Effect of Whole Body Irradiation on Colony Production by Bone Marrow Cells *in vitro*.* (32099)

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The production of colonies by bone marrow and other hematopoietic tissues *in vitro* using an agar method of culture has been described(1,2). It has been shown that the colonies formed from bone marrow cells in this system are mixed populations of the granulocytic series in varying stages of differentiation and phagocytic monocuclear cells (2). However, the type of marrow cell or cells responsible for colony production is uncertain. The present study was undertaken in an attempt to define the radiation sensitivity of colony forming cells and correlate changes in frequency distribution of marrow cells after various doses of irradiation with any changes in the ability of these same cell populations to initiate colony development *in vitro*.

Materials and methods. Two- to three-months-old male C57Bl mice were used in all experiments.

Radiation. For whole body irradiation 6 mice were placed in a perspex box, measuring 16.3 × 12 × 5.4 cm. The irradiation box was surrounded with bolus to give full back scatter. The X-ray tube operated at 235 KV, 15 mA and with an added filter of 0.25 mm Cu, plus 1.0 mm Al to give a half value layer of 1.0 mm Cu, the dose rate in air being 65 rads per minute. The FSD used was 50 cm and the mean tissue dose was calculated at a depth of 2.9 cm from the upper body surface of the animals in the box. The time required to give 100 rads mean whole body dose was 55 seconds. All mice were used 48 hours after irradiation.

Culture. The technique for the colony growth of bone marrow cells on feeder layers has been described in detail elsewhere(2). Kidneys from 8-day-old C57Bl mice were trypsinized, washed, resuspended and counted in a hemocytometer. The appropriate number of cells was then mixed with equal volumes of double strength of Eagle's minimum essential medium (supplemented by 10% fetal calf serum and 10% trypticase soy broth—E1010) and 1% agar. Five ml aliquots of this containing 5×10^6 kidney cells were then plated into 60 mm glass Petri dishes. Pools of bone marrow cells were collected from the femurs of three animals per group in Eagle's media and prepared as a single cell suspension by gentle pipetting. Viable, nucleated cell counts were done in a hemocytometer using nigrosin staining and appropriate dilutions made. Cells were then plated out at 1×10^6, 2.5×10^5 or 62,500 viable cells in 2 ml of equal volumes of double strength E1010 and 0.6% agar on top of the previously prepared kidney feeder layers. Triplicate sets of plates were prepared at each cell concentration. The agar was allowed to gel and the plates incubated at 37°C in a humidified incubator with a constant flow of 5% CO_2 in air.

Cell studies. Bone marrow cell size frequency distribution patterns were determined using a Coulter Model B attached to an automatic plotter Model J (Coulter Electronics, Hialeah, Fla.). Bone marrow cells were suspended in Eagle's medium with 10% fetal calf serum and cell debris removed by centrifugation at 200 *g* for 10 minutes. The cells were resuspended in phosphate buffered saline pH 7.0. The aperture used was 50 μ (settings; amplification 2, aperture current 2) and the counter calibrated for cell volumes using rat red cells. Percentage frequency distribution curves were constructed for cells having volumes between 102-317 μ^3 (windows 8-25).

Bone marrow differential cell counts were done on Wright's stained preparations of direct bone marrow smears and smears of marrow pools under oil immersion at × 1000 magnifications. Scoring of smears was done "blind," using coded smears.

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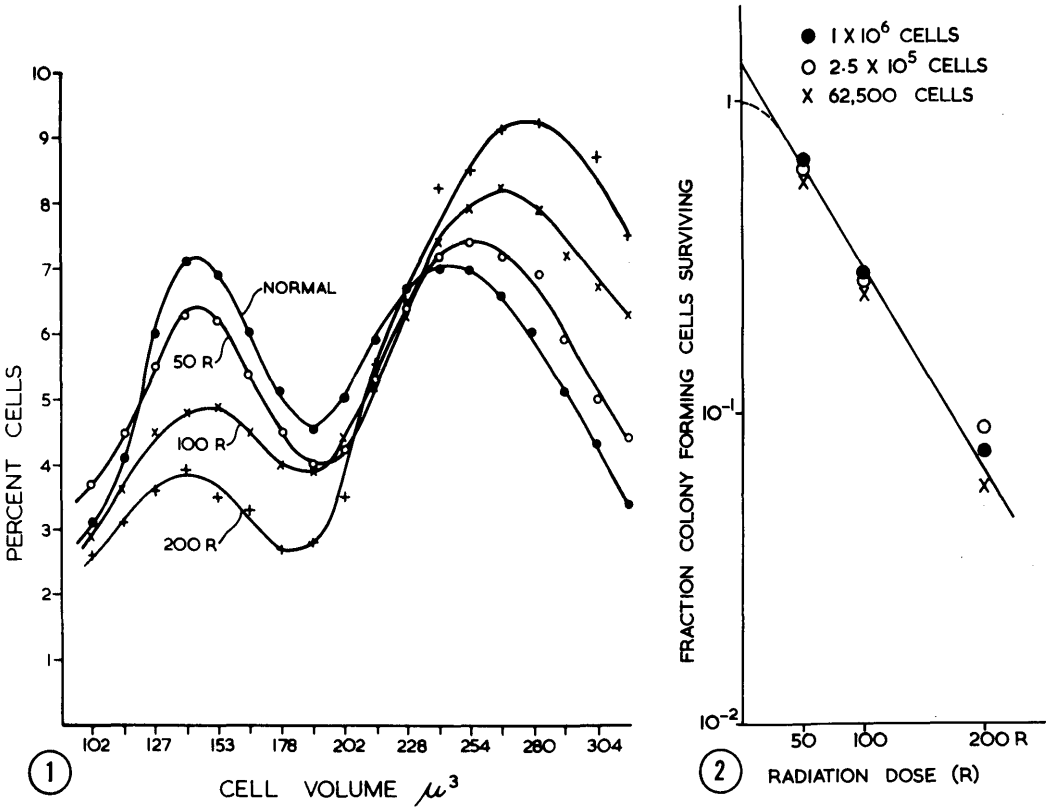

FIG. 1. Cell size distribution patterns of 3-months-old C57Bl bone marrow cells. Note the progressive fall in the peak of small cells with increasing doses of whole body irradiation.

FIG. 2. Radiation dose inactivation curve for bone marrow colony forming cells. Points are plotted as fraction n/n_o where n = number of colonies after varying doses of irradiation, n_o = number of colonies from non-irradiated bone marrow = 1. Curve is mean for the 3 cell dilutions used.

Results. The frequency distribution of bone marrow cells from normal C57Bl mice and C57Bl mice 48 hours following various doses of irradiation is seen in Fig. 1. Normal bone marrow cells produced a characteristic bimodal cell size distribution pattern. With increasing doses of irradiation there was a progressive fall in the percentage of bone marrow cells making up the small cell peak (cell volumes 102 μ^3 to 190 μ^3; peak at 150 μ^3) and a corresponding increase in the percentage of cells greater than 190 μ^3 in size.

Differential counts on stained smears of the irradiated and normal bone marrow cells from the same animals (Table I) indicated that this fall in the percentage of small cells was due largely to a progressive fall in the percentage of "small lymphocytes" (*i.e.*, mononuclear cells with nuclei less than 7 μ in diameter) in the marrow after irradiation.

The fall in colony forming ability of bone marrow cells from irradiated mice is seen in Fig. 2. The points plotted represent the means of 3 or 4 experiments (each on 3 mice) at each dosage of irradiation and dilution of bone marrow cells used. The incidence of colony forming cells in the bone marrow of non-irradiated mice (1 per 2000) is in good agreement with estimates obtained in previous experiments(2).

The shape of the radiation dose effect curve is similar to that obtained by other workers studying the effects of X-rays on the colony forming ability of mammalian cells *in vitro* and *in vivo*(3,4), with a shoulder at lower doses of irradiation followed by a linear exponent characteristic of a multi-hit system, the 0.37 survival dose being approximately 85

TABLE I. Changes in Bone Marrow Cell Populations 48 Hours Following Whole Body Irradiation.

Radiation Dosage	PERCENTAGE DISTRIBUTION OF Erythroblast	"Small Lymphocyte"	Myeloblast and Promyelocyte	Myelocyte and Polymorph
Normal Non-Irradiated	25	22	6	47
50R	24	15	7	54
100R	33	11	4	52
200R	31	8	4	57

rads. The similarity of the survival values obtained at three different marrow cell dilutions indicates that the system is quantitatively reliable over the range of cell dilutions plated ($62,500 - 1 \times 10^6$ per dish).

Discussion. The present data have demonstrated, as has been shown by others(5), that following whole body irradiation of mice there is a marked depletion of bone marrow "small lymphocytes," probably by pyknotic cell death(6), and a corresponding rise in the percentage of larger cells of the marrow. The 0.37 survival dose for bone marrow small lymphocytes obtained in the present study agrees well with an estimate of 75 R by Haot, Haughton and Revesz(5). In the present study this change in population distribution was correlated with a fall in the colony forming ability of marrow from irradiated animals in the *in vitro* system used, suggesting that colony forming cells are members of the "small lymphocyte" group of cells. If the colony forming cells belonged to one of the other cell populations in the bone marrow, then the number of colonies formed per thousand bone marrow cells should have increased rather than decreased following irradiation. However, the damaging effects of radiation on cells are undoubtedly more subtle than can be demonstrated by gross estimations of changes in cell morphology, size and viability as demonstrated by supra vital staining. As has been pointed out(4), more subtle cell damage may itself manifest only when the altered cells lose or gain potential for proliferation. Thus it is conceivable that the bone marrow colony forming cells in this system may not be members of the small lymphocyte population but cells of some other type which although appearing intact morphologically may have been rendered incapable of proliferation *in vitro*.

The findings are of considerable interest in view of earlier evidence(7) that some bone marrow "small lymphocytes" may be hematopoietic stem cells, capable of forming cell colonies in the spleen of irradiated recipients. In the present *in vitro* system only granulocytic and mononuclear cells were present in developing colonies and no erythroid, megakaryocytic or lymphoid cells were identified. It is uncertain therefore whether the *in vitro* colony forming cells in the present experiments are identical with the multipotential *in vivo* spleen colony forming cells. It may be, however, that current *in vitro* culture methods allow only certain types of cellular differentiation and that under other conditions the colony forming cells may have the potential for differentiation into cell types other than those that have been observed thus far.

Summary. With increasing doses of whole body irradiation there was an exponential decrease in the percentage of cells in the bone marrow capable of *in vitro* colony formation. This decrease was correlated with a fall in the percentage of small lymphocytes in the bone marrow suggesting that the *in vitro* colony forming cells are members of the small lymphocyte population.

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1. Pluznik, D., Sachs, L., J. Cell & Comp. Physiol., 1965, v66, 319.

2. Bradley, T. R., Metcalf, D., Aust. J. Exp. Biol. & Med. Sci., 1966, v44, 287.

3. Puck, T. T., Marcus, P. I., J. Exp. Med., 1956, v103, 653.

4. Till, J. E., McCullough, E. A., Radiation Research, 1961, v14, 213.

5. Haot, J., Haughton, G., Revesz, L., European

J. Cancer, 1967, v3, 67.
6. Trowell, O. A., J. Path. Bact., 1952, v64, 687.
7. Cudkowitz, G., Upton, A. C., Smith, L. H., Goslee, D. G., Hughes, W. L., Ann. N. Y. Acad. Sci., 1964, v114, 571.

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Experimental Herpes Simplex Infection in the Owl Monkey.* (32100)

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Characteristic of the herpesvirus group is the wide range of infectivity for animals. Thus, *H. hominis* infects the rabbit, guinea pig, mouse, hamster and cotton rat when inoculated by various parenteral routes(1). After ocular inoculation the usual strain of *H. hominis* infects the cornea of the rabbit and guinea pig, produces keratitis and fails to spread to the central nervous system or systemically. Intracerebral inoculation usually results in encephalitis. Certain neurotropic strains spread to the CNS after ocular inoculation.

A number of early studies showed that *H. hominis* was infectious also for Old World monkeys, by intracutaneous or intracerebral inoculation(2,3). Infection of the squirrel monkey by ocular inoculation resulting in keratitis, was reported recently(4). The susceptibility of other small New World monkeys is apparently unknown.

Herpesvirus simiae (Herpes B) represents the natural herpesvirus infection of the Rhesus monkey and is infectious for the human as well as a variety of laboratory animals. In the human, *H. simiae* is typically neurotropic and infection results in extensive encephalitis. Widespread systemic disease, with involvement of various organs, including the liver, spleen, kidneys and adrenals may also occur(5).

A new simian herpesvirus has recently been identified. The new virus, designated *Herpesvirus tamarinus* was isolated from marmoset monkeys during an outbreak of disease among newly formed colonies in this country. In these small New World primates *H. tamarinus* infections were frequently fatal. Characteristically, infection involved the liver and adrenal(6). Other small primates, such as the squirrel monkey *(Saimiri sciureus)* apparently develop subclinical infections, become viral excretors and may represent a reservoir of infection to a colony of susceptible animals(7).

During experiments designed to study the effects upon the cornea of ocular inoculations of *H. hominis*, it was observed that the owl monkey *(Aotus trivirgatus)* was markedly susceptible to infection. This report describes various clinical, virologic and pathologic events occurring during a generalized infection of the owl monkey with *H. hominis*.

Material and methods. Monkeys. The owl monkeys *(Aotus trivirgatus)* employed in these experiments were adult males and females weighing between 0.6 and 0.8 kg. These animals were captured wild in the jungles of Colombia, and were quarantined in mosquito-proof cages for 14 days prior to shipment to the United States. At an animal farm in this country they were housed separately, except for an occasional contact with Capuchins *(Cebus capucinus)*.

In the laboratory, the monkeys were caged

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