FROM THE DEPARTMENT OF RADIATION HYGIENE, INSTITUTE OF HYGIENE AND EPIDEMIOLOGY, PRAGUE, CZECHOSLOVAKIA.

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

V. KLENER and V. SVOBODA

The spleen is a component of the hemopoietic system which differs in many respects from bone marrow. JACOBSON et coll. (1949), FRIED et coll. (1966) and NILSSON (1970, 1971) have all reported that the spleen is an important source of compensatory and auxiliary hemopoiesis after external and internal irradiation.

An investigation of hemopoietic stem cells by means of a colony forming test has already been performed in mice given ^{226}Ra (0.03 μCi/g) intraperitoneally (SVOBODA & KLENER 1972). At the later stage of the experiment changes appeared in both the number of colony forming units of the spleen and the cellularity, which could be considered as manifestations of compensatory blood forming activity following the impairment of the marrow hemopoiesis.

The colony forming units of femoral bone marrow and other hematologic parameters have now been investigated in mice splenectomized two weeks

Submitted for publication 1 March 1972.

before the intraperitoneal injection of ^{226}Ra, the radiation from which failed to influence the survival of animals in the course of the experiment. The results obtained could be compared with the data of the previous investigation in nonsplenectomized mice, contributing thus to the evaluation of the role of the spleen in the hemopoiesis of intact and irradiated mice.

Material and Methods. The experiment was carried out in female random-bred H-mice weighing approximately 25 g and housed in groups of five under specific pathogene-free conditions. Splenectomy was performed in mice 8 weeks old under hexobarbital anesthesia (0.15 mg/g body weight). Two weeks later, one group of these mice was injected intraperitoneally with 0.03 μCi ^{226}Ra/g body weight, another group being maintained as control.

Peripheral blood counts and blood smears were performed at intervals of 5, 9, 14, 18 and 40 weeks after the injection, and the whole-body activity was measured by the method of LENGER (1971); five mice were examined at each interval. The animals were killed by cervical transection and the right femora dissected. The suspension of marrow cells was prepared by grinding the femora in a mortar, and washing the cells out of the bones with 10 ml Tyrode solution. The cellularity of the pooled femoral marrow was then determined.

Recipients of the same sex, weight and origin and all 10 weeks of age were exposed to 800 R whole-body irradiation at 60 R/min, HVL 1.0 mm Cu and a target distance of 50 cm with a TUR roentgen unit. The animals were placed in the homogenized field of a rotating plastic container and the system was monitored with an Integron-Victoreen ionization chamber. Immediately after exposure the lateral tail veins were injected with 1×10^5 marrow cells of the tested mice. One group received the cells from ^{226}Ra treated mice and another group those from non-irradiated controls.

The recipient mice were killed on the ninth day, 18 hours before which they were injected intraperitoneally with 1 μCi of ^{59}Fe (as ferric citrate). The spleens as well as both femora were extirpated, fixed in Bouin's solution, and counted in a well-type gamma detector. The uptake of ^{59}Fe in the spleens and femora was expressed as a percentage of the activity administered. Spleen colonies were counted independently by three observers 24 hours later. The total colony forming units per femur of the donor mice were calculated on the basis of the relative colony forming units and the total nucleated cell content in the femur.

The experiment was supplemented with histology of the liver and of certain other tissues carried out in 7 splenectomized mice one year later, 3 of these having received ^{226}Ra and 4 control animals. The liver of these mice was also examined cytologically.

Table 1

Colony formation and ^{59}Fe uptake in spleens and femora of recipient mice after injection of 1×10^5 pooled bone marrow cells from 5 splenectomized donors in each group

Weeks after ^{226}Ra injection	Group of tested mice	No. of recipient mice	No. of colonies (mean ± SE)	Per cent 18 hour ^{59}Fe uptake into recipient spleens (mean ± SE)	Per cent 18 hour ^{59}Fe uptake into 2 recipient femora (mean ± SE)
5	^{226}Ra	—	—	—	—
	Controls	12	18.2 ± 2.5	2.65 ± 0.38	0.38 ± 0.03
9	^{226}Ra	12	11.5 ± 2.3	1.20 ± 0.19	0.44 ± 0.04
	Controls	—	—	—	—
14	^{226}Ra	15	9.2 ± 0.8**	0.87 ± 0.07	0.33 ± 0.03
	Controls	16	21.4 ± 1.9	1.86 ± 0.26	0.39 ± 0.04
18	^{226}Ra	20	12.7 ± 1.5	1.27 ± 0.18*	0.29 ± 0.03
	Controls	16	20.0 ± 2.8	2.38 ± 0.39	0.38 ± 0.03
40	^{226}Ra	19	8.5 ± 0.9**	0.85 ± 0.09**	0.33 ± 0.01
	Controls	16	16.4 ± 1.3	1.50 ± 0.13	0.37 ± 0.05

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

Table 2

Peripheral blood counts, femoral marrow cellularity and femoral colony forming units in splenectomized mice (5 animals in each group)

Weeks after ^{226}Ra injection	Group of tested mice	Erythrocytes/mm^3 (mean ± SE) $\times 10^6$	Leukocytes/mm^3 (mean ± SE) $\times 10^3$	Nucleated marrow cells per femur $\times 10^6$	Total units per femur $\times 10^3$
5	^{226}Ra	9.7 ± 0.4	16.3 ± 2.4	26.4	—
	Controls	9.9 ± 0.5	9.2 ± 0.6	24.0	4.4
9	^{226}Ra	9.7 ± 0.2	9.9 ± 0.7	19.4	2.2
	Controls	9.7 ± 0.5	12.5 ± 0.8	26.0	—
14	^{226}Ra	9.2 ± 0.3	9.7 ± 0.9	22.6	2.1
	Controls	9.2 ± 0.3	10.6 ± 1.3	21.4	4.6
18	^{226}Ra	8.5 ± 0.2	10.0 ± 1.0	22.6	2.9
	Controls	8.5 ± 0.1	14.8 ± 1.4	31.6	6.3
40	^{226}Ra	9.3 ± 0.5	9.6 ± 0.5	26.2	2.2
	Controls	9.5 ± 0.2	8.0 ± 1.2	37.8	6.2

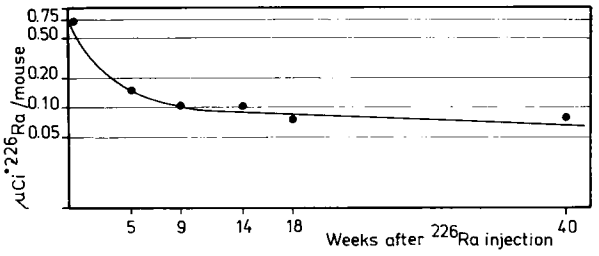

Fig. 1. Retention curve for splenectomized mice after single administration of ^{226}Ra.

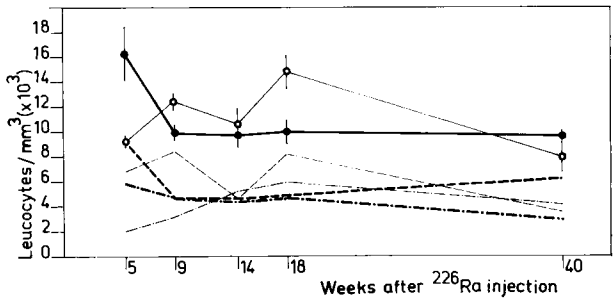

Fig. 2. Course in time of white blood count in irradiated (——— total counts, —·— granulocytes, — — — lymphocytes) and control splenectomized mice (——— total counts, —·— granulocytes, — — — lymphocytes).

Results

The results are summarized in Tables 1 and 2, while the values of the whole-body measurements taken in the course of the experiment appear in Fig. 1.

The counts of superficial spleen colonies were significantly lower in recipients injected with 1×10^5 marrow cells from splenectomized ^{226}Ra treated mice than in those administered with cells from non-irradiated but splenectomized mice. Evaluation of the splenic uptake of ^{59}Fe in recipient mice revealed similar significant differences. It is noteworthy that analogous results were not evident in the case of femoral ^{59}Fe incorporation. Under the given conditions the femoral iron uptake was almost as low as in recipient mice subjected only to roentgen irradiation.

The differential blood counts per mm^3 were obtained from blood smear evaluations and total leukocyte counts. When comparing the mice given ^{226}Ra with the controls, no significant difference was evident in the granulocyte, lymphocyte and monocyte counts (Fig. 2). Similarly the red blood count did not change significantly throughout the experimental period of 40 weeks. Femoral cellularity of both the radium injected and control mice was maintained at between 20 and 25 million from the fifth to the fourteenth week of the experiment. In the following two intervals the cellularity in ^{226}Ra given mice remained unchanged, while that of controls exceeded the level of 30 million.

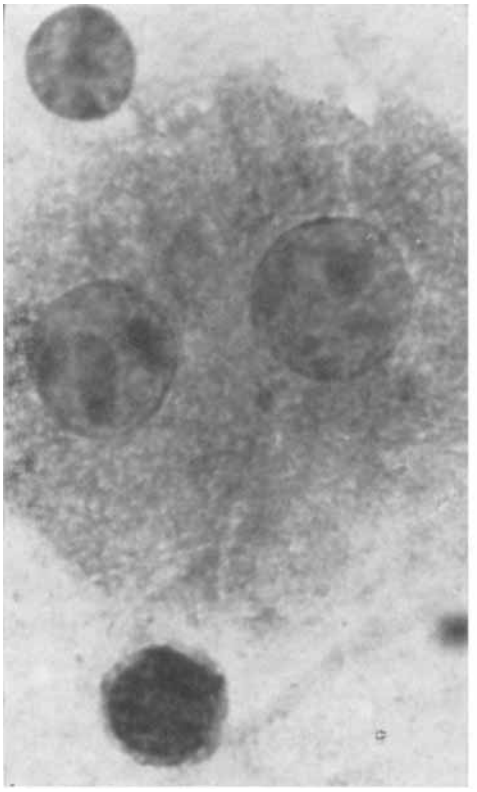

Fig. 3. Liver imprint with binuclear hepatocyte and intermediate erythroblast (splenectomized mouse one year after ^{226}Ra injection).

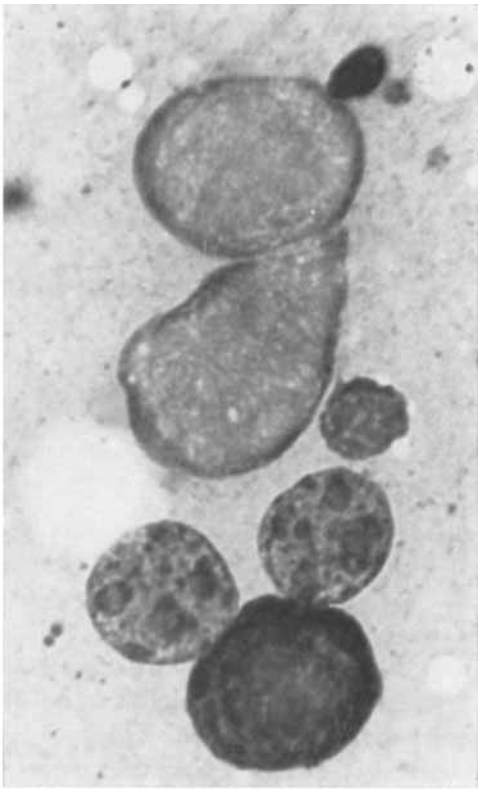

Fig. 4. Early precursors of red and white series in liver imprint (splenectomized mouse one year after ^{226}Ra injection).

The number of femoral colony forming units in mice exposed to ^{226}Ra was significantly lower than in the controls during the whole experimental period. The difference was manifested more clearly in the two last intervals.

Histology produced evidence of hemopoiesis in the liver of three splenectomized mice killed one year after ^{226}Ra application. Young granulocytes and erythroblasts were present in liver imprints stained with May-Grünwald-Giemsa (Figs 3, 4). In organs and tissues such as lymph nodes, thymus, kidneys, suprarenals, and lungs the hemopoiesis was absent. The femoral marrow cells were seriously depressed in these three mice. The early hemopoietic precursors were, however, not present in the liver of four splenctomized controls killed at the same age, only sporadic cells in more mature stage being evident. In no other organ or tissue was ectopic hemopoisis observed.

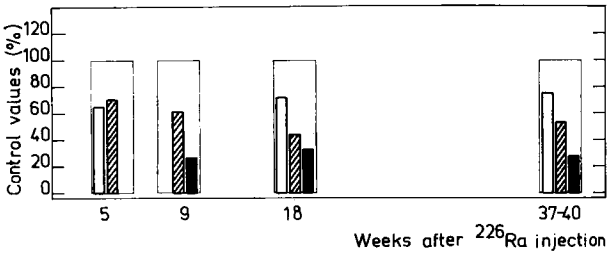

Fig. 5. Colony forming units in femoral bone marrow of ^{226}Ra injected splenectomized (black bar), ^{226}Ra injected nonsplenectomized (shaded bar), and nonirradiated splenectomized mice (heavy-line bar) as compared with respective values for intact controls (100 %) (light-line bar).

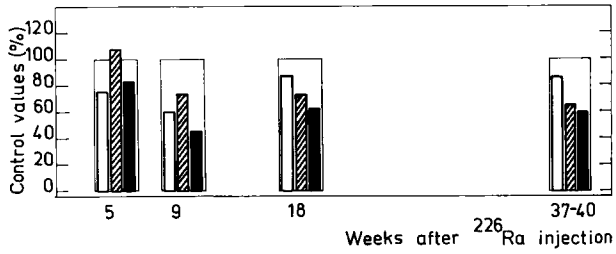

Fig. 6. Cellularity of femoral bone marrow of ^{226}Ra injected splenectomiezd (black bar), ^{226}Ra injected nonsplenectomized (shaded bar), and nonirradiated splenectomized mice (heavy-line bar) as compared with respective values for intact controls (100 %) (light-line bar).

Discussion

The present investigation in splenectomized mice given ^{226}Ra was carried out under the same conditions as in the previous experiment in the similarly treated nonsplenectomized animals (SVOBODA & KLENER). It proved useful to summarize the data from both experiments and to compare the corresponding groups (Figs 5, 6). The number of femoral colony forming units in the splenectomized, as compared with nonsplenectomized controls, fell during the whole experimental period and amounted in the last two intervals to 0.71 and 0.75 of their reference values. A comparison of the splenectomized and nonsplenectomized mice given ^{226}Ra produced analogous differences, these being most marked in the last interval (factor 0.5). The evaluation of femoral marrow cellularity revealed similar changes although of a lesser degree than with the colony forming units. Peripheral blood counts remained at the normal level in all groups examined.

The experiment indicated that the femoral compartment of pluripotent stem cells and of early differentiated progenitors capable of colony formation was impaired more seriously in mice with eliminated splenic hemopoiesis and exposed to ^{226}Ra. Nevertheless, this compartment did not fail and amounted to 0.36 to 0.50 of the control values in splenectomized but non-irradiated animals. Such a depression was not observed in the femoral cellularity of splenectomized and radium injected mice in which only at the last two intervals the respective

values decreased to 0.70. No significant changes occurred in the red and white peripheral blood counts during the experiment. This means that the normal level of the peripheral blood count can be maintained by hemopoiesis continuously damaged by the internally deposited ^{226}Ra and weakened by the elimination of spleen blood forming function. This observation seems to be in agreement with the findings of LISI et coll. (1969) in BALB/c female mice, indicating that the normal spleen has little influence on overall murine erythropoiesis.

It is however recognized that latent splenic factors exist in mouse hemopoiesis and become evident under stress conditions of acute and subacute irradiation. Classic experiments by JACOBSON et coll. (1949, 1950) indicated that the compensatory blood formation appeared in the spleen shielded during whole-body exposure to 600 or 1025 R roentgen irradiation. FRIED et coll. (1966) found that damage to bone marrow in ^{89}Sr applied mice was followed by the proliferation of spleen hemopoietic stem cells.

Noncellular factors producing postirradiation recovery of hemopoiesis were isolated both from the nonirradiated and irradiated spleen. Experiments by FORD et coll. (1968) in mice revealed a cell-free splenic extract effective upon the postirradiation survival. KNOSPE et coll. (1970) investigated a noncellular substance prepared from irradiated spleen and inducing stem cell proliferation in CAF_1 and CF_1 mice exposed to roentgen irradiation. Certain investigators (BURGER et coll. 1969, ŠMÍD & ŠIMŠA 1971) identified alpha-2 globulin of splenic origin as being capable of stimulating the hemopoietic recovery after irradiation. According to TWENTYMAN & BLACKETT (1970) spleen might be responsible for additional erythropoiesis and simultaneously for the maintenance of normal marrow erythropoiesis. The absence of the spleen may lead to changes in iron kinetics. Thus, both cellular and noncellular protective mechanisms were demonstrated in the spleen without excluding one another.

The present long-term experiments also revealed that a certain positive relationship between spleen and bone marrow hemopoiesis existed under normal conditions. If the relation is interrupted by splenectomy the number of both colony forming units and nucleated bone marrow cells is decreased and a new equilibrium state is supposed to be established. The same is also evident in mice, the bone marrow of which is being continuously exposed to radiation from incorporated ^{226}Ra. The splenic mechanisms influencing favourably the bone marrow hemopoiesis seem to be radiation resistant as in other homeostatic systems.

During the experimental period the absence of the spleen in ^{226}Ra injected mice did not interfere with the compensatory mechanisms of marrow hemopoiesis to such an extent that the production of blood cells became insufficient. The

ectopic hemopoiesis was observed one year after radium injection, which fact could be explained by the increasing inability of the bone marrow to produce the required amount of peripheral blood elements.

An intact spleen may form the centre for the compensatory blood forming activity. Preliminary results of the experiment with higher ^{226}Ra activities suggest that with heavily damaged marrow the spleen hemopoiesis is activated although unable to prevent the failure of overall hemopoiesis.

The results are worth comparing with the findings in C3H mice of KRETCHMAR & CONOVER (1970) who reported seven times higher concentration of colony forming units in the bone marrow than in the spleen. They assumed in addition that the ratio of colony forming units having pluripotent properties to all other such units including 'early differentiated precursors' is three times higher in the bone marrow than in the spleen. RENCRICCA et coll. (1970) demonstrated in mice with phenylhydrazine induced anemia that the units migrate from the bone marrow into the spleen rather than proliferate locally. These facts indicate a restricted ability of the spleen of the mouse to compensate for the damaged function of the bone marrow, and thus illustrate some important differences in the hemopoietic capacity between the spleen and bone marrow.

An additive hemopoietic function of the spleen in relation to the bone marrow is presumed as being effective both in non-irradiated and radium injected mice. The participation of such a splenic factor remains unchanged as long as the bone marrow maintains the production of differentiated elements. In the case of marrow decompensation signs of increased spleen hemopoiesis proportional to the marrow damage may occur. If the spleen be removed the ectopic blood formation in other tissues is established as the only available but not fully effective compensatory mechanism. The spleen is an important organ for hemopoiesis in mouse under both normal and stress conditions. The parts played by the complementary cellular proliferation and the homeostatic mechanisms remain, however, to be elucidated in greater detail.

Acknowledgements

The authors wish to thank Mrs R. Housová and Mrs Z. Kotašková for their technical assistance.

SUMMARY

Hematologic data obtained in splenectomized mice for long periods after the intraperitoneal injection of ^{226}Ra (0.03 μCi/g) are reported. The colony forming units in the bone marrow were reduced throughout the whole experimental period. The peripheral blood counts were not changed significantly.

ZUSAMMENFASSUNG

Es wird über hämatologische Daten von splenektomierten Mäusen während langer Zeiträume nach intraperitonealer Injektion von ^{226}Ra (0,03 μCi/g) berichtet. Die Koloniebildenden Einheiten des Knochenmarks waren während der gesamten Versuchszeit herabgesetzt. Die peripheren Blutwerte waren nicht signifikant verändert.

RÉSUMÉ

Présentation des données hématologiques obtenues sur des souris splénectomisées au cours de longues périodes suivant l'injection intrapéritonéale de ^{226}Ra (0,03 μCi/g). Les unités formatrices de colonie dans la moelle osseuse sont en nombre réduit tout au long de la période expérimentale. Les numérations du sang périphérique ne sont pas significativement modifiées.

REFERENCES

BURGER M., KNYSZYNSKI A. and BERENBLUM I.: Stimulation of thymic and bone marrow regeneration in irradiated mice by protein fractions of human serum and sheep spleen. Radiat. Res. 40 (1969), 193.

FORD L. C., DONALDSON D. M. and ALLEN A. L.: Protection of mice by postirradiation treatment with a cell-free component of spleen. Proc. Soc. exp. Biol. 127 (1968), 286.

FRIED W., GURNEY C. W. and SWATEK M.: The effect of strontium-89 on the stem cell compartment of the spleen. Radiat. Res. 29 (1966), 50.

JACOBSON L. O., MARKS E. K., GASTON E. O., ROBSON M. and ZIRKLE R. E.: The role of the spleen in radiation injury. Proc. Soc. exp. Biol. 70 (1949), 740.

— SIMMONS E. L., BETHARD W. F., MARKS E. K. and ROBSON M. J.: The influence of the spleen on hematopoietic recovery after irradiation injury. Proc. Soc. Exp. Biol. 73 (1950), 455.

KNOSPE W. H., FRIED W., GREGORY S. A., SASSETI R. J. and TROBAUGH F. E. JR: Effects of noncellular spleen derived factor on recovery of hematopoietic stem cells from irradiation. J. Lab. clin. Med. 76 (1970), 584.

KRETCHMAR A. L. and CONOVER W. R.: A difference between spleen-derived and bone marrow-derived colony-forming units in ability to protect lethally irradiated mice. Blood 36 (1970), 772.

LENGER V.: Measurement of ^{226}Ra in mice. Acta radiol. Ther. Phys. Biol. 10 (1971), 488.

LISI L., BRADY W. L. W., BRODSKY I. and RUGGIERI S. T.: Erythropoietic response to total body irradiation in splenectomized mice. Radiology 93 (1969), 682.

NILSSON A.: Pathologic effects of different doses of radiostrontium in mice. Changes in the haematopoietic system. Acta radiol. Ther. Phys. Biol. 9 (1970), 528.

— Pathologic effects of different doses of radiostrontium in mice. Development and incidence of leukaemia. Acta radiol. Ther. Phys. Biol. 10 (1971), 115.

RENCRICCA N. J., RIZZOLI V., HOWARD D., DUFFY P. and STOHLMAN F. JR: Stem cell migration and proliferation after severe anemia. Blood 36 (1970), 764.

SVOBODA V. and KLENER V.: Effect of incorporated ^{226}Ra on colony forming units of bone marrow and spleen in mice. Acta radiol. Ther. Phys. Biol. 11 (1972), 472.

ŠMID A. and ŠIMŠA J.: Stimulation of hemopoietic recovery in irradiated and/or contamined rats induced by 19 S α-2 macroglobulin (α-2 MA). *In:* Proceedings of 4th Conference of Radiation Hygiene, ČSSR, part II, p. 335. Edited by Purkyně Medical Research and Postgraduate Institute Press, Hradec Králové 1971.

TWENTYMAN P. R. and BLACKETT N. M.: Red cell production in the continuously irradiated mouse. Brit. J. Radiol. 43 (1970), 898.