

The Pattern of Stem Cell Repopulation in Heavily Irradiated Mice Receiving Transplants of Fetal Liver

By BERNHARD KUBANEK, NICHOLAS RENCRICCA, ADOLFO PORCELLINI,
DONALD HOWARD AND FREDERICK STOHLMAN, JR.

ERYTHROPOIESIS IN THE NEWBORN RAT is in many respects analogous to that seen during the fifth–sixth month of gestation in human beings. Thus, up until birth, the red cell production in the rodent is predominantly hepatic and only at birth does significant myeloid erythropoiesis commence. This appeared to offer the opportunity to evaluate regulation of fetal red cell production. Initially we observed that bilateral nephrectomy in the neonatal rat does not produce the erythroid aplasia characteristic of the renoprival adult rat.¹ This, in part, might be explained by extrarenal erythropoietin production which has been recently demonstrated in the newborn rat.² The failure of plethora and starvation³ to produce in the newborn animal the degree of erythroid aplasia seen in the adult might be accounted for by the failure to significantly alter the O₂ supply–demand relationship due to the high metabolic rate seen in the growing animal. In an effort to circumvent these problems we turned to transplantation of fetal tissues in a heavily irradiated adult animal. We have observed a difference in the recovery pattern of erythropoiesis in the recipients of fetal liver as compared with those of adult bone marrow.⁴ In part, these differences might be attributed to a difference in the generation time. Accordingly, we have compared the growth curve of hematopoietic stem cells derived from fetal liver with that of adult bone marrow.

MATERIALS AND METHODS

Virgin mice of the CF₁ strain were mated at 10–12 weeks of age. 16½ days later they were sacrificed and fetal liver suspensions were prepared as previously described.⁴ Adult marrow suspensions were made from the femora of 10–12-week-old CF₁ mice.⁵ To measure the number of colony forming units (CFU) present in the suspensions, 6×10^4 bone marrow cells or 4×10^5 fetal liver cells were given to 10-week-old CF₁ mice immediately after they were heavily irradiated (800 r.). The animals were sacrificed on the ninth day, the spleen fixed in Bouins, and the colonies counted independently by two observers. The plating efficiency (f value) and growth curve were measured by the technic of Siminovitch et al.⁶ For these measurements 1.8×10^6 bone marrow or 12×10^6 fetal liver cells were given immediately after irradiation. The f value was determined two hours later. The numbers of nucleated cells/spleen and the numbers of CFU were measured at intervals after transplantation.

*From St. Elizabeth's Hospital, Tufts University School of Medicine, Boston, Mass.
First submitted June 18, 1969; accepted for publication August 12, 1969.*

Supported in part by USPHS Grants HE-7542 and HE-5600, National Heart Institute.

BERNHARD KUBANEK, M.D.: *Research Fellow, St. Elizabeth's Hospital.* NICHOLAS RENCRICCA, PH.D.: *Research Fellow, St. Elizabeth's Hospital.* ADOLFO PORCELLINI, M.D.: *NATO Fellow, St. Elizabeth's Hospital.* DONALD HOWARD: *Research Associate, St. Elizabeth's Hospital.* FREDERICK STOHLMAN, JR., M.D.: *Director of Medicine and Research, St. Elizabeth's Hospital, and Professor of Medicine, Tufts University School of Medicine.*

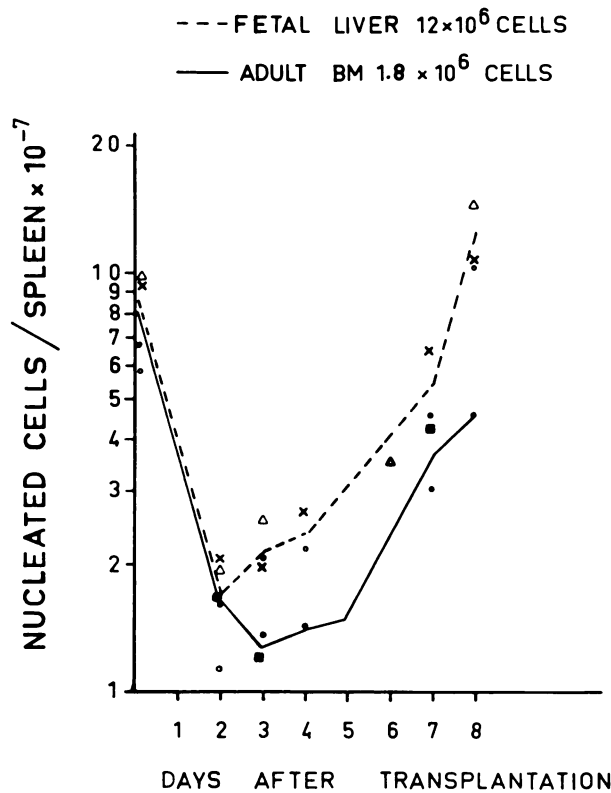


Fig. 1.—Numbers of nucleated cells present in spleen of heavily irradiated animals at intervals after transplantation with 16½-day-old fetal liver or adult bone marrow. Each point is value from pooled suspension of five spleens. Various symbols represent different experiments. Solid circle and square are adult bone marrow.

RESULTS

The numbers of CFU found in fetal liver suspensions at 16½ days was estimated at 1767 ± 151 based on six experiments. The mean f value in four experiments with fetal liver was 9.6 per cent with a range of 3.5–14.6 per cent. This value was somewhat lower than that for adult marrow in which a mean value of 14 per cent with a range of 13–15 was derived from three experiments. Using this f value the total number of colony forming cells in the fetal liver was estimated to be 18,410. The increase in the numbers of nucleated cells of the spleen is shown in Fig. 1, and the growth curve of CFU derived from three experiments each for fetal liver and adult bone marrow are shown in Fig. 2.

DISCUSSION

The f value for fetal liver in these experiments was somewhat lower than that observed in adult bone marrow of mice of the same strain. The f value for the 16½-day-old fetal liver in our experiments was comparable to that reported by Silini et al.⁷ These authors observed f values of 10.6 and 8.5 in hybrid fetuses at 15 and 17 days of gestation, respectively. In Swiss mice a value of 9.4 per cent was observed using liver obtained from fetuses at the seventeenth day of gestation.

The doubling time for transplanted fetal liver cells was shorter than that

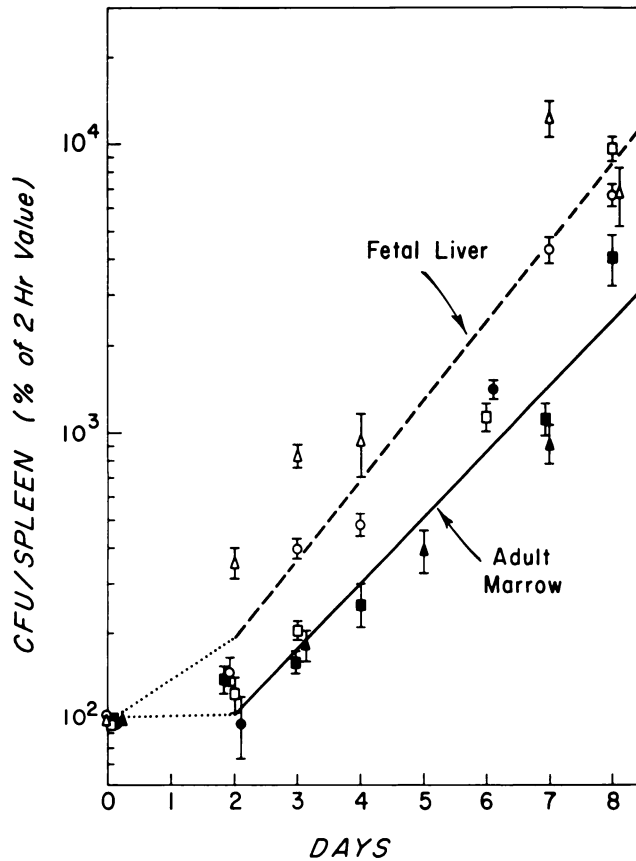


Fig. 2.—Growth curve of splenic CFU in heavily irradiated animals receiving 16½-day-old fetal liver or a adult bone marrow cells. Each point represents value ± 1 SE from individual experiment, and is mean number of colonies from 8–10 recipients. Curves fitted by least squares.

for adult cells, the values being approximately 24 hours in fetal tissue and 32 hours in adult hemopoietic tissue. Moreover, the CFU in the spleen of recipients of fetal liver increased somewhat earlier, and after two days there were significantly more stem cells present in the spleens of recipients of fetal liver than those of adult marrow. The doubling time of splenic CFU's represent a "net generation time" rather than a true generation time, since some cells are lost through differentiation and hence are not included in the measurements. Differentiation occurs earlier in recipients of fetal liver tissue than in recipients of adult bone marrow as indicated by the nucleated cell counts (Fig. 1) as well as the more rapid recovery of erythropoiesis.⁴ From this it can be appreciated that the doubling time of CFU recorded herein minimizes the differences in generation time between transplanted fetal and adult tissue. It might be further suggested that the shorter generation time of pluripotential cells derived from fetal liver accounts in significant measure for the earlier recovery of erythropoiesis after transplantation into heavily irradiated mice.

SUMMARY

Pluripotential cells derived from fetal liver had a lower plating efficiency than adult marrow cells, but estimates of the generation time derived from

the growth curve are significantly shorter and may account for the earlier erythroid population.

REFERENCES

1. Lucarelli, G., Howard, D., and Stohlman, F., Jr.: Regulation of erythropoiesis XV. Neonatal erythropoiesis and the effect of nephrectomy. *J. Clin. Invest.* 43:11, 1964.
2. Carmena, A. O., Howard, D., and Stohlman, F., Jr.: Regulation of erythropoiesis XXII. Erythropoietin production in the newborn animal. *Blood* 32:376, 1968.
3. Lucarelli, G., Porcellini, A., Carnevali, C., Carmena, A., and Stohlman, F., Jr.: Fetal and neonatal erythropoiesis. *Ann. N.Y. Acad. Sci.* 149:544, 1968.
4. Kubanek, B., Rencricca, N., Howard, D., and Stohlman, F., Jr.: The pattern of recovery of erythropoiesis in heavily irradiated mice receiving transplants of fetal liver. Unpublished.
5. Kubanek, B., Tyler, W. S., Ferrari, L., Porcellini, A., Howard, D., and Stohlman, F., Jr.: Regulation of erythropoiesis XXI. The effect of erythropoietin on the stem cell. *Proc. Soc. Exp. Biol. Med.* 127:770, 1968.
6. Siminovitch, L., McCulloch, E. A., and Till, J. E.: The distribution of colony forming cells among spleen colonies. *J. Cell Comp. Physiol.* 62:327, 1963.
7. Silini, G., Pozzi, L. V., and Pons, S.: Studies on the haemopoietic stem cells of mouse foetal liver. *J. Embryol. Exp. Morph.* 17:303, 1967.