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EFFECT OF INCORPORATED ^{226}Ra ON COLONY FORMING UNITS OF BONE MARROW AND SPLEEN IN MICE

by

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Reports on investigations of the long-term effect of ionizing radiation on hematopoiesis are not numerous. LAMERTON (1962) observed the response of peripheral rat blood elements and hematopoietic tissues to continuous external gamma irradiation at dose rates of from 176 to 4 rad per day and reported that 50 rad was the highest daily dose at which the peripheral blood count was steadily maintained. BLACKETT (1967) stated that under similar conditions of irradiation production of erythrocytes remained normal in rats, an ability explained by TARBUTT (1969) as due to changed cellular kinetics of the erythroid precursors. TWENTYMAN & BLACKETT (1970) applied continuous irradiation (40 rad/day) in mice and demonstrated that although the erythropoietic system did not break down it failed to prevent a considerable degree of anaemia caused by increased blood loss from thrombocytopenia. The histologic changes in bone marrow in mice produced by ^{90}Sr were described by NILSSON (1970). The kinetic investigation by FRIED et coll. (1966) dealt with the relation of the spleen stem cell compartment to the bone marrow damaged by ^{89}Sr radiation.

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The present work was directed to the stem cell compartment in marrow hematopoiesis in mice surviving for a long period after ^{226}Ra incorporation. Predominantly alpha particles participate in the biologic effect. The distribution of radium is not uniform in the bone; the arrangement of the bone structures delimiting the marrow spaces differs in various types of bones and need not even be the same in the various parts of a single bone. Furthermore the incorporated nuclide induces also the rearrangement of the bone tissue. The situation is further complicated by the retention of a fraction of radium-daughters. These conditions suggest that data may be obtained on the changes in the stem cell compartment as a whole although it may be extremely difficult to analyse such findings in relation to the spatial dose distribution in the bone marrow volume.

The colony forming units of medullar and splenic hematopoiesis in irradiated mice and controls were assessed by the method of TILL & MCCULLOCH (1961), the basis being the accepted view that these units have the properties of the hematopoietic stem cells that form the spleen colonies of clonal cell populations. Stem cells in the present report are the self-replicating cells capable of progressive differentiation into one or more types of mature cells.

Material and Methods. The experiment was carried out in female random-bred mice of H strain caged in groups of five under standard conditions. ^{226}Ra was injected intraperitoneally (0.03/μCi/g body weight) in a group aged 10 weeks, mean weight 25 g, another group with the same parameters being kept as a control. The mice were killed at intervals of 5, 9, 14, 18 and 37 weeks after injection, and at which peripheral blood counts and blood smears were carried out and the whole body activity was measured. The right femora were dissected, the bones ground in a mortar (CARSTEN & BOND 1968), and the pooled marrow cells washed in 10 ml Tyrode solution. The suspension obtained afforded the optimal number of bone marrow cells to comply with the experimental intentions.

The spleens were removed and homogenized in a tuberculin syringe with 3 ml Tyrode solution, the homogenate being filtered through a nylon gauze with further Tyrode solution to make up a volume of 50 ml. The suspensions were prepared under aseptic conditions at a temperature of melting ice. The cellularity of the pooled spleens was determined.

Recipients of the same origin, sex and weight and aged 10 weeks were irradiated with a TUR roentgen unit in a rotating plastic box with a homogenized field under monitoring control with a chamber Integron—Victoreen. The mice were exposed to 800 R whole-body irradiation at a rate of 60 R/min, a HVL of 1.0 mm Cu and a focus-object distance of 50 cm. The endogenous spleen colonies amounted to less than 0.5 colony per spleen. One group of mice received 10^5 nucleated bone marrow cells in a volume of 0.2 ml intravenously, and in another

Table 1

Colony formation and spleen ^{59}Fe uptake after injection of bone marrow and spleen cells of tested mice ($n=5$)

Weeks after ^{226}Ra application	Group of tested mice	No. of recipient mice		No. of colonies per 10^5 bone marrow cells administered (mean ± SE)	No. of colonies per 10^6 spleen cells administered (mean ± SE)	Per cent 18 hour ^{59}Fe uptake into recipient spleens	
		Bone marrow	Spleen			After 10^5 bone marrow cells administered (mean ± SE)	After 10^6 spleen cells administered (mean ± SE)
5	Controls	19	16	21.5 ± 1.5	26.4 ± 2.0	2.9 ± 0.3	3.8 ± 0.3
	^{226}Ra	8	16	14.0 ± 1.8**	36.6 ± 1.8	1.7 ± 0.2**	4.2 ± 0.3
9	Controls	19		19.7 ± 0.9		2.4 ± 0.2	
	^{226}Ra	20	18	16.1 ± 0.9*	30.9 ± 1.6	1.3 ± 0.1**	2.9 ± 0.3
14	Controls		22		35.9 ± 1.3		3.8 ± 0.3
	^{226}Ra		17		28.6 ± 1.5		3.3 ± 0.3
18	Controls	16	16	24.7 ± 1.2	40.0 ± 2.2	2.6 ± 0.2	3.5 ± 0.3
	^{226}Ra	18	17	16.1 ± 1.4**	25.3 ± 1.7	1.8 ± 0.2*	1.9 ± 0.2
37	Controls	19	21	18.5 ± 0.8	21.9 ± 0.7	2.0 ± 0.2	1.5 ± 0.1
	^{226}Ra	19	20	14.9 ± 0.9**	19.3 ± 1.3	1.6 ± 0.1	1.7 ± 0.1

* Significantly different from control value at 95 % level
** Significantly different at 99 % level (t-test)

group 10^6 spleen cells were injected under the same conditions. The animals were killed by transection of the cervical cord on the ninth day, 18 hours before which 1 μCi of ^{59}Fe was injected intraperitoneally. The spleens that were removed were fixed in Bouin's solution, the relative activity being ascertained from the incorporated ^{59}Fe (SMITH 1964 a, b). The colonies were counted at double magnification at 24 hours. The control experiment was performed under the same conditions. The number of colony forming units in the femoral bone marrow or the spleen were reached from the cellularity and colony forming ability (KNOSPE et coll. 1970). The results were statistically evaluated.

Results

Double the number or even more marrow cells and double the number of colony forming units were obtained in the femora of intact animals by the above technique as compared with flushing only the shaft cavity (cf. PLAYFAIR & COLE

Table 2

Peripheral blood counts, femoral and spleen cellularity and total colony forming units of tested mice

Weeks after ^{226}Ra injection	Group of tested mice	Erythrocytes/mm^3 (mean ± SE) × 10^6	Leucocytes/mm^3 (mean ± SE) × 10^3	Nucleated marrow cells per femur × 10^6	Nucleated cells per spleen × 10^6	Total colony forming units per femur × 10^3	Total colony forming units per spleen × 10^3
5	Controls	10.7 ± 0.6	12.8 ± 0.8	31.8	183	6.8	4.8
	^{226}Ra	9.6 ± 0.5	9.3 ± 0.8	34.0	155	4.8	5.7
9	Controls	10.2 ± 0.4	12.0 ± 1.1	43.4	162	8.5	
	^{226}Ra	10.0 ± 0.1	14.0 ± 0.7	32.2	233	5.2	7.2
14	Controls	9.9 ± 0.5	12.0 ± 2.5		123		4.4
	^{226}Ra	10.0 ± 0.3	11.5 ± 1.0		79		2.3
18	Controls	10.9 ± 0.4	14.0 ± 1.1	36.1	88	8.9	3.9
	^{226}Ra	10.2 ± 0.4	11.8 ± 0.7	26.4	115	4.2	2.9
37	Controls	10.2 ± 0.5	10.5 ± 1.8	44.2	81	8.2	1.8
	^{226}Ra	9.5 ± 0.3	8.2 ± 0.7	28.7	150	4.3	2.9

1965). About 30 to 40 × 10^6 marrow cells per femur and about 20 spleen colonies per 10^5 applied marrow cells were recorded. CARSTEN & BOND (1968) also obtained a greater yield of colony forming units if they prepared the cellular suspension by chapping and washing off the bones.

The number of macroscopic spleen colonies and the ^{59}Fe uptake in the spleens of recipients after the intravenous injection of 10^5 marrow cells and 10^6 spleen cells are presented in Table 1. The number of colonies per 10^5 marrow cells in mice given ^{226}Ra during the experimental period was lower than in the control groups, the ratio of these values remaining however comparable. The corresponding results were obtained by measuring the ^{59}Fe uptake in spleens colonised by irradiated bone marrow cells.

After injection of 10^6 spleen cells from ^{226}Ra treated mice the yield of spleen colonies was the highest in the first interval and decreased in further intervals whereas the number of colonies in the controls rose. The ^{59}Fe incorporation into the spleens of recipients given spleen cells from ^{226}Ra exposed mice was also higher in the first interval and subsequently decreased below the control values.

Further data in tested animals are summarized in Table 2. The colony forming units in femora of mice given ^{226}Ra amounted to 48 to 69 per cent of the control values, which corresponded to the decreased stem cell compartment. At the

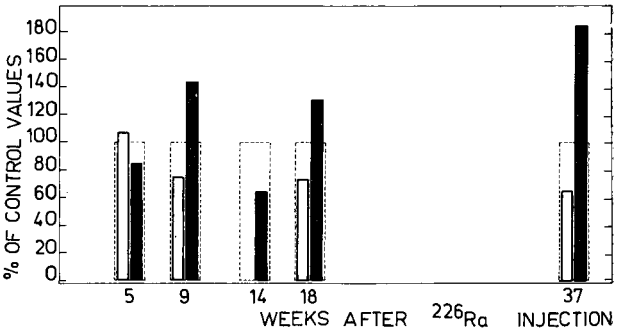

Fig. 1. Femoral bone marrow (solid-line bar) and spleen (black bar) cellularity of ^{226}Ra treated mice as percentage of control values (broken-line bar) in the respective intervals.

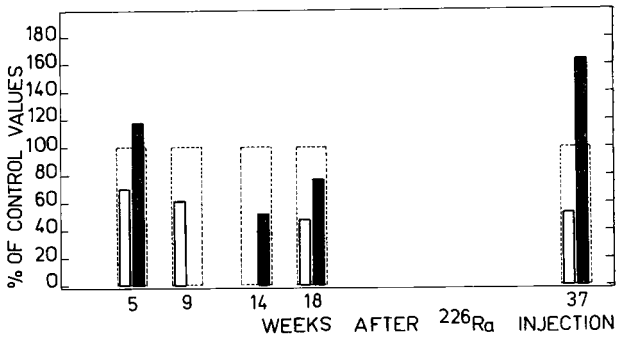

Fig. 2. Colony forming units of femoral bone marrow (solid-line bar) and spleen (black bar) of ^{226}Ra treated mice as percentage of control values (broken-line bar) in the respective intervals.

beginning of the experimental series the femoral marrow cellularity in the radium treated mice failed to differ from the control value; in the intervals that followed the number of marrow cells in intact mice increased and reached the level of forty million, while in those given ^{226}Ra it amounted to thirty million. At the 9th, 18th and 37th week after radium injection the femoral marrow cells in the tested mice were reduced to 74, 73 and 65 per cent of the controls. The femoral marrow cells in intact animals amounted to 1.1×10^6 per gram body weight, this being in good agreement with the results reported by Bozzini et coll. (1970); this figure in ^{226}Ra treated mice was 0.88×10^6 cells per gram body weight, the difference being statistically significant ($p < 0.05$). The bone marrow in mice given radium was thus hypoplastic and this condition was maintained during the whole experimental period.

The cellularity of the spleen in ^{226}Ra treated mice changed only slightly in the first two intervals exceeding even in the 9th week the respective control value and decreasing to a minimum in the 14th week. The number of spleen cells again increased in the subsequent intervals so that in the 37th week it was double as

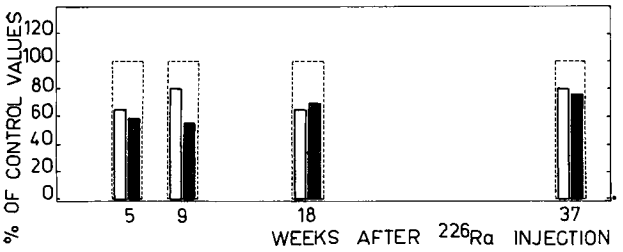

Fig. 3. Spleen colonies (solid-line bar) and spleen ^{59}Fe uptake (black bar) in recipient mice after administration of 10^5 nucleated bone marrow cells of ^{226}Ra treated mice, expressed as percentage of control values (broken-line bar).

compared with the controls. The colony forming units in spleens of animals exposed to radium changed in a similar manner.

The cellularity of the femoral bone marrow and of the spleen in mice with ^{226}Ra is presented in Fig. 1 as a percentage of the control values. The colony forming units appear in Fig. 2. The continuous decrease of cellularity and slower disappearance of the units from the control spleens with age, were noteworthy. Likewise as in the experiments reported by COGGLE & PROUKAKIS (1970) neither the number of femoral marrow cells in ageing mice nor the size of the stem cell compartement, as determined by an exocolonizing assay, decreased.

Discussion

The radiation effect upon femoral hematopoiesis in the period of 5 to 37 weeks following the intraperitoneal administration of 0.75 μCi ^{226}Ra per mouse was manifested by diminution of the stem cell compartment. A less significant decrease of bone marrow cellularity also occurred while the number of peripheral erythrocytes and leucocytes was fully compensated during the entire period of observation.

The ^{59}Fe incorporation into spleens of mice receiving bone marrow cell suspension from those exposed expressed as percentages of control values has been compared with the decrease in the colony forming units similarly recorded (Fig. 3). The results in the first two intervals may suggest a decreased erythroid differentiation. Nevertheless this comparison as a whole does not definitely indicate a change in the differentiating capacity of stem cells continuously irradiated by incorporated ^{226}Ra; no evidence on a different sensitivity of perhaps already committed elements with maintained repopulating ability appears to exist.

BLOOM (1948) observed after the intraperitoneally injected radium in the range of 0.17 to 1.0 μCi/g body weight a maximal depletion of marrow cells in the metaphyses and epiphyses of the femora of mice, i.e. in regions with trabecular bone and large endosteal surfaces as well as along the endosteum of the

shaft. It is probable that even with the smaller activities injected a greater hazard to the marrow cells exists in these critical paraendosteal volumes than in the remaining bone marrow. Assuming that the distribution of stem cells in the bone marrow be uniform, the decrease in the femoral colony forming units observed in the present experiment is inadequate for the size of the critical volume. This suggests that more stem cells are available close to the endosteum.

Due to the known effect of bone-seeking nuclides upon bone tissue, the possibility that depletion of the mesenchymal cells of this tissue participates, at least indirectly, in the diminution of the hematopoietic stem cell compartment cannot be excluded. This conception is to a certain extent supported by the recent findings of PATT & MALONEY (1970) and FONG et coll. (1971). These authors proved that the regenerative process in bone marrow after localized depletion originates in uncommitted mesenchymal cells in the connective tissue of the Haversian canals of the adjacent bone. No less interesting were the investigations by CROSBY (1970), TAVASSOLI & CROSBY (1970) and AMSEL et coll. (1969) which demonstrated that the stroma cells of bone marrow transplanted into extramedullary sites have the properties of primitive cells. This would appear to be similar to the behaviour of the above mentioned mesenchymal cells in bone tissue.

The colony forming units and cellularity in mice exposed to ^{226}Ra were changed in the same way as reported by FRIED et coll. (1966) who investigated mice given ^{89}Sr. These changes may be understood as manifestations of compensatory blood-forming activity in an irradiated organ following the impairment of bone marrow hematopoiesis. The importance of the spleen as a source of compensatory hematopoiesis with an increasing ^{90}Sr dose is obvious (NILSSON 1971). Considering that the irradiation from ^{226}Ra incorporated in mice failed to change the peripheral blood count for a considerable time, it would appear that effective reparatory and compensatory mechanisms did much to neutralize the damage to the bone marrow.

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SUMMARY

Data on hematopoiesis in mice were obtained over a period of 5 to 37 weeks after the intraperitoneal injection of ^{226}Ra. The repopulation ability of bone marrow cells as measured by the ^{59}Fe uptake in the recipient spleens changed in step with the colony forming units. The effects on the latter and the cellularity of the spleen may express the reaction of the irradiated organ to impairment of the blood-forming capacity of the marrow.

ZUSAMMENFASSUNG

Daten zur Hämatopoese der Maus während einer Zeit von 5 bis 37 Wochen nach intraperitonealer Injektion von ^{226}Ra wurden erhalten. Das Repopulationsvermögen von Knochenmarkzellen, gemessen durch die ^{59}Fe-Aufnahme der Milz von Rezipienten, folgte den Veränderungen der koloniebildenden Einheiten. Der Effekt auf letztere und die Zellularität der Milz mögen die Reaktion des bestrahlten Organismus auf die herabgesetzte Blutbildende Kapazität des Knochenmarks zum Ausdruck bringen.

RÉSUMÉ

Les auteurs ont étudié l'hématopoïèse de souris au cours d'une période de 5 à 37 semaines suivant l'injection intra-péritonéale de ^{226}Ra. L'aptitude au repeuplement des cellules de la moelle osseuse mesurée par la fixation de ^{59}Fe dans les rates receveuses change en fonction des unités formatrices de colonies. Les effets sur le repeuplement et sur la cellularité de la rate peuvent exprimer la réaction de l'organe irradié à la diminution du pouvoir hématopoïétique de la moelle.

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