Radiosensitivity of the Colony-Forming Cells of the Mouse Bone Marrow (34785)

JEAN F. DUPLAN AND LUDWIG E. FEINENDEGEN
(Introduced by George Brecher)
Laboratoire Pasteur, Paris, France; and Institut für Medizin, KFA Jülich, Germany

Since Till and McCulloch (1) described the spleen colony assay, radiosensitivity of colony-forming cells (CFC) has been the object of several investigations (2, 3). For the CFC of the bone marrow of the adult mouse, values for D_0 ranged from 65 to 100 R and extrapolation numbers between 1 and 2. It has recently been demonstrated (4) that less than 10% of CFC present in a suspension of normal bone marrow were susceptible *in vitro* to the lethal effect of tritiated thymidine of high specific activity. The "suicide" effect presumably eliminates, selectively, cells in DNA synthesis. From these data, the CFC of the adult bone marrow represent a relatively homogeneous population as far as their position in the generative cycle is concerned. It was therefore logical to investigate what modification of radiosensitivity would ensue if a significant number of this population was induced to enter DNA synthesis (5). To this end, experiments were undertaken on bone marrow suspensions from mice previously submitted to a significant bleeding.

Materials and Methods. The experimental animals were male and female mice of the purebred line XVII belonging to the F_{52} and subsequent generations of our colony. Recipients of bone marrow suspensions were 80 to 100 days old and donors 55 to 65 days. Donor animals were bled from a retro-orbital vein; the amount of blood removed varied between 0.35 and 0.45 ml. After an interval indicated by the protocol, microhematocrit determinations were performed on tail blood and the mice were killed. Bone marrow from several femurs were suspended in enough Tyrode's solution to produce suspensions of approximately 10^7 nucleated cells/ml. The suspension was divided into two aliquots. To one was added 3 to 4 μg of thymidine/ml, and to the other, 350 to 400 μCi of tritiated thymidine (sp act of 11–16 Ci/mmole). Both suspensions were incubated for 20 min at 37° and then diluted 1:10 with Tyrode's containing 3 μg of thymidine/ml. The number of nucleated cells were counted in a hemacytometer and both suspensions were adjusted to the desired concentrations. The recipients were divided into 5 groups of 12 to 16 mice and exposed to X-irradiation of 800, 700, 650, 600, and 500 R, respectively. Immediately after the initial irradiation each group was divided into two lots of 6 to 8 animals: one received an intravenous injection of the marrow cells incubated with cold thymidine; the other, the suspension incubated with tritiated thymidine. Thereafter, the individual groups received their second irradiation of 100, 150, 200, and 300 R, respectively. Only the group that had initially received 800 R was not exposed to a complementary second dose. In this fashion the cells of each suspension received *in vivo* X-ray doses of between 0 and 300 R while the recipients received total doses of 800 R, thus assuring that no endogenous CFC survived.

At the end of 8 or 9 days the recipients were killed, their spleens were removed and fixed in Bouin solution for 4 to 6 hr. The spleen colonies were enumerated under a 2.5× magnifying lens. Conforming to the generally accepted notion, each nodule was presumed to correspond to a colony-forming unit and to represent a fixed fraction of the injected CFC.

Results. The method outlined permits determination of the radiosensitivity of CFC in a population and simultaneously the radiosensitivity of a fraction of the same population which have survived the suicidal consequence of tritiated thymidine incorporation.

TABLE I. Comparative Radiosensitivity of CFC in Normal and Bled Mice With and Without Prior Thymidine.

Group (hr)	No. of mice	Hematocrit (%)	Suicide (%)	D_0 (R)	
				CT	T^3H
Control	8	45 ± 2[a]	0.9–11[b]	62 ± 5[a]	60 ± 6[a]
Bled, 6	4	28–31[b]	2 –12	59	63
24	3	29–32	8 –17	67	62
	5	30–32	21 –28	60	72
	6	28–32	30 –63	65	80
48	3	36–39	0 – 2	54	55

[a] 95% confidence limit; the 95% confidence limits were computed for the controls only, because of the small number of animals in the experimental groups. Confidence limits for D_0 of the one experimental group containing 6 animals is given in the text.

[b] Minimum and maximum values.

Table I indicates that bleeding had the desired and expected effect of increasing the number of CFC in S as determined by the higher suicide effects in bled animals, when their marrows were used 24 hr after bleeding. At that time three different pools of femoral marrow had suicide rates of 11, 26, and 43% compared to the control value of 6%. In contrast, the suicide effect was not increased at 6 and 48 hr after bleeding although at both times the hematocrits were significantly low.

Notwithstanding the significant shift toward S in the CFC of bled mice, the D_0 and extrapolation number remaining were unchanged. The values were 65 ± 6 R and 1.9 ± 4 for the bled animals and 62 ± 5 and 1.7 ± 0.2 for the controls. In contrast, the survivors of the thymidine suicide effect showed an increased D_0 in those pools in which the shift toward S was marked. Note that in the pool with a 26% suicide effect the D_0 was 72 and in the pool with a 43% suicide effect it was 80 ± 10 R. The difference between that value and the control of 65 R is significant at the 0.05 level (Fig. 1).

Discussion. The data presented confirmed that most of the CFC of the bone marrow are in a quiescent state since less than 10% are in S as indicated by their ability to incorporate tritiated thymidine. They must be presumed to be in G_0. By bleeding mice of approximately one third of their blood volume one induces not only anemia but also leukopenia which is followed, as a compensatory mechanism, by the multiplication of stem cells and increase of CFC in S. One would therefore expect to find some suitable interval at which a significant number of stem cells would become more sensitive to the lethal effect of tritiated thymidine. This

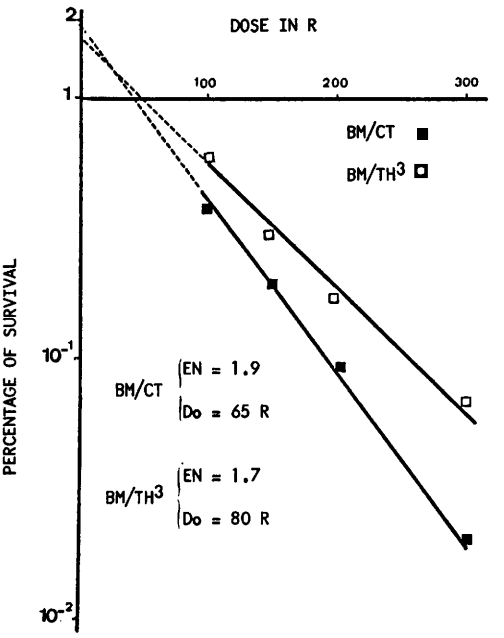

FIG. 1. Survival curves of CFC from bone marrow of bled mice with and without prior thymidine.

interval in our experiments was 24 hr, but in retrospect it is likely that a maximum effect might have been reached at an earlier time, had we had additional points of observation between the 6th and 24th hours.

The radiosensitivity of the normal population of medullary stem cells must therefore correspond to the G_0 and G_1 state because one can estimate the proportion of cells which are neither in S nor in G_2 nor in mitosis at approximately 80%. This estimate is based on the evidence that less than 10% of cells are in S, hence cells in mitosis and G_2 must also represent less than 10%. If 43% of the CFC are in S the radiosensitivity is unchanged but if one eliminates by thymidine suicide the portion of the population in S, the remaining fraction has a definitely lessened radiosensitivity with an increase of the D_0 to 80 R. Since the cells in mitosis are included in that surviving fraction and certainly have an increased radiosensitivity, there must be a large population with a relatively low radiosensitivity corresponding to G_1 or G_2 to result in the overall D_0 of 80 R. Another consequence of the observation is that the cells in S must be more radiosensitive than G_0 (or G_1) because their elimination results immediately in an increase in D_0. However, since one does not know the relative proportion of cells in G_0, G_1, G_2, and M in the population, one cannot compute a specific value for the D_0 of cells in S. One can only state, that the D_0 of S must be significantly less than 65 R and that of G_1 and G_2 higher than 80 R. It is noteworthy in this context, that this distribution of relative radiosensitivities in different parts of the cell cycle does not hold universally. For the cells of the Chinese hamster and human Hela cells irradiated *in vitro,* the S phase is relatively little radiosensitive while the G_2 phase seems to be the most radioresistant in the L cells of the mouse (5).

Finally, the observation that the overall sensitivity of CFC to X-irradiation remains unchanged after bleeding even though there is a significant change toward S (which is more radiosensitive than G_0) clearly indicates that bleeding does not induce synchronization.

Summary. Using the tritiated thymidine suicide technique to identify the proportion of a cell population in S, the prior observation that colony-forming cells of adult mouse bone marrow are predominantly in a quiescent state (G_0) was confirmed. When bone marrow was assayed 24 hr after bleeding, up to 43% of colony-forming units had entered S but their overall radiosensitivity was unchanged. However, survivors of the thymidine suicide among CFC had a significantly lower radiosensitivity, indicating that the CFC in S are more radiosensitive than in G_0. Moreover, cells in either G_1 or G_2 must have a lessened radiosensitivity to account for the maintenance of an unchanged D_0 of the cell population that had entered the multiplication cycle following bleeding and from which cells in S had not been eliminated.

1. Till, J. E., and McCulloch, E. A., Radiat. Res. **14**, 213 (1961).

2. McCulloch, E. A., and Till, J. E., Radiat. Res. **16**, 822 (1962).

3. Fred, S. S., and Smith, W. W., Radiat. Res. **32**, 314 (1967).

4. Becker, A. J., McCulloch, E. A., Siminovitch, L., and Till, J. E., Blood **26**, 296 (1965).

5. Sinclair, W. K., Radiat. Res. **33**, 620 (1968).

Received Sept. 22, 1969. P.S.E.B.M., 1970, Vol. 134.