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PATHOLOGIC EFFECTS OF DIFFERENT DOSES OF RADIOSTRONTIUM IN MICE

Development and incidence of leukaemia

by

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Interest in radiation-induced leukaemia has mostly been concentrated on leukaemia developing after external irradiation. It has, however, been reported (1—4, 8—11, 15—18) in different animal species that internal emitters can also induce various types of leukaemia. Internal emitters, such as radiostrontium, have different consequences for the haematopoietic system compared with those of external irradiation (10, 14). Radiostrontium rapidly concentrates in the skeleton and will thus for a considerable time almost exclusively irradiate the bone marrow. Extraskeletal parts of the haematopoietic tissue are on the other hand only slightly, and mainly indirectly, influenced by radiostrontium. These facts seem to offer problems of interest, particularly concerning the site of origin, development and relation between dose and incidence of leukaemia.

The present report deals with some of these aspects following the administration of different doses of ^{90}Sr to mice.

Material and Methods. The investigation comprised 1 430 CBA mice, 75 ± 5 days old; they were divided into two main series, series I and series II. The mice in each series were separated into different groups and were injected intraperi-

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Table 1

Experimental conditions and the ^{90}Sr doses employed

Dose of ^{90}Sr μCi/g body weight	Total number of mice investigated	Number of mice killed in groups of five	Last day of sacrifice	Number of mice that died before sacrifice was due
Series I				
1.6	120*	65	300	50
0.8	121	75	360	46
0.4	122	95	480	27
0.2	122**	103***	540	17
Control	95	94****	570	1
Series II				
1.2	50	—	—	50
0.8	100	—		100
0.7	400	—		400
0.6	50	—		50
0.4	100	—		100
0.2	50	—		50
Control	100	—		100

Of these animals 5* and 2** were lost during the experiment; only 3*** and 4**** animals, respectively, were sacrificed in the last test group.

toneally with various doses of $^{90}Sr(NO_3)_2$, in accordance with the data given in Table 1.

All the mice were kept in plastic cages, ten mice in each, and given a standard diet.

In series I, five mice from each of the different dose groups and from the untreated control group were sacrificed after 7, 14, 21 and 30 days, and then at monthly intervals until all mice were used up. Many mice in this series died however before their time of sacrifice (Table 1). The mice before sacrifice were anaesthetized for blood analysis, the blood being obtained with a Pasteur pipette from the medial venous plexus of the eye. The femora, tibiae and humeri, as well as the pelvic bones, spine, thymus, spleen, mesenteric lymph node, liver, kidneys and the brain, for the histologic investigation, were fixed in Stieve's fluid. They were prepared according to conventional histologic methods, stained routinely by the van Gieson method, haematoxylin and eosin, Lillie's azure-eosinate and PAS according to Hotchkiss.

The mice in series II were collected from earlier ^{90}Sr experiments devised to investigate long-term effects of ^{90}Sr. The number of mice and the doses of ^{90}Sr

Table 2

Type and frequency of leukaemia induced by the different doses of ^{90}Sr

Dose μCi/g	Number of mice	Thymic lympho-mas	Bone mar-row lym-phomas	Generalized* lymphomas	Myeloid leukaemia	Chronic lymphocytic leukaemia	Frequency of leukae-mia %
Series I							
1.6	115	1	1				1.7
0.8	121	—	3	—	1	—	3.3
0.4	122	2	4	5	—	1	9.8
0.2	120	2	2	—	—	—	3.3
0.0	95	—	—	—	—	—	
Series II							
1.2	50	2	—	2	—	—	8.0
0.8	100	1	6	—	—	—	7.0
0.7	400	9	8	5	—	—	5.5
0.6	50	1	5	—	—	—	12.0
0.4	100	3	20	—			23.0
0.2	50	—	11	3	—	—	28.0
0.0	100	—	—	1	—	—	1.0

* Site of origin could not be determined.

employed are given in Table 1. The mice in this series were allowed to survive for their total life span and special care was taken to detect leukaemia. All the mice were examined post mortem and investigated by conventional histologic and haematologic methods.

Results

Incidence of leukaemia

In series I, in spite of the great number of mice sacrificed in each of the different dose groups (Table 1), only four cases of leukaemia were found, two in each of the 1.6 and 0.4 μCi groups. Among a total of 140 mice in series I which died before sacrifice, 18 cases of leukaemia were detected. It is notable that among the 50 mice in the 1.6 μCi group which died before sacrifice none died from leukaemia. In the other groups, four of 46, ten of 27, and four of 17 developed leukaemia in the 0.8, 0.4 and 0.2 μCi groups, respectively. No leukaemia was observed in the control group.

In series II, a total of 76 mice developed leukaemia. The highest frequency (28 %) occurred among animals in the 0.2 μCi group and a slightly lower

Table 3

Data on the mice from which blood samples were obtained

Series	Type of leukaemia and dose employed μCi/g body weight	Mouse number	Weight of		Bone marrow: degree of leukaemia proliferation	Peripheral blood	
			Spleen mg	Thymus mg		Number of white cells	Haemoglobin g/100 ml blood
	Bone marrow lymphoma						
I (sacrificed)	1.6	I: 55	83.5	31.3	1	1 000	10.6
I (died) in agonae	0.4	I: B: 6	82.4	6.7	2	660	10.4
I (died)	0.4	I: B: 2	128.6	73.8	3	230	4.3
I (sacrificed)	0.4	I: 40b	224.0	26.0	2	5 320	7.8
II (died)	0.7	II: 32	882.4	28.3	3	28 720	5.9
	Thymic lymphoma						
I (sacrificed)	1.6	I: 57	227.9	181.2	0	1 240	9.9
II (died)	0.7	II: 75	645.6	350.4	0	8 260	7.2
	Generalized lymphoma						
I (died)	0.4	I: B: 7	264.2	93.5	3	7 340	12.6
II (died)	0.7	II: 122	1 080.1	301.2	3	30 100	4.8

0— No signs of leukaemia proliferation; 1— only thoracic vertebrae involved; 2— most marrow cavities involved; 3— most marrow cavities involved, infiltration lymphoma around thoracic vertebrae.

figure (23 %) was arrived at for the 0.4 μCi group (Table 2). One case of leukaemia was detected in the control material.

Type of leukaemia

Of the total of 98 cases of leukaemia in series I and II, 97 probably arose from the lymphatic cell series. These were defined according to their location and probable site of origin either as bone marrow lymphomas or thymic lymphomas. Cases in which it was impossible to determine the site of origin on account of widespread infiltration in various tissues were designated as generalized lymphoma. Sixty were bone marrow lymphomas, twenty-one thymic lymphomas and fifteen generalized lymphomas. One case of chronic lymphatic leukaemia and one of myeloid leukaemia were also recorded.

Bone marrow lymphoma. Only two cases were observed among the sacrificed mice in series I, one each in the 1.6 and 0.4 μCi groups. Among the mice that

Fig. 1. Sections from mouse of series I, killed 240 days after injection of 1.6 μCi ^{90}Sr/g body weight. Lymphoma arising from marrow in thoracic vertebra; lymphoid cell proliferation also in two other thoracic vertebrae. van Gieson $\times$ 50. (Weight of spleen 83.5 mg, of thymus 31.3 mg, number of leukocytes 1000/mm^3, haemoglobin 10.6 g/100 ml.)

died before sacrifice, eight bone marrow lymphomas were detected in series I and fifty in series II (Table 2).

The primary site of leukaemic proliferation was almost exclusively inside the bone marrow. Proliferation of lymphoid elements was detected predominantly within the thoracic vertebrae and the sternum, generally in areas inside one or more of the former (Fig. 1). Other parts of the marrow were probably not involved, but proliferation occurred successively in most parts of the marrow (Fig. 2), as well as infiltration into extramedullary tissues. This was the case particularly around the thoracic vertebrae, where lymphomas were common (Fig. 3a). The lymph nodes were usually unaffected but could sometimes be infiltrated and enlarged to a varying degree.

The thymus was not infiltrated with leukaemic cells (Figs 2b and 3b), or only slightly to moderately so, histologically giving the impression of being metastases. The mean weight of the thymus was 33.9 $\pm$ 4.44 mg. The normal mean weight of the thymus among the non-leukaemic mice in the corresponding age groups was in the 1.6 μCi group 6.8 $\pm$ 1.6 mg, in the 0.8 μCi group 12.8 $\pm$ 1.9 mg, in the 0.4 μCi group 15.5 $\pm$ 1.9 mg, and in the 0.2 μCi group 16.0 $\pm$ 1.7 mg. The thymus in 61.7 % of the 60 cases of bone marrow lymphomas weighed less than 16.0 mg, in 18.3 % between 16.1 and 48.0 mg, and in 20 % between 48.1 and 95.1 mg.

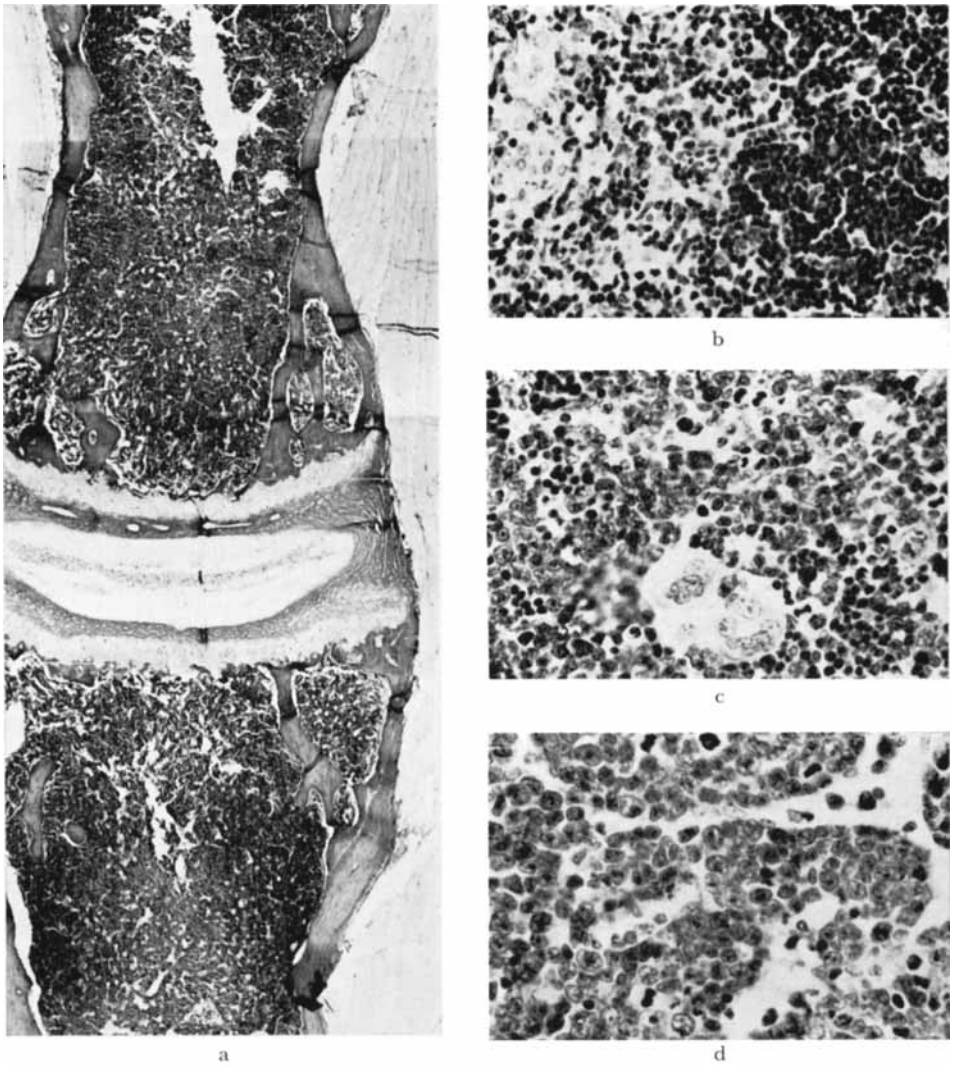


Fig. 2. Sections from mouse of series I, killed 150 days after injection of 0.4 μCi ^{90}Sr/g body weight. Most marrow cavities filled with proliferating lymphoid cells. (Weight of spleen 244.0 mg, of thymus 26.0 mg, number of leukocytes 5320/mm^3, haemoglobin 7.8 g/100 ml.) a) Lumbar vertebrae with tightly packed lymphoid cells. No infiltration of cells outside the marrow cavities. van Gieson $\times 50$. b) Thymus with normal histologic appearance. H. & E. $\times 500$. c) Spleen with heavy proliferation of lymphoid cells. Megakaryocytes and erythroid elements still remain. H. & E. $\times 500$. d) Magnification of marrow from same detail as in (a). Actively proliferating lymphoid cells. H. & E. $\times 1250$.

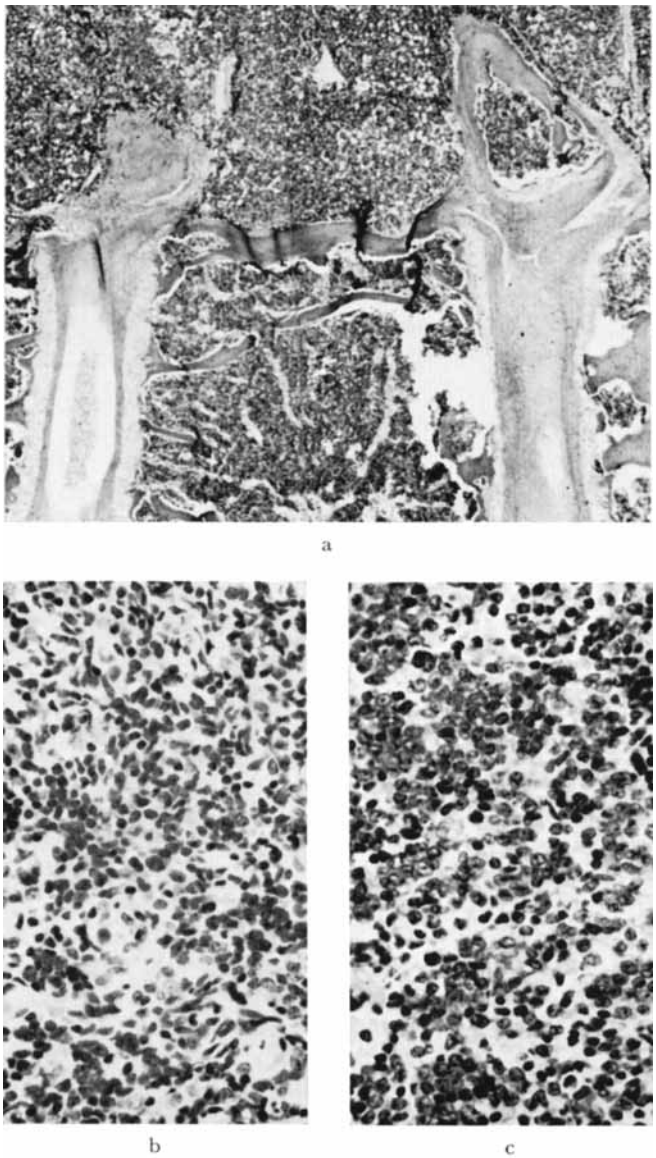


Fig. 3. Sections from mouse of series I that died 216 days after injection of 0.4 μCi ^{90}Sr/g body weight. All marrow cavities filled with proliferating lymphoid cells. (Weight of spleen 82.4 mg, of thymus 6.7 mg, number of leukocytes 660/mm^3, haemoglobin 10.4 g/100 ml.) a) Lymphoma infiltrating the tissues outside a thoracic vertebra. van Gieson $\times$50. b) Thymus. Marked lymphocyte depletion. H. & E. $\times$500. c) Spleen. Extensive lymphoid cell proliferation. H. & E. $\times$500.

The spleen was enlarged by increasing lymphoid proliferation in the red pulp but extensive extramedullary haematopoiesis usually existed (Figs 1, 2 and 3). The mean weight was 301.8 ± 24.3 mg (range 82.4—1 044.3 mg) compared with a mean weight for non-leukaemic mice of the same age of 91.3 ± 5.8 mg in the 1.6 μCi group, 65.8 ± 3.7 mg in the 0.8, 71.4 ± 7.8 mg in the 0.4, and 62.7 ± 2.8 mg in the 0.2 μCi group.

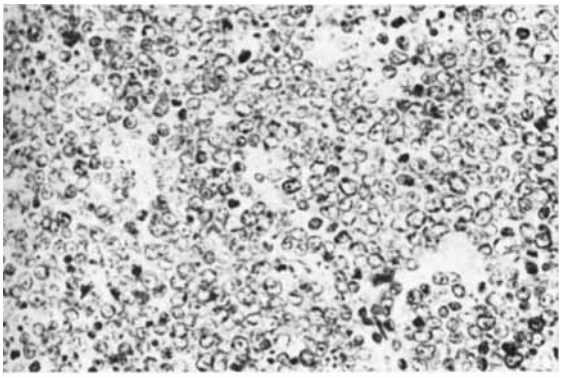
Apart from the two sacrificed mice, blood samples were obtained only from a few mice killed in agonae (Table 3).

Thymic lymphoma. Twenty-one of this type were observed, only one of which was among the sacrificed mice (Table 2). The primary leukaemic process seemed to have its site in the thymus (Fig. 4). In contrast to the bone marrow lymphomas, the thymus was generally much enlarged; usually it was the size of a hazelnut or larger and was adherent to the structures of the thoracic cavity. Its mean weight was 308.8 ± 69.7 mg (range 22.1—830.6). The spleen was often enlarged (mean weight 282.5 ± 35.0, range 72.0—518.4) and infiltrated with lymphoid cell elements. Islands of compensatory haematopoietic activity generally remained, however. The bone marrow and lymph nodes were infiltrated to varying degrees. One blood sample was obtained from a mouse killed in agonae (Table 3).

The spleen was slightly affected or unaffected (mean weight 72.2 mg) in two cases, both in series II; in three other cases (series I and II) it was only slightly infiltrated (mean weight 121.1 mg). The bone marrow was not infiltrated in these cases.

Generalized lymphoma. It was impossible to determine the site of origin of the leukaemic process in 15 cases (five among the mice which died before sacrifice in series I, and the remainder in series II). The process was generalized to many organs (liver, kidneys, brain, spinal cord, muscles) outside the haematopoietic system. A heavy proliferation of lymphoid cells was seen in the bone marrow, as well as infiltration, particularly of the muscles surrounding the thoracic spine. The thymus was enlarged (mean weight 131.7 ± 24.4 mg, range 18.3—320.3 mg) together with the spleen (mean weight 564.4 ± 98.5 mg, range 178.6—1 832.0 mg) and lymph nodes. Blood samples were obtained from two mice killed in agonae (Table 3).

One case of *chronic lymphocytic leukaemia* occurred among the sacrificed mice in the 0.4 μCi group 420 days after ^{90}Sr injection. The spleen was infiltrated with lymphoid elements. In all the bone marrow cavities, a large number of small circumscript foci of lymphoid cells occurred, scattered in an otherwise markedly hypoplastic marrow. Many mastocytes were present in the marrow as well as in



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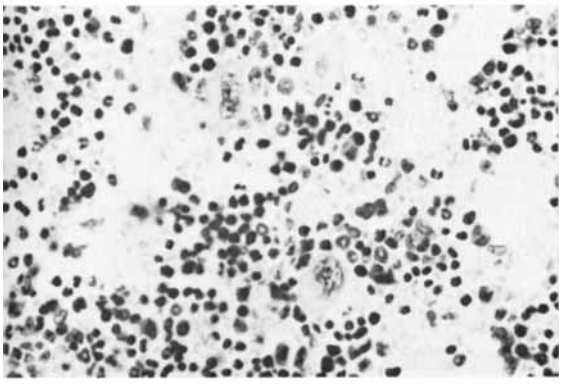

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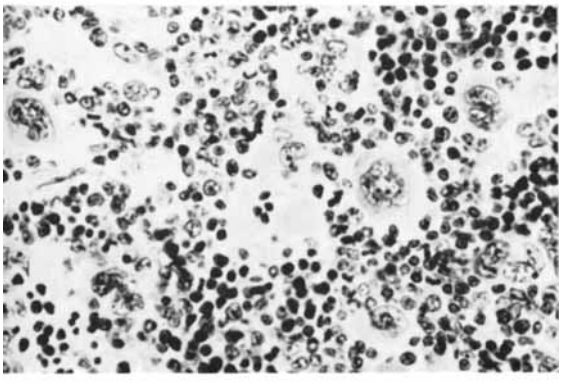

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Fig. 4. Sections from mouse of series II that died 150 days after injection of 0.7 μCi ^{90}Sr/g body weight. No lymphoid cell proliferation in the bone marrow or spleen. (Weight of spleen 85.4 mg, of thymus 22.1 mg.) a) Thymic lymphoma with heavy, uniform proliferation of lymphoid cells. H. & E. $\times$500. b) Bone marrow of thoracic vertebrae. Hypoplasia and no lymphoid cells. van Gieson $\times$500. c) Spleen. Intense erythro- and megakaryocytopoiesis. H. & E. $\times$500.

Table 4

Latency time for lymphoma development

Dose μCi/g body weight	Number of mice with lymphoma	Latency time days	Latency time, days and total number of:			
			Lymphomas n: 96	Bone marrow lymphomas n: 60	Thymic lymphomas n: 21	Generalized lymphomas n: 15
Series I						
1.6	2	255				
0.8	3	250				
0.4	11	240 $\pm$ 20.9				
0.2	4	252				
Control	0	—				
			237 $\pm$ 5.8	221.2 $\pm$ 7.6	254.3 $\pm$ 10.1	243.5 $\pm$ 14.1
Series II						
1.2	4	285				
0.8	7	200				
0.7	22	221 $\pm$ 9.2				
0.6	6	216				
0.4	23	222 $\pm$ 11.2				
0.2	14	262 $\pm$ 20.8				
Control	1	806				

other organs. The number of white cells in the peripheral blood was 46 500 (95 % lymphoid cells, 3 % monocytes and 2 % granulocytes).

One case of *myeloid leukaemia* was detected in a mice in the 1.6 μCi group (series I), one of those which died before sacrifice. Myeloid leukaemia was characterized by heavy proliferation of mostly mature granulocytic elements in the bone marrow, spleen and lymph nodes. The liver, muscles around the spine, and the membranes of the spinal cord were heavily infiltrated as well. The thymus, weighing only 1.4 mg, was not affected.

Latency time

The mean latency time for the total lymphoma material (n: 96) in series I and II was 237.8 $\pm$ 5.8 days. The case of myeloid leukaemia appeared at 352 days and the case of chronic lymphatic leukaemia at 420 days (Table 4).

The generalized lymphoma detected among the control animals in series II appeared after 806 days.

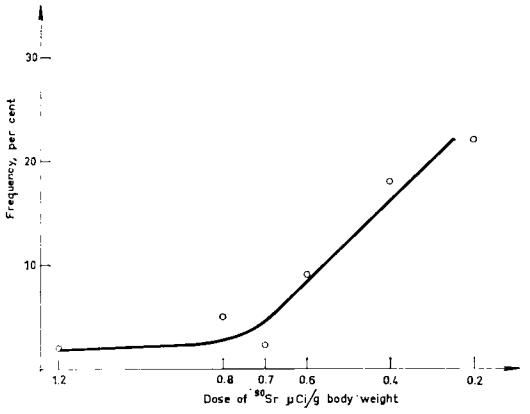

Fig. 5. Percentage of bone marrow lymphomas by dose in series II.

No relation between dose and latency time appeared to exist for lymphomas (Table 4). Nor was there any difference between thymic lymphoma (254.3 ± 10.1 days), bone marrow lymphoma (221.2 ± 7.6 days) and generalized lymphoma (243.5 ± 14.1 days).

Discussion

Leukaemia occurred in all of the dose groups employed in this investigation. Out of a total of 98 cases, 96 were lymphomas, one of myeloid leukaemia and one of chronic lymphatic leukaemia. Sixty (62.5 %) of the lymphomas seemed to have originated within the bone marrow, primarily as localized bone marrow lymphoma (Fig. 1). The occurrence of lymphomas in the bone marrow was inversely related to dose, since the frequency was less great in the higher than in the lower dose groups (Fig. 5).

Twenty-one (20.8 %) of the lymphomas detected appeared to arise in the thymus. No relation to dose seemed to exist but the material is too small to allow any definite conclusions. In 15 cases (15.6 %) of generalized lymphoma, it was impossible to decide from which tissue the lymphomas were derived.

The mean latency time in the various dose groups did not differ in this material (Table 4). Nor were there any differences in this respect between lymphomas arising in the bone marrow or in the thymus. Earlier work (14) has indicated that the highest doses of ^{90}Sr employed have a most destructive effect on the bone marrow. Most of the haematopoietic function is taken over by the spleen, and bone marrow regeneration occurs only to a limited extent, and particularly at certain sites, such as in the thoracic vertebrae and sternum, where the dose

absorbed is smaller than in larger bones, e.g. the long bones. In the lower dose groups the marrow is severely damaged initially but its capacity for regeneration is better preserved. Thus, with decreasing dose an increased number of living but damaged cells will occur. That the most favourable site for regeneration is in the smaller bones may therefore explain the start of lymphomas at multicentric sites, particularly within the thoracic vertebral and sternal marrow (Fig. 1).

WATANABE (18) in an earlier paper, assuming the development of leukaemia from the bone marrow, suggested that these changes take place first in certain parts of the bone marrow and thereafter spread to other parts and finally to other organs and tissues. He assumed that the most favourable sites for the start of leukaemia might be within the vertebral marrow. He also noticed a tendency to early infiltration of the tissues surrounding the vertebrae (17). Those suggestions and observations agree with the findings in the present investigation, though these could also indicate a multicentric genesis within the bone marrow, however.

The findings concerning the genesis of ^{90}Sr-induced leukaemia in this work are very different from those after fractionated, external, total body irradiation. Of the leukaemia cases described in the literature after fractionated, whole-body irradiation the majority represent lymphomas of the thymus, the latency time of which is much shorter than in those of non-thymic origin (6). This discrepancy may have been caused by differences in age and mode of irradiation. In the present investigation all the mice were 75 days old. Fractionated irradiation yields thymic lymphomas at an optimum rate only when young mice are irradiated. After injection of ^{90}Sr, the bone marrow is the haematopoietic tissue which is most subject to risk. The marrow is continuously irradiated but the degree of damage and regeneration in its various parts differ. Other parts of the haematopoietic system are on the other hand not damaged initially to the same extent as after external irradiation. Above all, the thymus is obviously not affected to the same degree as after external irradiation, and the regeneration is not so evident. Preliminary results with ^{90}Sr (7) have indicated that regeneration disturbances (early asymmetry) do not occur among young mice, which is of interest since these asymmetries have been assumed by JÄRPLID (6) to be phenomena associated with the induction of thymic lymphoma. That these changes did not appear in the present material may be connected with a lesser need of, and a better supply of, cells competent to repopulate the thymus.

The role of the spleen in relation to the bone marrow and the thymus in leukaemogenesis cannot be established from this investigation. The importance of the spleen as a source of compensatory haematopoiesis with increasing dose of ^{90}Sr is, however, apparent. It has also been shown by JACOBSON (5) that the haematopoietic reserve offered by the spleen is one factor determining the survival of the mice, since the mortality increases among splenectomized irradiated mice.

SUMMARY

Varying doses of ^{90}Sr were administered to 1 430 CBA mice, about 75 days old, to investigate the site of origin, development and relation between the dose and incidence of leukaemia. The significance of the bone marrow, particularly of the thoracic vertebrae, as the site of origin of leukaemia, and its relationship to the thymus and spleen in this respect as well as following irradiation, are considered in turn.

ZUSAMMENFASSUNG

Verschiedene Dosen von ^{90}Sr wurden an 1 430 75-tage alten CBA-Mäusen verabreicht um Ursprungsstelle, Entwicklung und Verhältnis zwischen der Dosis und der Entwicklung von Leukämie zu studieren. Die Bedeutung des Knochenmarkes, besonders in der dorsalen Wirbelsäule, als Ursprungsstelle der Leukämie und deren Verhältnis zu Thymus und Milz in dieser Hinsicht sowie nach Bestrahlung werden diskutiert.

RÉSUMÉ

L'auteur a administré des doses variées de ^{90}Sr à 1430 souris CBA âgées de 75 jours environ pour étudier le lieu de l'origine, le dévoloppement et la relation entre la dose et la fréquence de la leucémie. Il étudie l'importance de la moelle osseuse, en particulier celle des vertèbres dorsales, comme siège de l'origine de la leucémie et il étudie ses rapports avec le thymus et la rate à ce point de vue ainsi qu'après l'irradiation.

REFERENCES

1. Boegler F. und Kriegel H.: Leukämoide Reaktion nach 90-Strontium-Inkorporation bei Ratten. Blut 17 (1968), 345.
2. Casarett G. W., Tuttle L. W. and Baxter R. C.: Pathologic effects of ingested strontium-90 in rats and monkeys. UR-597. University of Rochester, New York 1961.
3. Cosgrove G. E. and Upton A. C.: Some late effects of radiostrontium in RF male mice. J. nucl. Med. 3 (1962), 293.
4. Ito T., Yokoro K., Ito A. and Nishihara E.: A comparative study of the leukemogenic effects of strontium-90 and X-Rays in mice (33 553). Proc. Soc. exp. Biol. 130 (1969), 345.
5. Jacobson L. O.: The hematologic effects of ionizing radiation. *In*: Radiation biology. Volume 1, p. 1029. Edited by A. Hollaender. McGraw-Hill Book Company, New York, Toronto, London, 1954.
6. Järplid B.: Radiation induced asymmetry and lymphoma of thymus in mice. Acta radiol. (1968) Suppl. No. 279.
7. — Personal communication (1969).
8. McClellan R. O.: Hematopoietic tissue neoplasms in animals administered 90-Sr. Hlth Phys. 12 (1966), 1362.
9. — and Rupprecht F. C.: Section I: Dose-effect studies. Toxicity of inhaled $^{90}SrCl_2$ in beagle dogs. LF-38. Lovelace Foundation, Albuquerque, New Mexico 1967.
10. Nilsson A.: Effects of radiostrontium on the blood and haematopoietic tissues of mice. Acta vet. scand. 3 (1962), 103.

11. — Influence of gestation and lactation on radiostrontium-induced malignancies in mice. I. Incidence, distribution and characteristics of ^{90}Sr-induced malignancies. Acta radiol. Ther. Phys. Biol. 6 (1967), 33.
12. — Pathologic effects of different doses of ^{90}Sr in mice. Development of carcinomas in the mucous membranes of the head. Acta radiol. Ther. Phys. Biol. 7 (1968), 27.
13. — Pathologic effects of different doses of radiostrontium in mice. Dose effect relationship in ^{90}Sr-induced bone tumours. Acta radiol. Ther. Phys. Biol. 9 (1970), 155.
14. — Pathologic effects of different doses of radiostrontium in mice. Changes in the haematopoietic system. Acta radiol. Ther. Phys. Biol. 9 (1970), 528.
15. VAN PUTTEN L. M. and DE VRIES M. J.: Strontium-90 toxicity in mice. J. nat. Cancer Inst. 28 (1962), 587.
16. THOMAS R. L. and SCOTT J. K.: Toxicity of inhaled ^{90}Sr in rats. Hlth Phys. 15 (1968), 173.
17. WATANABE S.: Leucémies expérimentales provoquées chez la souris par administration répétée de petites doses de certains radioisotopes (^{32}P, ^{89}Sr et ^{144}Ce). Pathologie-Biologie (Extrait de la Semaine des Hôpitaux) (1958), 34/23—24, 1833—48.
18. — Experimental studies on the development of leukemia in mice with frequent administration of small doses of some radioactive isotopes (^{32}P, ^{89}Sr, ^{144}Ce), September 1957.