Evidence for the Presence of Stem Cells in the Tail of the Mouse.* (30142)

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(Introduced by V. P. Bond)
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Several studies have shown that either shielding a portion of the mammalian hemopoietic system during irradiation, or post-irradiative infusion of hemopoietic tissue, results in subsequent regeneration of the hemopoietic system and increases survival. Jacobson and coworkers(1) demonstrated markedly increased survival in mice as a result of shielding the spleen at irradiation, even when splenectomy was performed as soon as one hour post-irradiation. Storer, Lushbaugh and Furchner(2) found an increased survival in rats from shielding tails in which red marrow had been induced, but not from shielding normal tails. Swift, Taketa and Bond(3) increased survival in mice by allowing a 90-minute interval to elapse between exposures of the upper and lower halves of the body. They also found(4) increased survival in rats when one hind leg was shielded during irradiation and was exposed later. The degree of protection was found to be a function of the interval between irradiations. Till and McCulloch(5) introduced a procedure for quantitative assay of the hemopoietic repopulation potential of cell suspensions in terms of the numbers of nodules formed in the spleens of irradiated recipient mice. Cudkowicz and coworkers(6) applied this procedure, and also one depending on splenic uptake of iododeoxyuridine(7), to studies of marrow repopulation in mice. More recently, using the spleen nodule technique, Trobaugh and Lewis (8) have measured the repopulation potential of the peripheral blood of mice, and Hanks (9) has measured rate of migration of repopulating factor from the shielded femur.

Earlier work at this laboratory(10) showed that shielding the tails of mice of the Hale-Stoner strain at irradiation resulted in regeneration of the spleen. No published studies of the effects of shielding the tails of mice are known to the authors. Mouse tail marrow, like that of rats, generally has been considered to be yellow and non-hemopoietic. The work described here was undertaken to confirm the earlier finding, elucidate further the mechanism of the effect, and evaluate the usefulness of the procedure as a radiobiological tool.

Materials and methods. Experimental. The mice used were males of the Hale-Stoner strain (albino Swiss), 6 to 8 weeks of age at time of irradiation. After irradiation the groups were housed separately, 10 or fewer mice to a cage, and kept under standard colony conditions until termination of the experiment.

Exposures were made in a 10-sectored cage placed 50 cm from the target of an X-ray machine operated at 250 Kvp and 30 ma with a Thoraeus III filter. The half-value layer of the radiation was 2.9 mm of Cu, and the rates were about 65 r/min for the body doses and 50 r/min for the tail. The exposures given in the tables are midline values measured with a calibrated Victoreen chamber. For the tail-shielded groups, the terminal ⅔ (or about 6 cm) of the length of the tail was shielded during the first irradiation with ⅛″ thicknesses of lead above and below; and during the second irradiation the remainder of the body was shielded with ¼″ of lead. The exposed regions overlapped so that every part of the body was irradiated at least once. The tail was held in position by taping to the lower half of the shield. The procedure does not cause undue stress and no anesthesia was used.

When IDU uptakes were to be measured, the mice were given a solution of 0.1% NaI for drinking water from the time of irradiation, following the procedure of Hughes and coworkers(7). On the final (5th,

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6th or 7th) day the mice were injected in the morning, and sacrificed 6 hours later. Each animal received by tail vein 0.2 cc of a solution containing both I^{131}-labeled iododeoxyuridine(7) (3 to 5 μc, carrier-free) and I^{125} as iodide (1 to 4 μc). The spleens were removed at sacrifice, weighed, and assayed for both isotopes. From the counts, a calculation was made of the fraction of IDU incorporated into the spleen, corrected for I^{131} as iodide (from breakdown of IDU).

For the experiment in which spleen nodules were counted, the method outlined by Till and McCulloch(5) was followed. Spleens were removed on the tenth day after irradiation and fixed in Bouin's solution.

Statistical procedures. The values tabulated for each group are the arithmetic means together with their 95% confidence intervals given by $\pm$ twice the unbiased estimates of their standard errors. In the case of the nodule counts, since a chi-square test on the two larger groups showed the distribution of counts to be consistent with Poisson statistics, the more appropriate asymmetric Poisson limits(11) (with equal 2½% outside probabilities) have been added in parentheses.

Anatomy and histology of the tail. Sagittal sections show the marrow spaces to be filled largely with fat cells, but the distribution of the scattered hemopoietic tissue to be relatively uniform throughout the marrow space of each bone. The ratio of the hemopoietic volume to that of fat appears approximately constant (about 1 to 5). Differential counts of nucleated hemopoietic cells were made from a femur and one of the larger tail vertebrae of a donor mouse. The ratio of myelocytic cells (exclusive of segmented polymorphonuclear cells) to round cells (lymphocytes and monocytes) were 2.0 for both tail and femur marrows, but those for myelocytic to erythrocytic cells were 2.0 for tail and 1.2 for femur. The erythrocytic component in tail marrow thus appears relatively reduced.

The total marrow volume shielded in these experiments (bones 10 through 30, counted from the pelvis) is estimated from sections to be about 8 mm^3. Counts of total nucleated hemopoietic cells in the 6 largest bones which were shielded, *i.e.*, numbers 10 through 15, in 2 unirradiated animals gave an average of 3.7×10^6. It is estimated from volume measurements that the rest of the shielded part of the tail contained an additional 15%, so that 4.3×10^6 nucleated hemopoietic cells per mouse were shielded. The concentration of nucleated cells in the tail marrow is thus 0.5×10^6 per mm^3. By comparison, a normal femur contains about 1.5×10^7 nucleated cells in a volume of about 5 mm^3, which is 3×10^6 per mm^3, or 6 times the concentration for tail marrow, a value compatible with the relative histological appearances of the two tissues.

Results. Table I lists IDU uptakes and spleen weights 5, 6 and 7 days post-irradiation. The spleens of the whole-body control animals are small, about ¼ normal weight, change little from day to day, and have low IDU uptakes. Spleen weights of the tail-shielded animals, after declining to a similar value, show a significant increase between 5 and 6 days post-irradiation, and a larger increase between 6 and 7 days. The corresponding IDU uptakes show a large increase between 5 and 6 days and a smaller one between 6 and 7 days. From the IDU data we concluded that either 6 or 7 days post-irradiation is a satisfactory time to measure

TABLE I. Spleen IDU Uptakes and Weights *vs* Time After Irradiation.

Irradiation	Days post-irradiation	No. mice	% IDU uptake mean $\pm$ 2 S.E.*	Spleen wt, mg mean $\pm$ 2 S.E.
675 r, tail-shielded	5	5	.09 $\pm$ .04	31 $\pm$ 6
" "	6	5	.45 $\pm$ .08	44 $\pm$ 6
" "	7	3	.56 $\pm$ .11	80 $\pm$12
675 r, whole-body	5	4	.00 $\pm$ .01	31 $\pm$ 5
" "	6	12	.01 $\pm$ .01	24 $\pm$ 2
" "	7	4	.01 $\pm$ .01	26 $\pm$ 3

* See *Statistical procedures.*

TABLE II. Spleen IDU Uptakes and Weights on 6th Day Post-Irradiation *vs* Time Interval Between Irradiations of 675 r to Body with Tail Shielded and 675 r to Tail Only.

Time interval between irradiations	No. mice	% IDU uptake ——means ± 2 S.E.——	Spleen wt, mg ——means ± 2 S.E.——
0 hr (whole-body)	3	.03 ± .01	31 ± 8
	12*	.01 ± .01	24 ± 2
.3 hr	9	.03 ± .01	30 ± 3
1.8 "	6	.06 ± .03	32 ± 4
3.4 "	5	.09 ± .03	33 ± 6
21 "	5	.32 ± .15	51 ±13
6 days (no tail exposure)	4	.45 ± .29	51 ±16
	5*	.45 ± .08	44 ± 6

* Corresponding data from experiment of Table I.

TABLE III. Spleen Nodule Counts and Weights on 10th Day Post-Irradiation *vs* Time Interval Between Irradiations of 780 r to Body with Tail Shielded and 780 r to Tail Only.

Time interval between irradiations	No. mice	Nodules per spleen ——means ± 2 S.E.——	Spleen wt, mg ——means ± 2 S.E.——
0 hr (whole-body)	10	.2 ± .3 (.04 to .63)*	24 ± 2
1.9 hr	22	8.0 ± 1.2 (6.9 to 9.3)	45 ± 5
3.6 "	21	11.0 ± 1.4 (9.7 to 12.6)	51 ± 7
10 days (no tail exposure)	5	>20†	115 ± 9
Unirradiated	2		107 ±10

* Poisson limits—see *Statistical procedures.*
† Numerous overlapping colonies, estimated more than 20 for each spleen.

uptake, whereas 5 days appears less desirable since the uptake is smaller and is probably affected more by variations in the exact time between irradiation and injection.

Table II and Fig. 1 show the dependence of IDU uptake measured on day 6, on the time interval between irradiation with tail shielded and irradiation of the tail only. The uptake values appear to increase at an approximately constant rate for the first day of time interval and more slowly thereafter.

Table III summarizes the results of a study of the dependence of spleen nodule counts on the interval between irradiations. The first 3 average nodule counts are well within the linear range of our counting technique which has been found to extend to 15 nodules per spleen as determined by injection of known numbers of marrow cells. The 4 extra days' regeneration time of Table III compared to Table II (10 days *vs* 6) is reflected in the greater spleen weights of the 2- and 3½-hour groups.

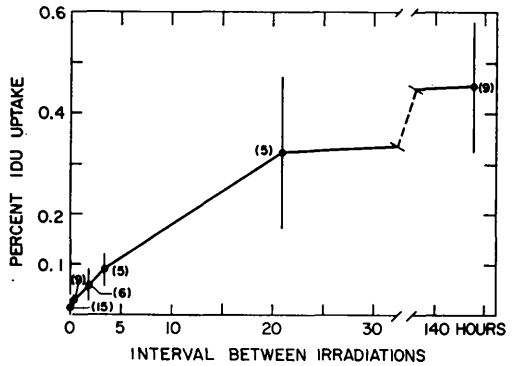

FIG. 1. Plot of IDU uptake data of Table II.

Effective suppression of the tail at the end of the interval between irradiations is shown by the low values of IDU uptake (Table II) and of nodule counts (Table III), respectively, for the zero-interval (whole body) control groups.

The gross appearance of these spleens is similar to that illustrated by Till and McCulloch(5), and is indistinguishable from that of spleens which we have obtained 10 days after infusion of femur marrow suspensions into irradiated mice.

Histological examination of H- and E-stained sections of 28 nodules in spleens of mice from the 1.9-hour group of Table III showed all nodules to be hemopoietic with many mitotic figures. Twenty-five appeared to be purely erthrocytic. (Studies of the histology of similar nodules have been reported by others(12,8).)

Discussion. The above results are taken to indicate that hemopoietic stem cells migrate from the shielded tail after the first irradiation and seed colonies in the spleen, and that such migration is effectively terminated by the second irradiation. The subsequent spleen regeneration is a measure of the number of stem cells which migrated during the interval between irradiations. In particular, the number of spleen nodules at sacrifice is proportional to this number of cells (within the limits of the counting method—see *Results*).

The IDU uptake is less simply related to

the stem cell migration. Like its structural analog thymidine, IDU is incorporated only into DNA. The amount of IDU incorporated into a tissue can thus be expected to reflect the rate at which that tissue is synthesizing DNA, and this has been shown to be the case by Hughes and coworkers(7). Since this rate is, in turn, a measure of cellular proliferation, it follows that IDU uptake depends not only on the number of colonies present, but also on their size and on the fraction of cells which are in the S-phase at the time. The rate of colony growth (estimated from nodule sizes to have a doubling time of about 12 hours) is such that colonies which are seeded early are much larger than those seeded late and hence incorporate most of the IDU. It is thus not unexpected that the probable uptake value for the 6-day interval (Table II, Fig. 1) is only a little higher than that for 21 hours.

The first 3 nodule counts given in Table III show a significantly greater average increase per hour in the first 1.9 hours than in the next 1.7 hours. The rates with 95% confidence limits are 4.0 ± 0.7 and 1.8 ± 1.1 nodules per hour, respectively. No significant part of this difference in rates can be an artifact of nodule counting since, as noted above (*Results*), the 3 counts are within the linear range of counting. Taking into consideration the IDU data of Table II and Fig. 1, it thus seems likely that a nodule curve for the first 20 hours of interval would show a steady rate approximating the lesser of the 2, *i.e.*, 1.8 per hour, during all except the first few hours. Such a curve would have a positive extrapolated value of about 4 to 6 on the nodule axis. This would appear to indicate a loosely-bound stem cell pool of corresponding size, which is depleted from the tail within this initial period. The existence of such a pool would be in agreement with two other findings. First, Hellman(13) found a strong inverse rate-dependence in the irradiative killing of stem cells in mice with one leg shielded, which corresponds to a loosely-bound pool in the leg capable of seeding about 10 spleen nodules. Second, in a preliminary experiment in which we irradiated the tail first, then the rest of the body and finally the tail again, we found that a significant number of stem cells apparently migrated from the body to the tail within a few hours and back to the spleen within a day. Hanks'(9) shielded femur data, on the other hand, gave no indication of such a pool.

For partial body shielding experiments, tail-shielding appears to have certain advantages over leg-shielding. As noted above (*Materials and methods*), no anesthesia is required. The tail is more readily accessible than the leg, and the amount shielded is subject to more precise control. The composition of the tail is more homogeneous and appears to be particularly well suited for studies of the effect of the proximity of bony structures on the dose delivered by X-rays to marrow.

Summary. In experiments in which the tails of mice were shielded during exposure to X-rays, colonization of the spleen occurred in excess of that found in whole-body irradiated control animals. This was the case whether the measure of effect was post-irradiative gain in spleen weight, uptake of IDU (5-iodo-2′-deoxyuridine), or nodule count. The degree of colonization was found to be a function of the length of time which elapsed between the original irradiation (with tail shielded) and a subsequent, suppressive irradiation of the tail. Histological examination showed that the spleen nodules were hemopoietic, and that normal tail vertebrae contained hemopoietic tissue.

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Some Cytologic Changes of Rat Liver in Experimental Porphyria.* (30143)

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Experimental porphyria refers to a condition in which one of the most prominent metabolic aberrations is an over-production of porphyrins in the liver. This condition was first induced experimentally with allylisopropylacetylcarbamide (Sedormid), but in recent years a wide variety of compounds has been found to induce the same metabolic changes. Most investigated among these has been the increased formation and accumulation of porphyrins and their precursors in the liver as well as their excretion in the urine(1,2). Not only is the entire heme biosynthetic pathway accelerated in this condition(3), but the effects of porphyria-inducing drugs are now known to encompass several areas of metabolism. In lieu of a thorough literature review suffice it to say that a great variety of enzyme activities, metabolic pathways, and physiological values have been reported to be abnormal in experimental porphyria as well as in the naturally occurring porphyrias.

The observation that livers of porphyric animals are enlarged has been known for several years. Yet, relatively little investigation has been directed toward this effect of the drugs. The present observations on cytologic changes occurring in liver cells were made to obtain a more thorough understanding of the nature of experimental porphyria.

Materials and methods. Sprague-Dawley female rats weighing 180-200 g were divided into 2 groups of 4 animals each. To induce porphyria 26 mg of allylisopropylacetamide (AIA) in a solution of 0.45% NaCl (20 mg AIA/ml) were injected subcutaneously twice daily for 3 consecutive days in the one group. The other group received saline injections. The rats were then sacrificed by decapitation and the livers immediately removed for study. To aid in consistent porphyria induction all animals were starved beginning one day prior to the first injection and throughout the experiment. They were given water *ad libitum*.

Liver volume was determined by water displacement. A portion of each liver was fixed in neutral formalin and stained with hematoxylin and eosin. The number of cells per unit volume of liver tissue was determined by counting the nuclei in the mounted and stained tissue sections using oil immersion (970 ×) in the following manner: The microscope ocular contained a calibrated grid 1 cm × 1 cm and subdivided into 0.1 cm units. The grid was placed over an area chosen at random in the tissue section. All of the nuclei within the perimeter of the grid and in the entire thickness of the section were counted. The thickness of the section was estimated using the fine focus adjustment on the microscope. This ranged from 4 to 7 μ. Thus, knowing the size of the grid,

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