Early Proliferation of Transplanted Spleen Colony-Forming Cells
II. Circulation of Cells* (33956)

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We presented evidence that transplanted spleen colony-forming cells are proliferating as early as 24 hr after transplantation (1). This is in agreement with some published results (2) but not with others (3, 4). Decrease (1, 2, 5) in the number of colony-forming cells in the spleen between 4 and 24 hr after transplantation may be a reason for this discrepancy and suggests that the techniques may grossly underestimate the number and growth rate of the colony-forming cells transplanted (1). Indeed, the doubling time of the population, based on transplantation data at 24 hr, was about 48 hr (1). On the other hand, our (6) preliminary estimate of the generation time of these cells, based on vinblastine sensitivity 24 hr after transplantation, suggests that they are dividing with a mean cell cycle time of about 14 hr.

Differentiation or death of some of the cells in the spleen of the primary recipient, during the first 24 hr, seems unlikely since the method of assay requires that these cells survive and form gross nodules in the spleens of secondary recipients; otherwise they would not have been counted. Thus, if death or differentiation of cells is suggested, one would have to explain how harvesting the cells from spleens of the primary recipient and injecting them into the secondary recipients prevented such loss.

Other possible explanations have been discussed (1, 7, 8). Dividing cells may be susceptible to trauma during extraction from the spleen and preparation of cell suspensions, changes in "transplantability" of cells may occur, and emigration of cells must be considered. The last suggestion is supported by a number of observations. Cells capable of forming colonies in the spleens of irradiated mice presumably circulate in the blood (9–11) and emigrate from bone marrow of femur, tibia, or tail (12, 13), and from the spleen since shielding this organ reduces mortality in irradiated animals (14). Thus, circulation of transplanted colony-forming cells may continuously occur and there may be a net emigration of cells from the spleen to other tissues during the first day following transplantation. The data summarized below suggest that circulation may be involved in the early decrease in number of transplanted colony-forming cells in the spleen of irradiated mice.

Materials and Methods. The mice used in these experiments and the irradiation factors and techniques of preparation and injection of cell suspensions were as described previously (1). Briefly, bone marrow cells were prepared in cold Tyrode's solution by flushing cells out of the femoral cavity. Suspensions of cells from lung and spleen were prepared from dissected organs by cutting the tissue into small pieces and passing cells through a fine mesh stainless steel gauze or by gentle disruption in a homogenizer with a loose-fitting Teflon pestle. Cells were counted in dilutions of these suspensions with a Coulter counter. Appropriate dilutions of all suspensions were made so that the desired number of cells was contained in 1 ml. This volume was then injected intravenously into each animal of appropriate groups of lethally-irradiated (875 R total body X-radiation) mice.

Results. If cells lodge temporarily in organs and subsequently emigrate and circulate, evidence for this phenomenon would be found in other tissues as well as in the spleen. Since bone marrow cells injected intravenously pass through the capillaries of the lung (15) and since hemopoiesis is char-

* Supported in part by NIH Contract AM 11894 and AEC Contract (40-1)-3635, and by the U. S. Atomic Energy Commission under contract with the Union Carbide Corporation.

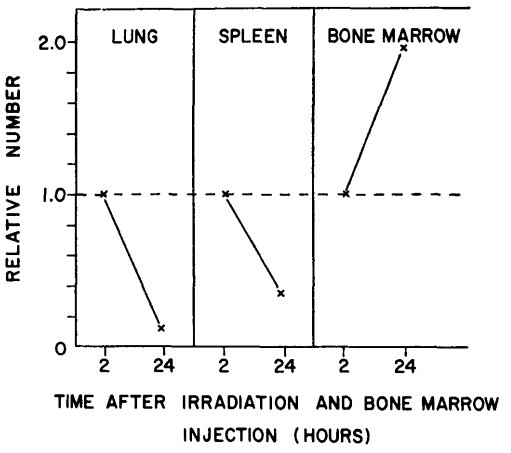

FIG. 1. Change in content of colony-forming cells in lung, spleen, and bone marrow: lethally-irradiated mice were given 20×10^6 syngeneic bone marrow cells. They were killed at 2 or 24 hr and the number of colony-forming cells in the lung was determined. Similar determinations were made for spleen and bone marrow taken from animals given 10^6 syngeneic bone marrow cells. The number of colony-forming cells found at 24 hr is shown normalized to the number at 2 hr. Each point is based on the average number of colonies determined by counting gross nodules in at least 12 spleens. Differences between content of colony-forming cells found at 2 and 24 hr was at least 3 times the standard error for each of the three tissues.

acteristically a process that proceeds in bone marrow tissue, a study of the lung and bone marrow was of particular interest. Accordingly, syngeneic bone marrow cells were injected into mice which had been given 875 R total body X-radiation. They were killed 2 or 24 hr later and separate cell suspensions were made from spleen, lung, and bone marrow. Aliquots of these suspensions were injected into irradiated recipients; one group was given spleen cells, another cells from lung, and a third bone marrow cells. The results are shown in Fig. 1. The decrease in number of colony-forming cells at 24 hr is evident in lung and spleen but in bone marrow the number doubled, suggesting that cells emigrate from lung and spleen and immigrate to the bone marrow. If these changes are due to circulation, cells would presumably pass primarily via the blood stream. Accordingly, syngeneic bone marrow cells were given intravenously to irradiated primary recipients and they were killed 2, 8, 16, or 24 hr later. Aliquots of blood, spleen cells, and bone marrow cells from these mice were injected into irradiated recipients as before. The number of gross splenic nodules found 9 days later is compared in Fig. 2. While there was a marked reduction in colony-forming cells in the spleen between 2 and 16 hr, the number of similar cells in the bone marrow decreased slightly at first but then increased. In the blood, the number decreased and then remained constant at about 1 colony-forming cell per 0.2 ml. The data suggest continuous circulation of cells since the number in the blood is constant after 8 hr.

Another possibility should be considered in

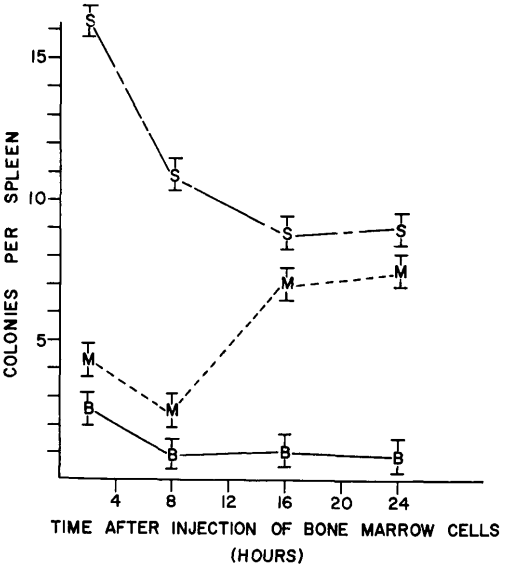

FIG. 2. Average number of colonies in the spleen of lethally-irradiated mice 9 days after injection of 0.2 ml of blood (B), suspension of spleen cells (0.1 spleen equivalent) (S), and bone marrow cells (½ femur equivalent), (M). The injected cells were prepared from donor mice which were irradiated, given 10×10^6 syngeneic bone marrow cells, and killed at the time indicated along the abscissa. The point for spleen taken from donors 2 hr after injection of bone marrow cells is too low; many of the spleens of the assay animals contained confluent colonies. Each point is the average colony count determined by counting gross nodules in from 14 to 40 spleens. The vertical lines approximately indicate standard error.

TABLE I. Fraction of Injected Cells, f, which Settle and Form Colonies in the Spleen of Irradiated Mice.[a]

Cell source	Expt. no.	CFU[c] injected	CFU found	f
Bone marrow	1	30	5.8 (13)[d]	0.193
	2	78	13.9 (27)	0.178
Spleen cells (4)[b]	1	82	17.9 (14)	0.218
	2	35	7.2 (11)	0.206
(24)[b]	1	100	11.9 (21)	0.119
	2	75	14.0 (22)	0.187
(96)[b]	1	62	5.2 (15)	0.084
	2	47	3.8 (15)	0.081

[a] Determined by the method of Siminovitch *et al.* (16).

[b] Hours cells had been in the primary recipient before spleens were harvested and cell suspensions made.

[c] CFU refers to colony-forming units.

[d] Numbers in parentheses indicate the number of spleens counted in the second group of recipients required by the procedure of Siminovitch *et al.* (16).

connection with the results (Figs. 1 and 2). Only a small fraction, f, of the cells that are injected lodge in the spleen and subsequently give rise to gross nodules (16). The properties of the injected cells and the factors in the host environment which determine f are unknown. Nevertheless, this fraction can be estimated by the procedure of Siminovitch *et al.* (16) which was used to obtain the values of f listed in Table I. Cells harvested from the spleen 4 hr after injection of syngeneic bone marrow cells settled in the spleen of secondary recipients in about the same proportions ($f = 0.21$) as normal bone marrow cells. However, $f = 0.15$ for cells harvested at 24 hr and only 0.08 at 96 hr after transplantation. The fraction of cells that settle and form colonies in the spleen of irradiated mice is clearly not constant for transplanted cells.

Discussion. Using these values for f as correction factors (16), we can correct the growth curve published in our previous report (1). The number of colony-forming cells in the spleen 4, 14, and 96 hr after injection of 10^6 syngeneic bone marrow cells would then be 153, 61, and 284, respectively. The loss in colony-forming cells in the spleen during the first 24 hr after transplantation that could be ascribed to circulation would thus be estimated to be about 92 of the 153 cells found at 4 hr. However, new cells have entered this population by proliferation, hence the net emigration of cells out of the spleen during the first 24 hr is uncertain but must be substantial [at least $(90/150) = (3/5)$].

The use of f as a correction factor may not be valid, however, if cells with spleen colony-forming capacity circulate in the host after transplantation, because f and the kinetics of circulation of cells cannot be independently measured by the transplantation methods used. The value of f appears to be a function of time after injection of the cells, one or more as yet unidentified characteristics of the cells that are injected (8), and factors related to the host environment, including age of host (17) into which the cells are injected; *i.e.*, $f = \mathrm{f}$ (time, cells, host).

We have no direct evidence that cells taken from the spleen are subject to variable loss due to the trauma of preparation of suspensions (7). However, the changes in the number of cells in the spleen might be explained on this basis. Cells could possibly be especially sensitive to disruption of the tissue in which they are growing during DNA synthesis. It was shown (18) that only a small fraction of colony-forming cells in the bone marrow of normal mice are synthesizing DNA, whereas a large fraction of these cells, proliferating in the spleen after transplantation, are synthesizing DNA. We had preliminary evidence (6) that the generation time of

transplanted colony-forming cells becomes progressively shorter during the first 4 days and this suggests that a progressively larger fraction of the cells are in DNA synthesis.

No change in f was found for transplanted cells growing in the bone marrow of irradiated mice (19). Cell suspensions from bone marrow of irradiated mice are readily prepared, however, and it is possible that proliferating cells in this milieu are not significantly traumatized.

Summary. Circulation of cells injected into lethally irradiated mice appears to be an important phenomenon in transplantation experiments. The number of colony-forming cells that can be recovered from the lung at 24 hr after transplantation is only about 0.1 the number recovered at 2–4 hr. In spleen this ratio is 0.3, whereas, in bone marrow it is 2. The change in number of transplanted cells in spleen, bone marrow, and blood suggests circulation of cells among the tissues of the host. The proportion of transplanted cells involved in this circulation was uncertain because of changes in the transplantation fraction, f, for cells recoverable from the spleen 4, 24, and 96 hr after transplantation. The changes in f were consistent with the assumption that cells are more susceptible to trauma when they are rapidly proliferating.

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Received Jan. 13, 1969. P.S.E.B.M., 1969, Vol. 131.