (4–5). Splenic implants regenerate from surviving connective tissue cells located at the surface of the implants and their viability is maintained by perfusion of necessary nutrients prior to vascularization of the implants. These connective tissue cells proliferate and differentiate to form splenic stroma which is later populated by circulating immunohemopoietic cells to form distinctive splenic tissue (3). It has been shown that ectopic splenic implants fail to thrive if part of the original spleen is left *in situ*, but do grow well following total removal of the original spleen (2, 3).

This study was undertaken to examine the radiosensitivity of those cells responsible for regeneration of splenic implants in rats splenectomized so that the implants were the only functional splenic tissue.

*Materials and Methods.* Male Wistar rats weighing 100–300 g were used throughout these experiments. The animals were kept under normal laboratory conditions and were fed purina lab chow and water *ad libitum*. All surgical procedures were carried out under aseptic conditions. Intraperitoneal sodium pentobarbitol (5 mg/100 g body wt) was used for anesthesia. Splenic tissue was irradiated from a $^{137}Cs$ source.

Splenectomy was carried out through a midline abdominal incision. The excised spleen was cut into eight approximately equal pieces and each piece was then weighed (weight range 40–80 mg). For irradiation, the tissue was placed in a sterile Petri dish containing a few drops of sterile saline. Using a pair of blunt-tipped scissors, pockets were made in the subcutaneous tissue of the abdomen on both sides of the midline incision. Irradiated pieces of spleen were implanted in the pockets on the right side of the midline incision, and for every irradiated specimen a nonirradiated piece was implanted on the left to serve as a control. After 6 wk, the implants were removed and reweighed. They were then fixed in 10% buffered formalin, sectioned at 6 $\mu$m and stained with hematoxylin-eosin, PAS-hematoxylin and reticulin stain. To study the sequence of events during regeneration, implants were removed at the following intervals: daily during the first week; twice weekly for the next 4 wk; and finally at 6 wk. Touch imprints were obtained from the implants and stained with Wright–Giemsa and the tissue was then fixed and processed for histological examination.

*Results.* Nonirradiated implants undergo a sequence of histogenetic events that results in complete regeneration of splenic tissue. During the first 2 days, there is no circulation through the implant as its vascular pathways are disconnected from the general circulation and, as a result, the red pulp appears relatively acellular except for stromal elements consisting of fixed reticular cells. By days 4 and 5, necrosis is seen in the entire implant excepting a peripheral shell bordering the subcutaneous tissue. The necrosis has the characteristics of "coagulation necrosis", the general architecture of the tissue is recognizable, the nuclei are pyknotic and the cells are no longer viable. This suggests that

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necrosis results from a lack of blood circulation through the tissue. By the end of the first week, the viable tissue in the peripheral layer is invaded by capillaries from the subcutaneous tissue that constitutes the supporting bed. The process of regeneration originates in this outer zone, consisting primarily of fixed connective tissue cells. Cytologically, on imprints, these cells are star-shaped or spindle-shaped with somewhat eccentric nuclei, loose chromatin pattern, unusually prominent nucleoli and frequent mitotic figures. The cytoplasm is basophilic and has small slender processes. During the second and third weeks, these cells appear to differentiate into splenic reticular cells that, on imprints, have a smaller nuclear–cytoplasmic ratio, less intense cytoplasmic basophilia and longer, but still slender, cytoplasmic processes. In preparations stained with reticulin stain the cells are seen to intersperse between silver staining reticulin fibers. As this tissue develops into splenic red pulp, the small arteries become surrounded by lymphocytes and these regions gradually attain the histologic features of white pulp. The outer regenerative zone, initially no more than a layer of 3–4 cells, gradually extends in centripetal direction to replace the necrotic zone in the central part of the implant. The outer zone of this regenerative shell is always more differentiated than is the central part and may have acquired a definitive splenic structure. The central part, however, bordering the necrotic zone, is no more than a layer of proliferating connective tissue cells. By the end of 6 wk the implant is completely regenerated.

Table I summarizes the effect on subsequent take of various doses of irradiation administered to the splenic tissue prior to implantation.

The control implants took successfully in all instances. It can be seen that irradiation up to 1500 rads does not significantly alter the percentage of take of splenic implants. There is, however, no take when the dose of radiation is increased to 1750 rads. Histologically, the successful implants, irradiated prior to implantation with up to 1500 rads appear similar to the controls (Fig. 1). The structure of both the red and white pulp is normal (Fig. 2). Fewer lymphatic nodules were seen in irradiated implants but morphometric studies were not done to quantitate the ratio of the white pulp in these implants. The unsuccessful implants become masses of necrotic tissue (Fig. 3) contained within a fibrous capsule. Resorption and replacement of this necrotic tissue by the surrounding supportive tissue was not observed when these implants were followed for up to 8 wk although autophagocytosis was commonly seen.

TABLE I. THE EFFECT OF *In Vitro* IRRADIATION ON THE SUBSEQUENT GROWTH OF ECTOPIC SPLENIC AUTOTRANSPLANTS.

| Dose in rads | Number of implants | Percentage of takes |
|---|---|---|
| 500 | 12 | 100 |
| 1000 | 16 | 100 |
| 1500 | 20 | 70 |
| 1750 | 16 | 0 |
| 2000 | 16 | 0 |
| 4000 | 20 | 0 |

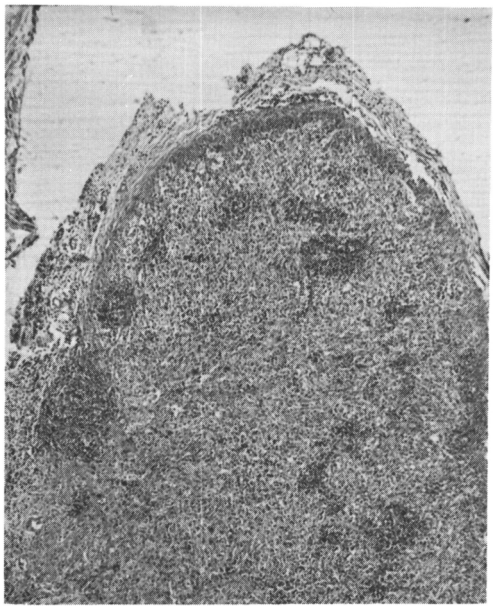

FIG. 1. Splenic autoimplant irradiated with 1000 rads prior to implantation. Both the red pulp and the white pulp appear to be normal. Note the peripheral parts of the implant are in a more advanced state of development than the center. Six weeks after implantation, hematoxylin-eosin. ×45.

*Discussion.* Like bone marrow, splenic tissue is made up of stroma and lympho-

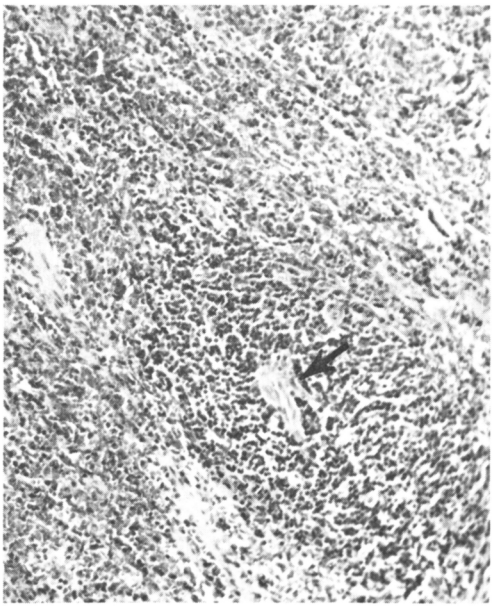

FIG. 2. Higher magnification—a lymphoid nodule dominates this figure of a splenic autotransplant irradiated with 1000 rads prior to implantation. Note the central artery (arrow) and the marginal zone of red pulp surrounding the nodule. Six weeks after implantation, hematoxylin-eosin. ×160.

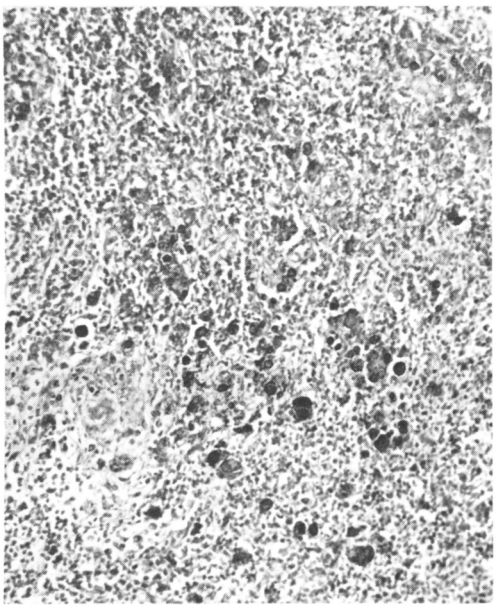

FIG. 3. Splenic implant irradiated with 4000 rads prior to implantation. The implant consists of a necrotic tissue without defined splenic structure. The dark masses are probably cellular debris. Six weeks after implantation, hematoxylin-eosin. ×160.

hemopoietic elements. Whereas the lymphohemopoietic elements of both marrow and spleen have been intensively studied, the stromal elements of these tissues have received less attention. The splenic stroma is comprised of endothelial cells of the splenic sinuses and fixed reticular cells that elaborate extracellular reticulum (6). In bone marrow, these cells form a reticular meshwork that traps lymphohemopoietic cells, providing a place for their interaction, division and differentiation (6). In both spleen and marrow, it is these stromal elements that are responsible for the regeneration of tissue in autotransplants placed in ectopic sites.

The regeneration of both splenic and marrow implants originates from proliferating, primitive-appearing, spindle-shaped cells that have a delicate chromatin pattern and a basophilic cytoplasm that displays slender branches (3, 7). We believe that these cells are primitive mesenchymal elements, or connective tissue stem cells (8), derived from reticular cells of the splenic stoma by the process of dedifferentiation, and that during implant regeneration they redifferentiate into reticular cells to form the splenic stroma. Once the stroma has been regenerated, circulating lymphohemopoietic cells may repopulate the implant and give rise to the definitive structure of spleen. Although direct evidence is lacking, we believe that 1500 rads should have been sufficient to destroy lymphoid elements within the splenic implants, suggesting that lymphoid reconstitution derives from extrinsic, circulating lymphoid elements.

It has previously been demonstrated that the stroma of bone marrow is more radioresistant than are the hemopoietic elements (9–10). A dose of 1000 rads destroys hemopoiesis but a greater dose is needed to destroy the marrow stroma. The findings in the present study indicate that the stromal elements of the spleen are also resistant to doses of irradiation as high as 1500 rads and, in this respect are similar to the stromal cells of bone marrow.

Information on the radiosensitivity of regenerating spleen tissue may have practical significance in a clinical context, where irradiation is used in an attempt to ablate

splenic function. Splenic irradiation has often been unsatisfactory in the management of hypersplenism (11). This study indicates that, to be effective, a single dose greater than 1500 rads may be required to control hypersplenism.

*Summary and Conclusions.* From these data we conclude:

1. *In vitro* irradiation of splenic fragments with doses less than 1500 rads does not prevent subsequent regeneration of splenic autoimplants in splenectomized rats.

2. The relative radioresistance of this regenerative process suggests that splenic regeneration is dependent upon the viability of stromal elements. Once the stroma is reconstituted, it is probably 'seeded' by circulating lymphohemopoietic cells to form recognizable splenic tissue. In this respect the process is similar to that seen in regenerating marrow tissue.

3. The relative radioresistance of splenic stroma must be taken into account when irradiation is considered as a therapeutic measure for management of splenic disorders.

1. Perla, D., Amer. J. Pathol. **12,** 665 (1936).
2. Cameron, G. R., and Rhee, K. S., J. Pathol. Bact. **78,** 335 (1959).
3. Tavassoli, M., Ratzan, R. J., and Crosby, W. H., Blood **41,** 701 (1973).
4. Stobie, G. H., Can. Med. Assoc. J. **56,** 374 (1947).
5. Loeb, W., Seaman, W. B., and Moore, C. V., Blood **7,** 904 (1952).
6. Weiss, L., in "The Cells and Tissues in the Immune System" p. 3. Prentice-Hall, Englewood Cliffs, N. J. (1972).
7. Tavassoli, M., and Crosby, W. H., Science **161,** 54 (1968).
8. Huggins, C., Wiseman, S., and Reddi, A. H., J. Exp. Med. **132,** 1250 (1970).
9. Knospe, W. H., Blom, J., and Crosby, W. H., Blood **28,** 398 (1966).
10. Maniatis, A., Tavassoli, M., and Crosby, W. H., Blood **38,** 569 (1971).
11. Comas, F. V., Andrews, G. A., and Nelson, B., Amer. J. Roentgenol. **104,** 668 (1968).

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