

Viability of Stored Bone Marrow Colony Forming Units

THE continued use of bone marrow transplantation¹ in disease states and after accidental or therapeutic exposure to radiation has led to an increasing number of *in vitro* and *in vivo* studies of the viability of human and animal bone marrow. Among the more recent methods for quantitative studies of animal marrow is the mouse spleen colony technique. Till and McCulloch² were the first to show that syngeneic mouse bone marrow, when injected into animals which had received a lethal dose of radiation, will cause the formation of macroscopic spleen nodules in a number directly proportional to the number of injected cells. These discrete colonies are assumed to arise from single marrow cells, which are generally described as colony forming units (CFU) and are often equated with the marrow stem cell. This method of studying colony formation has been used to study stem cell kinetics³, cellular radiation sensitivity², immune reactions⁴, anaemic mutants⁵, and various phases of haematopoietic repopulation in the normal and post-irradiation states.

In spite of the wide use of these techniques in studies which involve a delay between collection of the marrow cells and reinjection, little attention has been given to the effect of temperature, storage and suspending media on the viability of CFUs. A series of determinations was therefore made to evaluate the viability of the bone marrow CFU after suspension in two common suspending media at room and ice temperatures. The suspending media used were CMRL₁₀₆₆ (ref. 2) and Puck's saline G (ref. 6), which is a buffered nutrient saline mixture. For each determination, primary cell suspensions were obtained by collecting the marrow cells from the femurs and tibiae of two donor mice. In all cases donor and recipient mice were 8 week old white males of the Hale-Stoner Brookhaven strain. The bone marrow cells were collected by the Stoner and Bond method⁷. This involved thorough grinding with a pestle and mortar of the entire femur and tibia in a small amount of the suspending medium. This method not only shortened the preparation time but also gave a higher yield of CFUs than did the aspiration technique in which portions of the femur and the entire tibia were not used. After the two primary cell solutions had been obtained they were divided into equal portions

with one portion of each being maintained at room temperature while the other was placed in an ice bath. The cellular content of the solutions was determined by counting an aliquot treated with saponin in a Coulter counter with a 100 μ aperture. Using the proper medium, the standard solutions were then diluted to a concentration of 6×10^5 cells/ml. At the time intervals indicated, 1/10 ml. (60,000 cells) were injected, via the tail vein, into each animal which had received a whole body exposure of 700 rads of 250 kVp X-rays during the hour before the injection. After 7 days the recipient animals were killed, and their spleens placed in Bouin's solution for 24 h, after which the macroscopic colonies were counted using a times three magnifier. Three replications of the entire procedure were carried out to produce six donor and twenty-five recipient animals at each of the points indicated in Fig. 1.

Two facts are immediately apparent from the data expressed in Fig. 1. First, maintenance of cell solutions at the temperature of ice preserves their viability for longer periods than does maintenance at room temperature. Second, viability of the cells depends on which of the two media is used. The saline G medium can maintain cells for longer and the most marked differences between the media appear at the shortest time interval (30 min). This early effect suggests that there is a sub-population of cells which are immediately rendered non-viable in the CMRL₁₀₆₆ solution.

Wide variations in CFU content of the mouse marrow have been reported, and by determinations on three different mouse strains we have shown strain dependence to be a factor. Our results also show that some of the variation in the efficiency of marrow to form colonies may be due to such simple factors as suspending medium, temperature, and delay between the harvesting of marrow and the injection of cells.

The close control of handling techniques may be even more important when investigating the type as well as the number of colonies formed. The histological nature of spleen colonies has been studied quantitatively⁸⁻¹⁰. The difference in type of resultant colony is usually attributed to either a different type of original colony forming unit or to the microenvironment in which the injected cell finds itself. If the original cell determined the later colony type, consideration must be given to the possible selective sensitivity of the original cell types to the suspending media, temperatures, storage time and so on. We are now investigating the extent to which the ratio of myelocytic to erythrocytic colonies might be altered by these factors.

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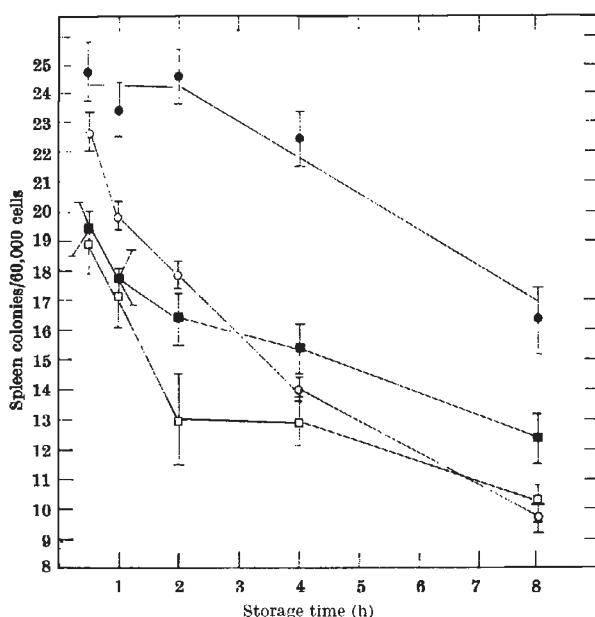


Fig. 1. Viability of stored colony forming units. ●—●, Saline G, ice temperature; ○—○, saline G, room temperature; ■—■, CMRL₁₀₆₆, ice temperature; □—□, CMRL₁₀₆₆, room temperature.