FACTORS INFLUENCING HEMATOPOIETIC SPLEEN COLONY FORMATION IN IRRADIATED MICE

I. The Normal Pattern of Endogenous Colony Formation*

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Hematopoietic nodules visible in the gross have been noted on the spleens of mice exposed to irradiation 10 days previously, and their numbers have been shown to decrease with increasing irradiation dose (1, 2). It remained, however, for Till and McCulloch (3) to demonstrate that the number of such "colonies" declined exponentially with increasing irradiation dose. Similar colonies are formed when heavily irradiated (more than 800 R) mice are given a sufficient number of bone marrow, spleen, blood, or peritoneal fluid cells (4). The colonies formed in the "exogenous" or "transplant" system have been shown to be clonal (5), i.e., formed from single precursor cells which are thought to be stem cells since the numbers of colonies formed is directly proportional to the number of cells injected (4). Considerable information has been obtained with respect to the exogenous system, including changes in colony number (4), spleen weight (6), splenic iron uptake (7), iron incorporation into red cells (8), and splenic uptake of iododeoxyuridine (IUdR) as an index of DNA synthesis (9, 10). The endogenous system, however, in which spleen colonies presumably develop from the surviving stem cells of a less heavily irradiated mouse, has received less attention in the literature and, although isolated data are available, there is little unified information.

The endogenous system has certain advantages over the exogenous transplant system. These include simplicity, rapidity, and perhaps most importantly, in vitro manipulation of cells is avoided. The use of the transplant system as a measure of stem cell activity has been questioned, since the kinetics of the stem cell recovery pattern, using transplanted cells, differed markedly from those observed using certain other systems thought to reflect endogenous stem cell activity (11).

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We undertook the study of patterns of endogenous colony formation hoping to use this system as an assay for factors influencing the rate of growth and direction of differentiation of hematopoietic tissue. The following paper indicates that the system can indeed be influenced profoundly by various stimuli. In this report, we are concerned with characteristics of the normal pattern of spleen colony formation in irradiated mice. The relationship of colony numbers, spleen weight, splenic iron and IUdR uptake to irradiation exposure, and the interrelationships of these measurements are reported.

Materials and Methods

Mice.—B6D2F1 mice (C57 BL/6J females × DBA/2J males) were purchased from Jackson Laboratories, Bar Harbor, Maine, or raised in our laboratory from parental strains purchased from that source. Male mice were used except where indicated. Mice were irradiated when 7–11 wk of age and were caged in groups of 5–10, except after high doses of irradiation (800 R or more), when they were caged singly or in pairs. Purina Lab Chow and water were available ad lib.

Irradiation.—The source was a ^{60}Co irradiator equipped with a cylindrical elevator and an automatic timer (model GR 9, U.S. Nuclear Corp., Burbank, Calif.). It delivered approximately 900 R/min in air as measured with a Fricke ferrous sulfate dosimeter. Irradiation exposures were calculated assuming a half-life of 5.27 yr. Mice were irradiated five at a time in cylindrical ventilated cans, 8.5 cm in diameter.

Isotopes.—Ferric chloride-^{59}Fe, specific activity 7.2–18.2 mc/mg, and carrier-free sodium sulfate-^{35}S, 552 μc/ml, were purchased from Abbott Laboratories, North Chicago, Ill. Diisopropylfluorophosphate-^{32}P (DF-^{32}P), specific activity 0.144 mc/mg, and 5-iodo-2′-deoxyuridine-^{125}I (^{125}I-UdR), specific activity 2.3–4.8 mc/mg, were purchased from the Radiochemical Centre, Amersham, England.

Experimental Procedure.—Mice were killed 10 days after irradiation. 6 hr before killing, they were injected subcutaneously with 0.1 μc of ^{59}Fe citrate (7) in a volume of 0.1 ml. In certain experiments, mice were also injected subcutaneously on the opposite side with 0.1 ml of a saline solution of ^{125}I-UdR, 0.1 or 0.3 μc. The mice were weighed, the volume of packed red cells (VPRC) determined by orbital sinus puncture with a capillary tube (12), and killed by cervical dislocation. The spleens were removed, placed in Bouin's solution, weighed, and radioactivity determined in a well type scintillation counter. ^{125}I and ^{59}Fe were counted in channels adjusted so that there was no discernible activity due to iodine in the iron channel and a constant amount (6%) of iron detected in the iodine channel. No interference by either isotope with the uptake of the other by the spleen or with counting efficiency was observed in vivo or in vitro at the doses used. The ^{125}I-UdR uptake by normal spleens, expressed as cpm/mg, was linear with the dose administered over the range 0.1–2.0 μc. Within 24 hr, the spleens were removed from Bouin's solution, the number of macroscopic nodules counted, and the spleens were then stored in 70% ethyl alcohol. Histologic sections, stained with Giemsa and with hematoxylin and eosin, were prepared from selected spleens.

Calculations.—Data from many experiments were pooled, and the mean and standard error at each irradiation exposure plotted on semilogarithmic paper. Where appropriate, the regression lines were calculated by the method of least squares with the aid of a Control Data 3200 computer. The curve relating the number of colonies to irradiation exposure was calculated by entering the logarithm of the mean number of colonies into the equation as many times as there were spleens at that exposure. This made it possible to consider radiation exposure which included some spleens with no colonies. A similar procedure was followed

when values for spleens without colonies were subtracted from values for spleen weight, iron and IUdR uptake.

A standard expression relating the effect of irradiation upon cell survival is the term D_o. This is the dosage of irradiation required to reduce a cellular population to 37% of its original number (13). We have used D_o to describe the exponential portions of curves relating spleen

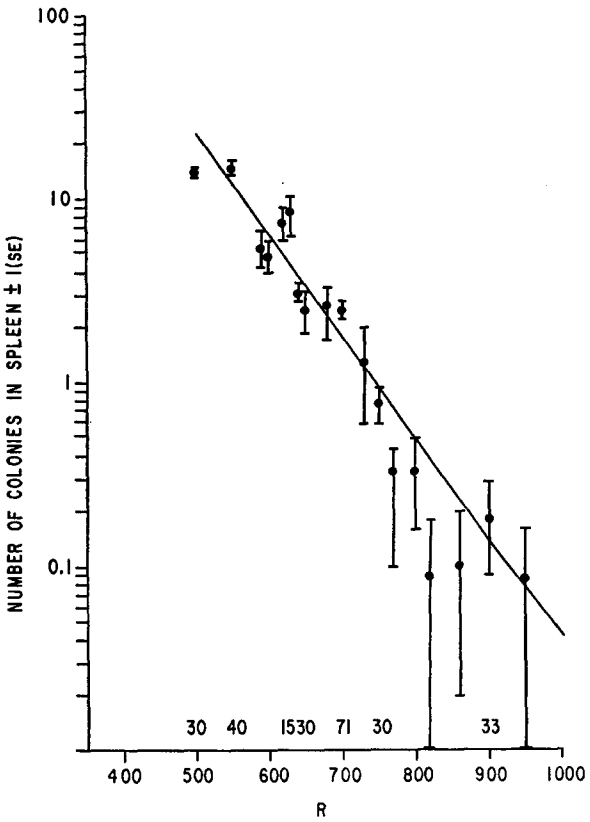

FIG. 1. The number of macroscopic hematopoietic spleen colonies as a function of the amount of irradiation. Each point is the mean of 10 mice, except as indicated at the bottom of the figure. Mice were killed 10 days after irradiation.

colony number, spleen weight, iron uptake, IUdR uptake, and VPRC to irradiation exposure. Irradiation exposure is expressed in roentgens (R).

RESULTS

Colony Formation as a Function of Irradiation Exposure.—The number of macroscopic colonies 10 days after irradiation decreased exponentially with increasing irradiation (Fig. 1). The D_o was 78 R. With less than 500 R, colonies were confluent and could not be counted accurately. This relationship obtained for groups of mice irradiated at different exposures on the same or on different

days. The theoretical extrapolated number of spleen colonies at zero irradiation was 13,770. The hematopoietic nature of the colonies was confirmed by histologic examination. The most prominent surface colonies were composed primarily of erythroid cells.

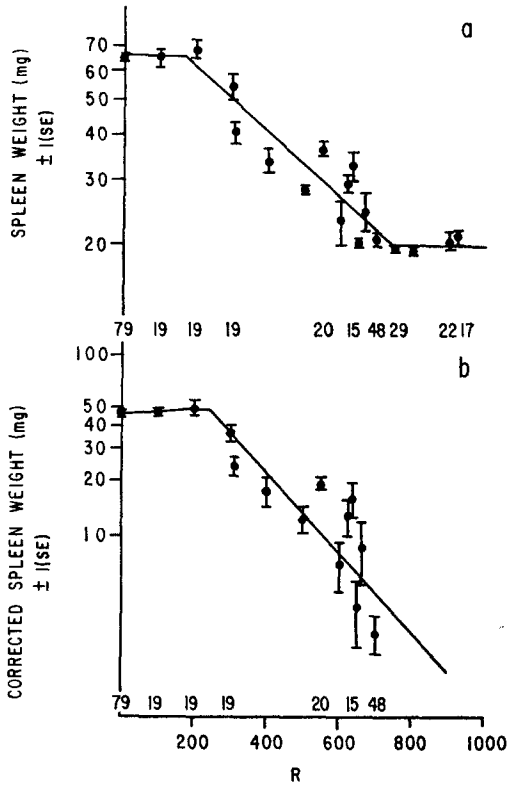

FIG. 2. Spleen weight as a function of the amount of irradiation. Each point is the mean of 10 mice, except as indicated at the bottom of the figure. Mice were killed 10 days after irradiation. The top figure (*a*) is the gross spleen weight. The bottom figure (*b*) is the corrected spleen weight. The mean weight of irradiated spleens without colonies (18 mg) has been subtracted from the gross spleen weight in the exponential range (200–700 R).

Spleen Weight.—Spleen weight changed very little at 100–200 R, and then decreased exponentially to 700 R where a plateau was reached (Fig. 2*a*). The calculated D_0 for the curve of decline observed between 200 and 700 R was 455 R. If the mean weight of irradiated spleens with no colonies (18 mg) was subtracted from the mean weight at each exposure, the D_0 of the resulting curve was 196 R (Fig. 2 *b*). This is probably a more accurate representation of the radiosensitivity of the spleen weight since it has been corrected for the irreducible minimum weight presumably due to radioresistant stroma.

Iron Uptake.—The iron uptake of the spleen at 6 hr after subcutaneous injection was expressed as a percentage of the uptake of the unirradiated control group (Fig. 3). It was corrected for the mean amount (5.9% of control) of iron taken up by the irradiated spleens without colonies. Iron uptake by spleens of groups of unirradiated mice ranged from 1.8 to 4.4% of the injected dose in

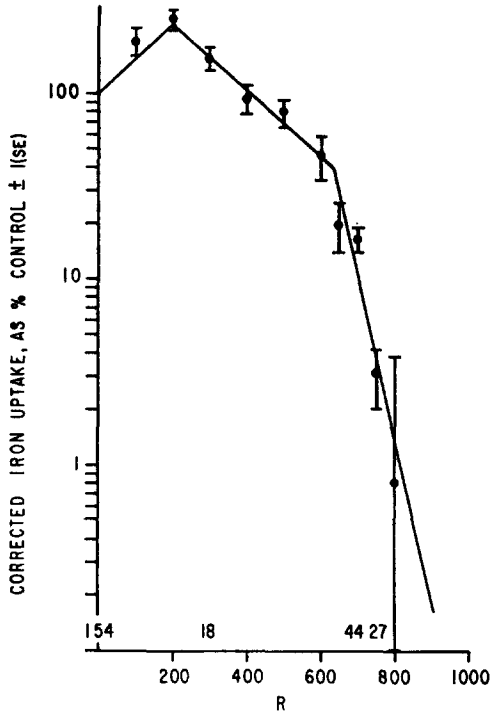

FIG. 3. Corrected 6 hr splenic iron uptake as a function of the amount of irradiation. Values are expressed as a percentage of the uptake of the unirradiated controls corrected for the uptake of the irradiated spleens without colonies. Each point is the mean of 10 mice, except as indicated at the bottom of the figure. Mice were killed 10 days after irradiation.

various experiments. The curve was complex, with an initial rise to over 250% at 200 R, followed by an exponential decline with D_o of 244 R from 200–600 R and an apparent change in slope from 600–800 R (D_o of 50 R). Curves with similar configurations were obtained at 9 and 13 days after irradiation as well as at 10 days, with iron given intraperitoneally rather than subcutaneously. The subcutaneous route of administration appeared preferable since the uptake by spleens without colonies was 40% less than it was after intraperitoneal administration.

Iododeoxyuridine Uptake.—The 6 hr IUdR uptake of the spleen was expressed as a percentage of the injected dose (Fig. 4). It was corrected for the mean

amount (0.071% of injected dose) taken up by the irradiated spleens without colonies. Between 200 and 800 R, there was an exponential decline with a D_o of 193 R.

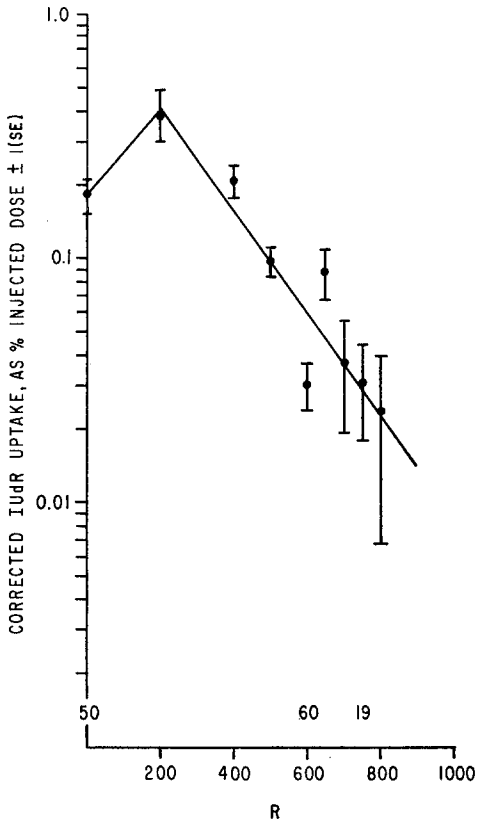

FIG. 4. Corrected 6 hr splenic iododeoxyuridine uptake as a function of the amount of irradiation. Values are expressed as a percentage of the injected dose corrected for the uptake of the irradiated spleens without colonies. Each point is the mean of 10 mice, except as indicated at the bottom of the figure. Mice were killed 10 days after irradiation.

Volume of Packed Red Cells (VPRC).—The fall in VPRC with increasing radiation dose could be approximated as a curve with two exponential slopes, with a change of slope at 400 R (Fig. 5). The more rapidly falling portion of the curve, with a D_o of 1111 R, is probably related, at least in part, to thrombocytopenic hemorrhage at high irradiation exposures. Hemorrhage was noted at the isotope injection site in many animals when they were killed 6 hr later.

Relationships of Spleen Colony Number, Weight, and Uptake of Iron and Iododeoxyuridine.—The slopes of the regression lines for number of visible

colonies and corrected iron uptake in the range 600–800 R are quite similar. As would be anticipated, there is a fairly good correlation ($r = +0.86$) between the mean iron uptake plotted against number of colonies (in the easily counted range) (Fig. 6).

The slopes of the regression lines for corrected spleen weight and corrected IUdR uptake are virtually the same (D_0 values of 196 R and 193 R, respectively). These slopes, however, are less than half as steep as those of colony number and iron uptake (D_0 values of 78 R and 50 R).

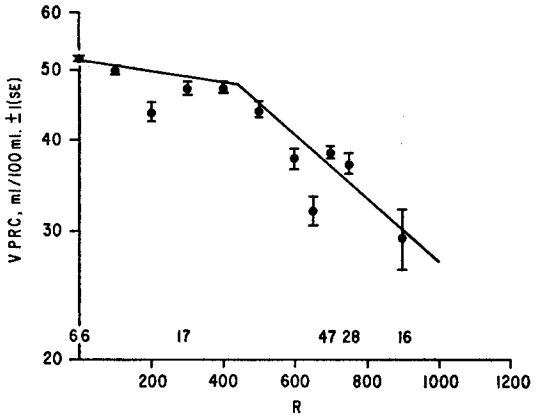

FIG. 5. Volume of packed red cells as a function of the amount of irradiation. Each point is the mean of 10 mice, except as indicated at the bottom of the figure. Mice were killed 10 days after irradiation.

The Effect of Age.—

50 female mice of the same age were divided into five equal groups. At 6 wk intervals a group was irradiated (600 R), killed 10 days later, and examined as described above.

Colony numbers, spleen weight, iron uptake, volume of packed red cells, and mouse weight tended to increase progressively with age (Table I).

Hypertransfusion.—To determine whether erythroid colonies could be eliminated from the spleens, studies were performed in mice hypertransfused before irradiation.

Blood was obtained from donor C57BL mice by decapitation and exsanguination into heparin, or obtained from Jackson Laboratories in heparin solution and used within 3 days of collection. The red cells were washed three times in saline and injected intraperitoneally as an 80% suspension in divided doses on each of 2 days, with a total dose of 1.5–1.7 ml.

The most apparent effect of hypertransfusion in irradiated and nonirradiated mice was to decrease iron uptake by the spleen (Table II). In plethoric mice, no decrease in the very low iron uptake per milligram of splenic tissue attended

irradiation. Spleen colonies in irradiated, plethoric mice were very small and difficult to count with accuracy but appeared to be reduced in number when compared to normal mice exposed to the same amount of irradiation. The colony numbers listed for the hypertransfused mice are, therefore, only approximations. Microscopic examination indicated that these small colonies were composed primarily of granulocytes and/or megakaryocytes. Erythroid colonies were not found. It was concluded that virtually no splenic erythropoiesis occurs in hypertransfused mice, even after irradiation.

Spleens of the unirradiated plethoric animals were heavier than those of

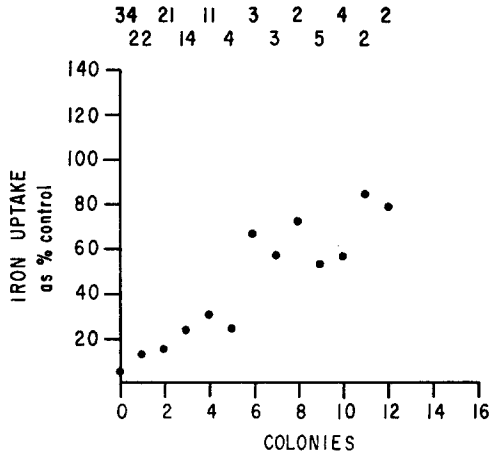

FIG. 6. 6 hr iron uptake as a function of colony number. Values are expressed as a percentage of the uptake of the unirradiated controls. Each point is the mean of the number of mice indicated at the top of the figure.

nonplethoric mice and appeared engorged with blood. 10 days after irradiation, however, the spleens from hypertransfused mice were not heavier than those from normal mice. IUdR uptake tended to decrease in all but the most heavily irradiated spleens when compared to spleens of normal irradiated mice at the same radiation exposure.

Uptake of Radioactive Diisopropylfluorophosphate and Sulfate by Irradiated Mouse Spleens.—DF-^{32}P labels neutrophilic granulocytes and, to a lesser extent, erythrocytes and platelets (14). An attempt was made to correlate spleen DF-^{32}P uptake with colony formation.

Two groups of 15 mice each were given 450 and 900 R, while a third group served as an unirradiated control. Nine days later, 5 mice in each group were given either 84, 42, or 8.4 μg (4.2, 2.1, or 0.42 mg/kg) of DF-^{32}P diluted in saline in a volume of 0.25 ml intraperitoneally. 6 hr later, the mice were killed and the radioactivity of the spleens determined by counting the whole spleen in an end-window Geiger-Mueller counter.

At the highest dose of DF-^{32}P considerable acute mortality was observed in proportion to the amount of irradiation given (Table III). At lower exposures, the uptake of DF-^{32}P was lower in the irradiated spleens than in the control

TABLE I
Effect of Age on Response to Irradiation

Age	No. of colonies*	Spleen weight*	Iron uptake* (% of inj. dose)	VPRC*	Mouse weight*
wk		*mg*		%	*g*
11	7.7 ± 2.3	23.2 ± 2.4	1.52 ± 0.39	34.3 ± 2.6	18.9 ± 0.8
17	12.2 ± 2.0	29.3 ± 1.9	1.49 ± 0.19	36.5 ± 0.9	22.0 ± 0.7
23	15.3 ± 3.4	34.0 ± 2.9	3.63 ± 0.71	40.1 ± 0.7	23.8 ± 0.6
29	>30‡	44.3 ± 3.6	4.00 ± 0.40	40.5 ± 0.7	24.0 ± 0.6
35	>30‡	39.0 ± 2.4	4.36 ± 0.83	40.7 ± 1.4	25.6 ± 0.4

* Mean values ± SE for 10 mice in each group, killed 10 days after 600 R. Iron injected 6 hr before killing.

‡ Confluent.

TABLE II
Effect of Hypertransfusion

Irradiation exposure	No. of mice	Amount of blood transfused per mouse	No. of colonies*	Spleen weight*	Iron uptake*	Iron uptake*	IUdR uptake*	VPRC*
R		*ml*		*mg*	*cpm*	*cpm/mg*	*cpm*	%
0	10	0		48.7 ± 1.4	112 ± 6	2.3 ± 0.6	484 ± 148	54.3 ± 0.3
0	9	1.7		84.3 ± 8.4	34 ± 18	0.4 ± 0.1	366 ± 35	64.6 ± 1.2
600	9	0	8.9 ± 1.4	31.7 ± 2.0	234 ± 59	7.4 ± 1.6	322 ± 38	38.0 ± 1.0
600	9	1.5	6.6 ± 1.1	28.6 ± 1.9	20 ± 1	0.7 ± 0.1	314 ± 35	59.0 ± 3.8
650	10	0	6.4 ± 1.5	27.5 ± 3.5	207 ± 3	7.8 ± 1.1	345 ± 53	36.1 ± 0.6
650	9	1.7	1.6 ± 0.5	22.9 ± 2.1	8 ± 4	0.4 ± 0.1	162 ± 19	51.5 ± 3.4
750	10	0	1.9 ± 1.1	20.3 ± 2.5	93 ± 36	4.7 ± 1.4	205 ± 52	32.9 ± 1.5
750	8	1.7	0.6 ± 0.4	21.0 ± 2.4	15 ± 3	0.8 ± 0.2	240 ± 51	43.1 ± 4.9

Mice, transfused i.p. with 80% red cells in saline 5–6 days before irradiation, were killed 10 days after irradiation. Isotopes given s.c. 6 hr before killing.

* Mean ± SE.

group, but there was little difference in uptake between the spleens with no colonies (900 R) and those expected to have confluent colonies (450 R). It was concluded that DF-^{32}P would not be a useful indicator of splenic hematopoiesis under the conditions employed.

Sodium sulfate-^{35}S is rapidly incorporated into rodent bone marrow (15) with a peak of radioactivity at 24 hr, and has been found by radioautographic

techniques to be incorporated almost exclusively into myeloid elements and megakaryocytes in the dog (16). The possibility of utilizing splenic uptake of ^{35}S as an indicator of nonerythroid hematopoiesis in spleen colonies was studied.

Four groups of 10 female mice were given varying amounts of irradiation, and another group served as an unirradiated control. 8 days later, five mice from each group received 10 μc of sodium sulfate-^{35}S diluted in saline in a volume of 0.5 ml, intraperitoneally. 24 hr later,

TABLE III

6 Hr Spleen DF-^{32}P Uptake at Different Irradiation Doses, 9 Days After Irradiation

Irradiation exposure	DF-^{32}P dose	Acute deaths	Mean cpm/spleen*
R	*mg/kg*		
0	4.2	1/5	2264
450	4.2	3/5	1995
900	4.2	5/5	—
0	2.1	0	1244
450	2.1	0	768
900	2.1	0	720
0	0.42	0	267
450	0.42	0	197
900	0.42	0	272

Only mice living at 6 hr were considered.

TABLE IV

24 Hr Spleen Sulfate-^{35}S Uptake at Different Irradiation Doses, 8 Days after Irradiation

Irradiation exposure	Mean No. of colonies	Mean cpm/spleen
R		
0		1382
450	14	847
525	7.2	747
700	1.0	623
850	0.2	641

the spleens were removed, dissolved in 1 ml of an organic base (NCS solubilizer, Nuclear-Chicago Corp., Des Plaines, Ill.) and counted in a liquid scintillation counting system made by adding 15 ml of toluene containing 4 g/liter of PPO 2,5-diphenyloxazole, and 0.05 g/liter of POPOP, *p*-bis[2-(5-phenyloxazolyl)]benzene (Pilot Chemicals Inc., Watertown, Mass.). Correction for quenching was made using an internal standard. The uninjected mice were killed at the same time and spleen colonies counted.

As the mean number of colonies per group decreased, there was a fall in radioactivity per spleen (Table IV). A plot of the logarithm of the radioactivity per spleen against the number of colonies was linear. The large amount of radioactivity in the heavily irradiated spleens with virtually no colonies, how-

ever, suggests that this method is at best an insensitive measure of hematopoiesis.

Attempts to Produce Spleen Colonies by Drug Administration.—Since sublethal doses of irradiation produce discrete hematopoietic spleen colonies 10 days later, a variety of drugs which are known to induce dose-related bone marrow depression was tried at different dose schedules and routes of administration.

Vinblastine, nitrogen mustard, methotrexate, and cyclophosphamide given at doses less than and exceeding the 10 day LD_{50} did not produce visible discrete spleen colonies in any of 242 drug-treated mice, although splenic hypertrophy was often observed. Spleen hypertrophy was particularly striking after vinblastine and microscopic examination showed diffuse hematopoiesis throughout the spleen.

DISCUSSION

The formation of hematopoietic colonies in the mouse spleen after irradiation is thought to be a function of surviving pluripotential stem cells, i.e., cells which respond to appropriate stimuli by differentiation into red cells, white cells, or platelets, and which are capable of self-replication. The sensitivity of colony-forming cells to irradiation in vivo (17) or in vitro (4), as assayed by transplantation into lethally irradiated hosts, is similar to that found for other mammalian cells irradiated and grown in vitro (18, 19), antibody-producing cells (20), erythropoietin-responsive cells (21), or cells of the spleen, thymus, and bone marrow (22). The D_o for endogenous colonies determined in this study (78 R) is similar to that reported for a less extensive irradiation range (3, 23, 24) and to that which may be calculated from other published data (6, 25–27). Others have reported colony numbers at isolated irradiation exposures with which our data is in fair agreement (28, 29).

Spleen uptake of iron and IUdR as well as spleen weight were measured in order to obtain ancillary information concerning splenic hematopoiesis.

If these measurements all reflect cell growth and function in colonies, the D_o values, describing radiation sensitivity, should be similar. The D_o for iron uptake was 50 R over the 600–800 R range while that for colony number was 78 R. The D_o for IUdR uptake (193 R) was virtually identical with that for spleen weight (196 R). However, the D_o for the latter two measurements was higher than those for the former two. The significance of these measurements will be considered in the following paragraphs.

The uptake of radioactive iron by spleens of irradiated mice was studied on the assumption that erythropoiesis in spleen colonies would thus be measured. Our data suggest that this assumption is valid over the same range of irradiation. The D_o for 6 hr iron uptake was 50 R, while that for colony number was 78 R. Furthermore, there was a good correlation between iron uptake and colony number ($r = +0.86$). Schooley (30) has reported a good correlation

between 24 hr spleen iron uptake and colony number in the transplant system. On histologic examination, large surface colonies appeared to be composed primarily of erythroid tissue, an observation in agreement with the detailed morphologic analysis carried out on spleen colonies derived from transplanted cells by Lewis and coworkers (31). Thus, the abolition of these large colonies by hypertransfusion was expected. Hypertransfusion reduced iron uptake by the spleen to that of an irradiated spleen with no colonies.

Using the endogenous system and measuring 6 hr iron uptake on postirradiation day 8, Smith (7) did not obtain values above those for unirradiated mice in the low irradiation range. Our higher values at 100–200 R are probably due to the measurement being made on day 10 since, in the transplant system, splenic iron uptake increases daily from 6 to 9 days for a given bone marrow dose (7). Smith reported a D_0 of 86 R in the 400–600 R range on day 8. He postulated that the depression of splenic iron uptake by high doses of bone marrow in the transplant system may be due to increased marrow red cell production in the recipient and regulatory suppression of splenic erythropoiesis. It is possible that a similar mechanism explains our higher value for D_0 at 200–600 R on day 10 (244 R), perhaps acting in conjunction with the time factor. Thus at this low range there is enough bone marrow erythropoiesis to decrease the splenic iron uptake somewhat, whereas at higher exposures the spleen may function alone.

Spleen weight changes were compared with colony number and iron uptake in order to determine whether an estimate of the weight of individual colonies could be obtained. The D_0 for spleen weight (196 R), however, was much higher than that for colony number (78 R) or iron uptake (50 R), even when corrected for the weight of the spleen with no colonies. A very similar D_0 may be calculated for 10 day postirradiation spleen weight from the data of Brues and Stroud (32). This probably represents the combination of the early loss of cells and the later proliferative response to irradiation. Spleen weight measurements 1–2 days after irradiation (32, 33), corrected for the residual stroma, measure cell loss. The D_0 values for such curves are 75–125 R, which is in the same range as that for colonies. Between 6 and 16 days after irradiation, spleen weight increases markedly, relatively more in the more heavily irradiated organs, accompanied by an increase in the mitotic index (32). Interrelationships between spleen weight and colony number in the transplant system have also been considered by Popp et al (6). Smith (7) found poor correlation between spleen weight and iron uptake in normal mice and in the transplant system. However, at a given radiation exposure, spleen weight may be a useful estimate of colony size. In a group of animals given 550 R, we observed a good correlation ($r = +0.75$) between colony number and spleen weight.

Radioactive iododeoxyuridine (IUdR) has been utilized as a measure of short-term DNA synthesis in mammalian cells in vitro, being incorporated in a

manner similar to thymidine with which it is competitive (34). The major advantage of ^{125}I-UdR is that it is a gamma emitter and thus technically is easier to detect than tritiated thymidine. The in vivo use of IUdR is less satisfactory because it is extensively deiodinated in the early hours after its administration (35, 36). Nevertheless, 6 hr splenic uptake of IUdR has been used by Cudkowicz et al. (10) to measure repopulation by transplanted bone marrow 5 days after irradiation. These authors found a linear relationship between the logarithm of the splenic uptake and bone marrow cell dose. Assuming that the bone marrow cell dose of Cudkowicz et al. can be converted into an irradiation exposure producing the same number of colonies in the endogenous system, a curve of the IUdR uptake against irradiation exposure yields a D_o of approximately 75 R. This is virtually identical to the D_o for colonies in our studies. Our IUdR uptake curve, based on data obtained on postirradiation day 10, had a D_o of 193 R, virtually the same as that for spleen weight, but much higher than that for colony number. The difference again is probably due to the time element, since by 10 days considerable differentiation into more mature hematopoietic cells has occurred, many of which do not synthesize DNA.

Many investigators have reported isolated points for hematocrit, hemoglobin, or red cell values in mice after varying amounts of irradiation (1, 37–44), with which our data are in fair agreement. The most comparable data are those of Ingram and Mason (45) who, in rats, also noted a sharp inflection at 400 R. This is probably due to hemorrhage resulting in part from thrombocytopenia and perhaps to increased capillary permeability as well (39, 46).

An increase in the number of endogenous spleen colonies was noted with increasing age. This may be a result of increasing spleen weight since spleen weight tended to parallel increase in colonies. Spleen weights and the percentage of splenic erythroid cells increase and then decline at various ages in different strains of normal unirradiated mice (47). If a constant number of stem cells were present regardless of age, increased numbers of spleen colonies might occur on the basis of increased spleen weight alone, because of the increased size of the "trapping area" for circulating colony-forming cells. On the other hand, there may well be an increase in the total number of stem cells occurring with increasing age, with a larger number (but the same fraction of the original) surviving a given irradiation dose. This is supported by studies which have demonstrated an increase in the LD_{50} of mice with increasing age, followed by a plateau or decline (48–51). The radioprotective effect of bone marrow transplanted into a lethally irradiated host reportedly increases with the age of the donor (50). The radiosensitivity of colony-forming cells in mouse bone marrow irradiated in vitro, however, has been reported to increase slightly with age (52).

Removal of the stimulus to erythropoiesis by hypertransfusion reduced the number of countable colonies. O'Grady et al. (53) reported that no increase

occurs in the number of nonerythroid colonies, evaluated histologically, in hypertransfused recipients of bone marrow cells, even though erythroid colonies are virtually abolished. These observations suggest that stem cells may differentiate into nonerythroid precursors at a fixed rate which is not influenced by a lesser demand for red cells. However, other observations suggest that the presence of a powerful stimulus to erythropoiesis may cause preferential differentiation of stem cells into erythrocytes at the expense of granulocytes and platelets (54).

The results of these studies suggest that the endogenous spleen colony method measures the same parameters as does the exogenous, transplant method. Since there are reproducible interrelations between spleen colonies, spleen weight, iron and IUdR uptake, the erythroid composition and proliferative capacities of the colonies can be measured by the simultaneous use of both methods.

SUMMARY

Data pertaining to the endogenous mouse spleen colony system, 10 days postirradiation, are presented. The D_0 for visible colonies is 78 R, while that for 6 hr iron uptake over a range of 600–800 R is 50 R. The D_0 for spleen weight is 196 R and that for IUdR uptake is 193 R. These measurements increase with the age of the mouse. Hypertransfusion decreases spleen iron uptake and colony number. DF-^{32}P and sodium sulfate-^{35}S are not useful indicators of splenic hematopoiesis in this system. Visible hematopoietic colonies in the spleen are not produced by vinblastine, nitrogen mustard, methotrexate, or cyclophosphamide.

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