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PATHOLOGIC EFFECTS OF DIFFERENT DOSES OF RADIOSTRONTIUM IN MICE

Dose effect relationship in ^{90}Sr-induced bone tumours

by

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In spite of a very extensive literature concerning the carcinogenic effect of radiostrontium there seem to be only a few reports dealing with the various problems related to dose dependency. Studies in this field (FINKEL et coll. 1957) have mostly been concerned with the relationship between dose and frequency of mice having overt macroscopically detectable osteosarcomas. Many questions still remain which seem to be of interest for a better understanding of the dynamics of tumour formation and its relation to dose and time. In a previous report the main attention was focussed on the ^{90}Sr-induced carcinomas and their localisation in the mucous membranes of the head, their development and relation to dose (NILSSON 1968). A more detailed study concerning the effect of blood and blood-forming tissues and their dependency on dose will be presented in a further paper.

The purpose of the present work was to obtain more information concerning the dose-dependent histologic changes in bone and their relation to frequency, location and type of bone tumours. The relation between dose, time of appear-

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Table 1

Experimental conditions and the ^{90}Sr doses employed

Dose of ^{90}Sr in μCi/g body weight	Total number of mice investigated	Number of mice killed in groups of five	Last day for sacrifice	Number of spontaneously dead mice
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

Out of these animals five* and two** were lost during the experiment; only three*** and four**** animals, respectively, were sacrificed in the last test group.

ance and type of histologic changes and fluctuations in the bone cell populations was studied, as well as their possible relation to later development of tumours. The influence of the ^{90}Sr dose on tumour growth rate and latency time was also taken into account.

Material and Methods. Four groups of CBA mice, 75 days old, were treated intraperitoneally with $^{90}Sr(NO_3)_2$. A group of 95 animals not treated with ^{90}Sr were used as control. At intervals of 7, 14, 21 and 30 days after injection of ^{90}Sr, and then at monthly intervals, five mice from each were selected at random and sacrificed, until all mice in each series had been utilized. The experimental conditions and the ^{90}Sr doses employed are recorded in Table 1. The animals were examined roentgenologically before being killed and the roentgenograms were used as a guide for locating tumours.

The mice were sacrificed by cervical dislocation. Both femurs, tibias and humerus, the spine, pelvic bones and the head were fixed in Stieve's fluid and decalcified in 20 % formic acid for histologic examination. Conventional methods were used, the sections being stained according to the van Gieson method, haematoxylin-eosin, Lillie's azure-eosinate, Masson's trichromemethod, PAS according to Hotchkiss and Foot, and Foot's silver method. The animals that died spontaneously were autopsied and treated in the same way.

The numbers of osteoblasts and osteoclasts were determined per millimeter endosteum lining the epiphyseal-metaphyseal part of the distal femur, at various intervals of time between 7 and 210 days after injection of ^{90}Sr (Fig. 1). The bone sections were investigated in a Wild microscope equipped with a Wild 'Zeichentubus'. The field was projected at a magnification of 1 250 diameters and the endosteum was measured with an odometer. At each interval, 10 fields

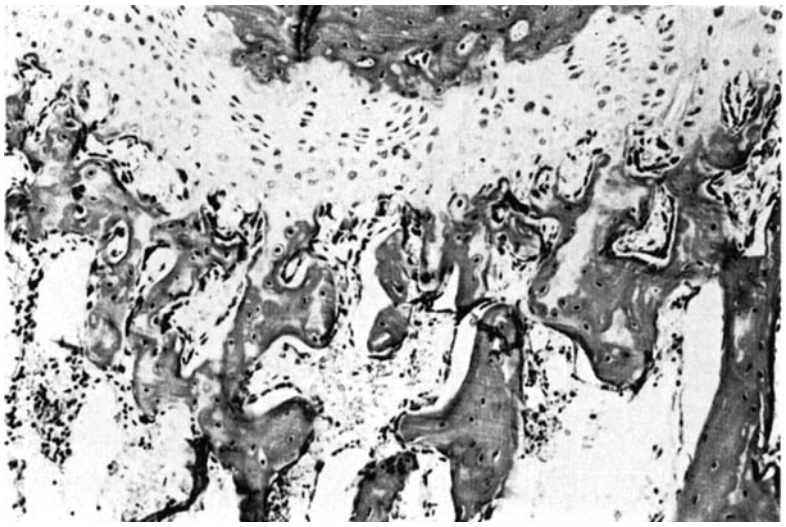

Fig. 1. Epiphyseal plate and metaphyseal bone of femur, 30 days after injection of 0.4 μCi ^{90}Sr/g body weight. Osteoblasts and osteclasts were counted in a zone immediately below the epiphyseal cartilage. van Gieson. × 125.

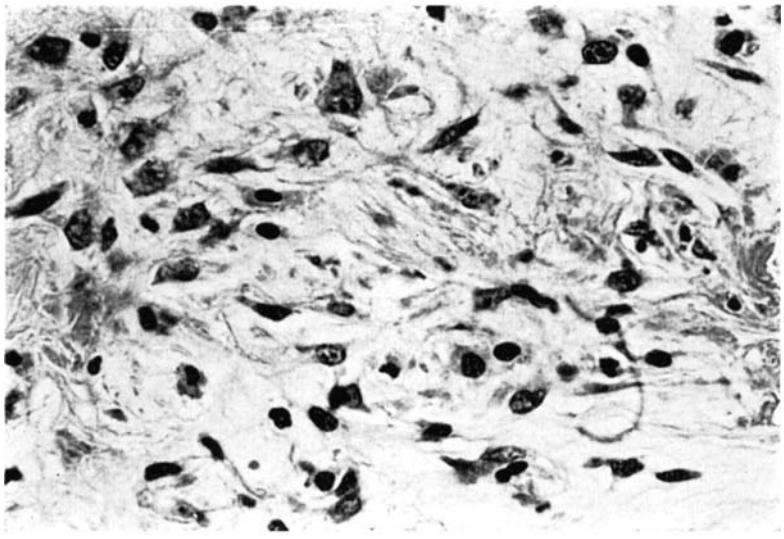

Fig. 2. Non-neoplastic, fibroblastic proliferation in bone marrow, femur, 210 days after injection of 0.8 μCi ^{90}Sr/g body weight. van Gieson. × 500.

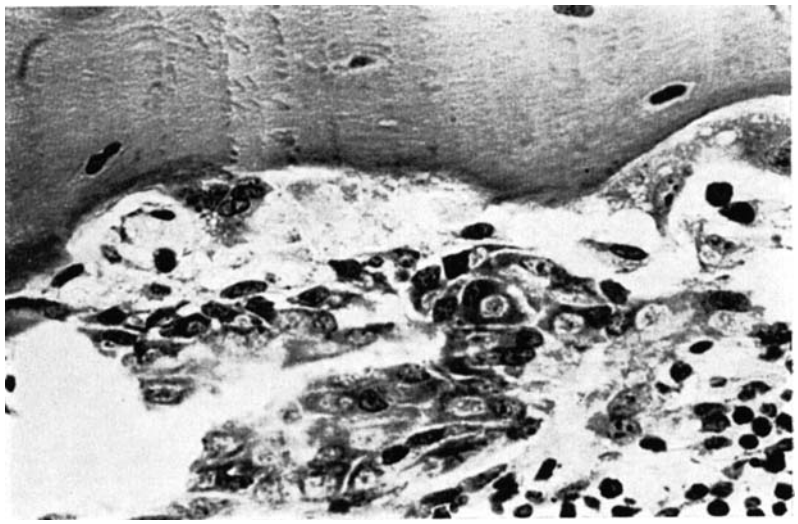

Fig. 3. Non-neoplastic focal proliferation of osteoblast-like cells and osteoclastic activity along the endosteal linings in diaphysis, tibia, 218 days after injection of 0.4 μCi ^{90}Sr/g body weight. van Gieson. × 500.

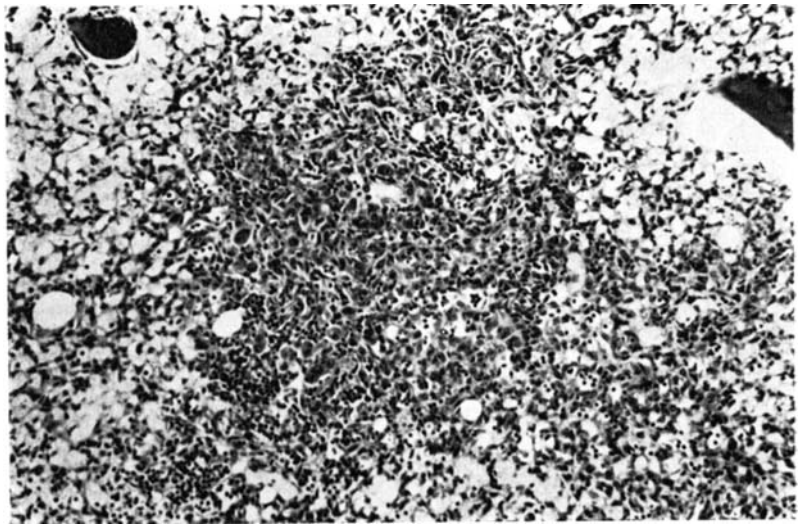

Fig. 4. Solitary neoplastic bud inside non-neoplastic fibroblastic tissue from lumbar vertebra, 300 days after injection of 0.8 μCi ^{90}Sr/g body weight. van Gieson. × 125.

Table 2

Cause of spontaneous death in relation to doses of ^{90}Sr administered per gram body weight

	1.6 μCi		0.8 μCi		0.4 μCi		0.2 μCi	
	No. of mice	Mean survival days	No. of mice	Mean survival days	No. of mice	Mean survival days	No. of mice	Mean survival days
Acute aplastic anaemia	6	18.2						
Chronic anaemia	9	263	5	226			1	358
Osteosarcoma	19	259.9	30	319.5	8	408.9		
Squamous cell carcinoma	16	282.5	3	338.3				
Leukaemia			3	250	11	264.5	4	251.3
Adenocarcinoma							1	402
Angiosarcoma			1	314			1	425
Fibrosarcoma							1	347
Liver rupture (hepatoma)							1	422
Liver rupture (carcinoma of the liver)							1	439
Not settled			4	350.0	8	293.8	7	383.4
Total	50	238.7	46	312.5	27	316.0	17	357.8

were counted in each of five femurs. The number of cells per millimeter of endosteum was determined by dividing the aggregate number of cells counted by the total length of the endosteum measured for the fifty fields. The cells supposed to be osteoblasts were characteristically arranged in a one-cell thick layer carpeting the bone surfaces. The cells were either columnar in shape, or they were thin, flattened, or, later on, fusiform elements. The osteoclasts were large, multi- or uni-nucleated cells, present on or near bone and in process of absorption.

The number of nucleated chondrocytes in the epiphyseal cartilage were determined at the same time intervals as the mean value of 10 unit areas of 6 400 μ^2 from both femurs of five mice. Chondrocytes consisted of single or multiple cells residing in lacunae in the intercellular substance of cartilage.

The mice were fed during the experiment on a standard diet ad libitum and kept in the same room under similar environmental conditions.

Histologic evaluation

The histologic events leading to overt paraosteal tumour growth in this material were defined as follows.

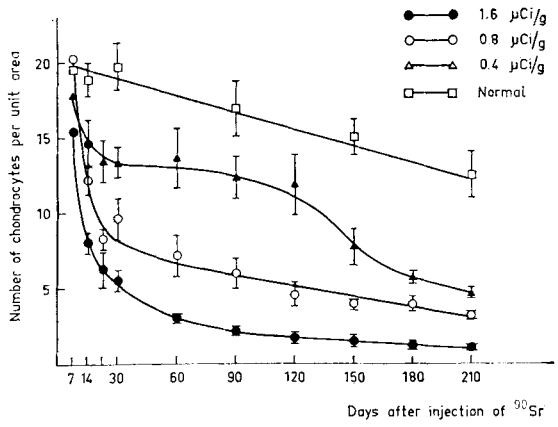

Fig. 5. Number of chondrocytes per unit area (6 400 μ^2) related to dose and time after injection of ^{90}Sr. Confidence limit 67 %.

Non-neoplastic proliferation. Distinct proliferation of uniform reticular cells with a histologic appearance of fibrosis (Fig. 2), with or without formation of foci of endosteal cell elements (Fig. 3), in both cases without neoplastic characteristics.

Osteosarcoma buds (microscopic tumours). Completely intramedullarily situated foci of proliferations containing pleomorphic cell elements (Fig. 4). Buds of this type have earlier been shown to be transplantable and to have malignant characteristics (NILSSON 1962).

Overt, macroscopic tumours. Tumours breaking through the compact bone with or without infiltrating the surrounding soft tissue.

Results

A survey of the pathologic findings among spontaneously dead animals in the various dose groups are listed in Table 2. Some mice in the 1.6 µCi group died early due to haematopoietic disorders. Of more interest is however that the animals succumbed from carcinomas nearly as often as from osteosarcomas. The cause of death in the 0.8 µCi group was predominantly osteosarcomas, and in the 0.4, and possibly also in the 0.2 µCi group, it was leukaemia. It should however be observed that many of the mice who died from carcinomas had osteosarcomas or leukaemia as well.

Pathologic features of the skeleton

Histologic appearances in relation to dose and time. The variations in the distal femur in histologic appearances in relation to dose were distinguished by an

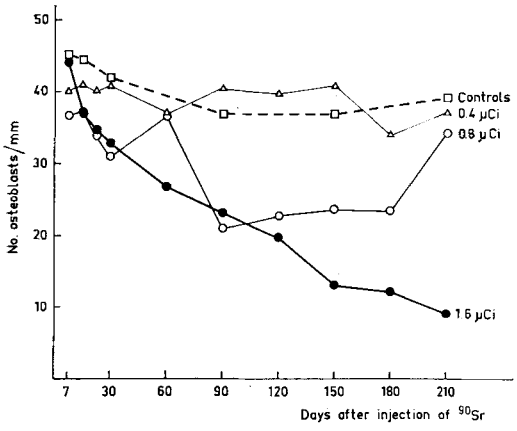

Fig. 6. Number of osteoblasts per millimeter endostium of distal metaphysis of femur related to dose and time. There was a significant difference between the 0.8 μCi and 1.6 μCi groups at 7 days ($t_{df\ 98}$ = =2.392, $p < 0.025$), 60 days ($t=3.560$; $p < 0.005$) and 150—210 days (t varied between 4.310 and 6.471, $p < 0.001$). The 0.4 μCi group differed significantly from the 0.8 μCi group at 21 days ($t=2.352$, $p < 0.01$), 30 days ($t= 3.421$, $p < 0.001$), 90—150 days (t varied between 4.585 and 6.670, $p < 0.001$) and 180 days ($t=2.204$, $p < 0.05$) after injection of ^{90}Sr.

earlier start and greater severity with increasing dose. The chondrocyte columns in the epiphyseal plate were disorganised and hypertrophic chondrocytes occurred, associated with a broadening of the plate. The dose effect on the number of nucleated cartilage cells in the epiphyseal plate is demonstrated in Fig. 5. The epiphyseal cartilage was strongly devitalized in the 1.6 μCi group already after 90 days. In the 0.4 μCi group, on the other hand, a tendency to regeneration was observed after 30—120 days.

It is hardly possible to draw up a general schedule for the early and intermediate changes in the osteoblast and osteoclast populations. Quantitatively there was a depletion of the osteoblast population, the size and duration of which was related to dose (Fig. 6). Quantitatively the osteoblasts were transformed into a more fusiform type of cells, more rapidly with increasing dose. Such cells appeared in the 1.6 μCi group after 7 days but not until about 60, 150 and 360 days in the respective 0.8, 0.4 and 0.2 μCi groups.

The number of osteoclasts increased in all dose groups during the first few months after ^{90}Sr injection (Fig. 7). The osteoclasts during the initial phase were localized mostly inside resorption cavities. Most of these cavities had lost their osteoclasts after 60—120 days. Instead, particularly after 150 days in the 1.6 μCi group, immature bone occurred in these cavities and along endosteal linings; this was a basophilic, PAS-positive, coarse-fibred bone, containing only a few fusiform, atypical osteoblast elements (Fig. 8). It is notable that these changes in the distal femur of the 1.6 μCi group seem to constitute a cessation of the cellular activity, as apparent from the lack of focal proliferations. Apposition of immature bone was seen to a much lesser extent in the 0.8 μCi group and in the other two groups it was quite insignificant. Synchronously with an increasing

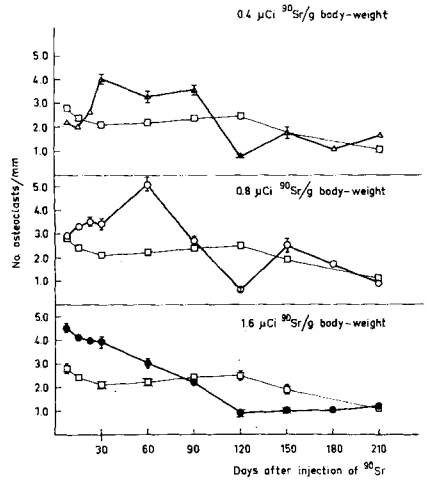

Fig. 7. Number of osteoclasts per millimeter of endostium in the distal metaphysis of femur related to dose and time in the controls.

activity of the osteoblast population, endosteal foci of proliferating osteoblast-like elements appeared. Such foci were seen in the 0.8 μCi group after 180—210 days but not until 300 and around 500 days, respectively, in the 0.4 and 0.2 μCi groups. These types of changes were not so striking in the 0.4 μCi group; instead, increased activity and proliferation of reticular cells predominated.

The histologic changes described were also studied in other bones. The destructive changes inside each group were generally of about the same magnitude in the tibia and femur but usually of much less severity in other bones, particularly in thoracic and cervical vertebrae.

Tumour development

Non-neoplastic proliferations. The occurrence of scattered, focally situated, proliferations of osteoblastic cell elements in resorption cavities or along endosteal linings (Fig. 8) as well as a diffuse increase of reticular cells (Fig. 2) could be detected in all the different dose groups. The relationship between dose and the first appearance and the rate of transformation of these changes into tumour entities was studied more thoroughly for the fibroblastic types of tumour, since the different stages of their development are more clearly defined and easier to follow than the osteoblastic types (SUNDELIN & NILSSON 1968). The frequency and start of these changes were dependent on dose and time after injection of ^{90}Sr (Fig. 9). In the 1.6 μCi group, these changes were usually most numerous in the lumbar vertebrae and pelvic bones. In the other groups the long bones, particularly the femur, were most often involved. The 0.2 and 1.6 μCi groups

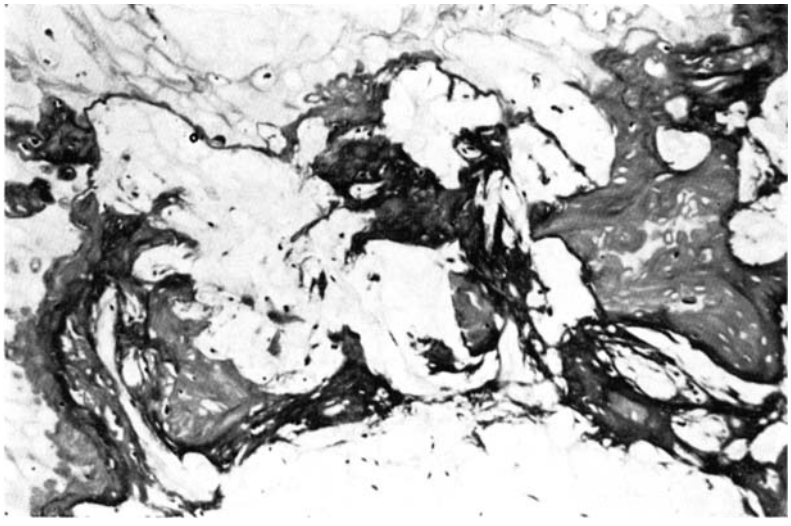

Fig. 8. Heavy destruction of epiphyseal plate and metaphyseal bone, apposition of basophilic, immature coarse-fibred bone, 270 days after injection of 1.6 μCi ^{90}Sr/g body weight. van Gieson. × 300.

show the lowest incidence of these changes in the femur, the 0.8 μCi group the highest, and the 0.4 group a frequency about four times as great as the 1.6 group.

Intramedullary tumour buds. The further development of the non-neoplastic proliferations into intramedullary tumours consists of a successive confluence of expanding, multicentric centres of proliferating osteoblast-like cell elements. These cells attain in time a more atypical appearance, lay down immature bone and acquire the histologic characteristics of an osteoblastic osteosarcoma. During neoplastic transformation of the fibroblastic type there seems to be constant formation of small solitary or multiple foci or accumulations of tightly packed, atypical cells inside the non-neoplastic fibrous tissue (Fig. 4). The induction time for intramedullary osteosarcomas was related to dose. Generally, the osteoblastic type appeared earlier than the fibroblastic buds. On the other hand, the mean induction time for the two tumour types did not differ significantly.

Rate of transformation. For fibroblastic osteosarcomas there existed a dose-related influence on the rate of transformation of non-neoplastic proliferations into intramedullary osteosarcoma buds and further into overt paraosteal tumours. The rate of development was significantly increased in the 1.6 μCi group as compared with the other series. No such relation was found for the osteoblastic type (Table 3).

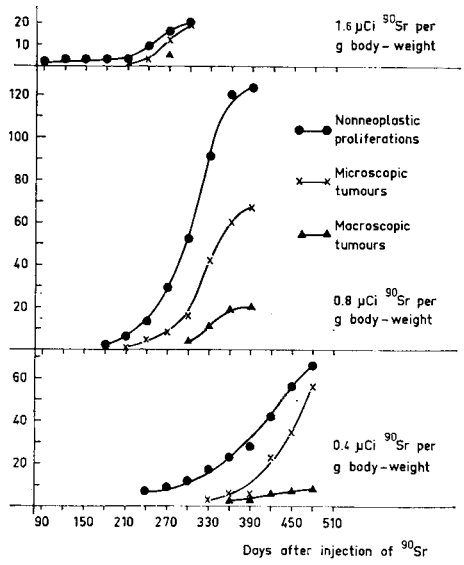

Fig. 9. Accumulated number of fibroblastic non-neoplastic proliferations, microscopically intramedullary, and macroscopically extramedullary fibroblastic osteosarcomas.

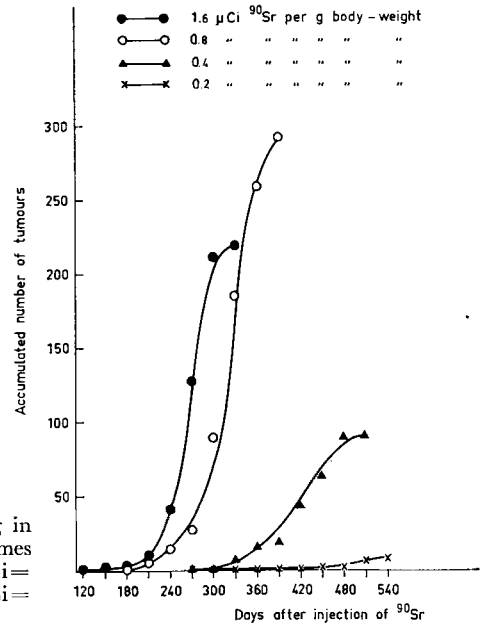

Fig. 10. Total number of tumours occurring in the different dose groups. The latency times were: 1.6 μCi group $=267.6 \pm 4.6$, 0.8 μCi $=320.7 \pm 5.4$, 0.4 μCi $=426.7 \pm 9.8$, 0.2 μCi $=485.1 \pm 37.8$.

Table 3

Influence of dosage on rate of tumour development calculated from curves in Fig. 8 by horizontal retrograde extrapolation from the points representing respectively detected osteosarcoma buds to the curves for detected non-neoplastic proliferations and from macroscopic tumours to the curves for detected tumour buds

Dose	Tumour	Horizontal retrograde extrapolation of time interval between	
		Detected tumour buds and curve representing non-neoplastic proliferations: days ± SE	Detected macroscopic tumours and curve representing tumour buds: days ± SE
1.6 μCi		21.3±4.3 (n: 19)	29.2±1.6 (n: 5)
0.8	Fibroblastic	58.1±0.9 (n: 66)	57.2±2.4 (n: 20)
0.4		66.1±3.9 (n: 50)	63.1±8.8 (n: 8)
1.6 μCi			53.3±2.7 (n: 30)
0.8	Osteoblastic		53.6±1.9 (n: 54)
0.4			63 ±17 (n: 6)
1.6 μCi versus 0.8 μCi	Fibroblastic	$t = 8.364$ ($p < 0.0005$)	9.722 ($p < 0.0005$)
0.8 μCi versus 0.4 μCi		$t = 1.995$ ($p < 0.025$)	0.648 (not significant)

Tumour-bearing animals, tumour incidence and tumour multiplicity. The highest frequency of tumour-bearing mice amongst both spontaneously dead (89.1 %) and sacrificed animals (76.7 %) was found in the 0.8 μCi group (see Table 4). More than half the number of mice in this group had macroscopic tumours, while considerably fewer macroscopic tumours were seen in the other groups.

The total number of tumours in the various groups can be directly compared since the total number of mice was the same in each group (Table 4, Fig. 10). A total of 609 tumours were found in the whole material. Of this total number 35.9 %, 47.9 %, 14.8 % and 1.3 % were located in the groups which started with the highest dose. Of these tumours, 37 (17.1 %) in the 1.6 μCi group and 79 (27.1 %), 17 (18.9 %) and 1 (12.5 %) in the lower dose series, respectively, were of macroscopic size. For all but the lowest dose group, a considerable tendency towards occurrence of multiple tumours was observed (see Table 5).

Table 4

Tumour frequency and total number of tumours in relation to dose of ^{90}Sr in μCi/g body weight and time

Spontaneously dead mice	1.6 μCi		
	Total number of mice	Number of mice with tumour	Total number of tumours
	50	42 (84.0 %)	132
Sacrificed mice			
Day 7—90	30	0	0
120	5	1	1
150	5	1	1
180	5	1	1
210	5	3	5
240	5	5	22
270	5	5	28
300	5	5	29
330			
360			
390			
420			
450			
480			
510			
540			
Total sacrificed mice	65	21 (32.3 %)	87
Per cent of sacrificed mice with tumours after appearance of first tumour		60.0	

Induction time. As may be seen from Fig. 10, the latency period was considerably longer with decreasing dose, though it may be difficult from an experiment of this type to calculate the true mean induction with certainty.

Tumour location. A definite correlation existed between dose and tumour location (Table 6). In the 0.8 μCi group and the 0.2 μCi group, more than 50 % of the tumours were located preferentially in the long bones, particularly the femur. In contrast, most tumours in the 1.6 μCi group were sited in the lumbar, sacral spine and pelvic bones (56 %). There was a preference with

Table 4 *(cont.)*

0.8 μCi			0.4 μCi			0.2 μCi		
Total number of mice	Number of mice with tumour	Total number of tumours	Total number of mice	Number of mice with tumour	Total numbar of tumours	Total number of mice	Number of mice with tumour	Total number of tumours
46	41 (89.1 %)	195	27	11 (40.7 %)	20	17	1 (5.9 %)	1
45	0	0						
5	2	5						
5	4	8	55	0	0			
5	3	7	5	1	1	60	0	0
5	5	17	5	0	0	5	1	1
5	4	20	5	3	6	5	0	0
5	5	40	5	2	3	5	0	0
			5	1	2	5	0	0
			5	5	20	5	0	0
			5	4	12	5	0	0
			5	5	26	5	0	0
						5	2	4
						3	2	2
75	23 (30.7 %)	97	95	21 (22.1 %)	70	103	5 (4.9 %)	8
	76.7			52.5			10.4	

increasing dose for tumour development also in the thoracic spine and the head. Inside the particular long bones there was a location pattern which was obviously related to dose. Most tumours in the 1.6 μCi group were located in the proximal ends of the femur, tibia and humerus. The same applied in the 0.8 μCi series as regards tibia and humerus but in the femur the distal metaphysis was the predominating site. In the 0.4 μCi group, on the other hand, the diaphysis of these bones was most often involved (Table 7).

The location of tumours in relation to the type of osteosarcoma differed with respect to their sites in the different parts of the skeleton. In all the dose

Table 5

Tumour multicentricity in relation to dose

Dose group μCi/g body weight	Number of osteosarcomas/mouse		
	Spontaneously dead	Sacrificed after appearance of first tumour	Whole experiment
1.6	2.6	2.5	2.6
0.8	4.2	3.2	3.8
0.4	0.74	1.8	1.3
0.2	0.06	0.16	0.13

Table 6

Anatomical distribution of osteosarcomas in relation to dose delivered in μCi/g body weight

Location	Per cent tumours in each dose group				Total number of tumours in each dose group			
	1.6 μCi	0.8 μCi	0.4 μCi	0.2 μCi	1.6 μCi	0.8 μCi	0.4 μCi	0.2 μCi
Femur	8.2	28.8	28.9	(25)	18	84	26	2
Tibia	5.9	13.4	14.4	(37.5)	13	39	13	3
Humerus	5.9	12.7	11.1	(12.5)	13	37	10	1
Total long bones	20.1	54.8	54.4	(75.0)	44	160	49	6
Pelvic bones	22.8	13.7	17.8		50	40	16	
Cervical spine	3.7	3.1	2.2		8	9	2	
Thoracic spine	6.8	2.1			15	6		
Lumbar spine	22.8	11.0	5.6		50	32	5	2
Sacral spine	10.5	5.5	11.1	(25.0)	23	16	10	
Lumbar and sacral spine	1.4	0.3			3	1		
Coccygeal spine	2.7				6			
Total vertebral column	47.9	21.9	18.9	(25.0)	105	64	17	2
Head	7.8	4.5	2.2		17	13	2	
Multiple site	1.4	5.1	6.7		3	15	6	
Total					219	292	90	8

Table 7

Distribution of tumours in various parts of the long bones in relation to the total number of tumours in each of the long bones within the dose group

μCi ^{90}Sr/g body weight	Total number of tumours	Distribution of tumours in					
		Distal epiphysis		Diaphysis		Proximal epiphysis	
		Number	Per cent	Number	Per cent	Number	Per cent
Femur							
1.6	18*	1	5.6	4	22.2	11	61.1
0.8	84**	38	45.2	22	26.2	17	20.2
0.4	26°°	4	15.4	15	57.7	4	15.4
Tibia							
1.6	13	2	15.4	7	53.8	4	30.8
0.8	39°	1	2.6	5	12.8	32	82.1
0.4	13*	0	0.0	8	61.5	3	23.1
Humerus							
1.6	13	3	23.1	1	7.7	9	69.2
0.8	37*	4	10.8	6	16.2	25	67.6
0.4	10°	0	0.0	9	100.0	0	0.0

The number of tumours that could not be located to a particular zone are indicated as follows one tumour (°), two tumours (*), three tumours (°°), seven tumours (**).

groups, the humerus and the lumbar and the sacral spine had the highest frequency of fibroblastic osteosarcoma. In the pelvic bones, there was a strong predominance of osteoblastic osteosarcoma in the 1.6 μCi group, whereas in the 0.4 μCi group it was of fibroblastic type. All tumours in the head were of the osteoblastic type. As regards the site inside particular bones, the fibroblastic intramedullary osteosarcoma buds in the 0.4 μCi group occurred to a much greater extent in the diaphyseal part of the long bones than did the osteoblastic tumour buds.

Tumour types. With decreasing dose, there was a shift from a prodominance of osteoblastic to fibroblastic osteosarcomas (Table 8).

Discussion

Relationship between dose and tumour frequency in various tissues. Each of the different ^{90}Sr doses administered in this investigation proved capable of inducing osteoarcomas. In previous studies (FINKEL 1958), the same was reported with

Table 8

Histological classification of bone tumours

Type of tumours	Percentage distribution by dose			
	1.6 μCi n: 219	0.8 μCi n: 292	0.4 μCi n: 90	0.2 μCi n: 8
Osteoblastic osteosarcomas	87.2	68.8	24.4	(50.0)
Fibroblastic osteosarcomas	11.0	29.8	71.1	(37.5)
Chondroblastic osteosarcomas	0.9	—	—	
Angiosarcomas	0.9	2.1	4.4	(12.5)

doses even lower than we used. The optimal dose, 0.8 μCi/g body weight, was in good agreement with earlier reports (FINKEL 1958, NILSSON 1962). The frequency of osteosarcomas was still very high in the 1.6 μCi series and, in addition, carcinomas occurred in the mucous membranes of the mouth and nose, as reported in a separate paper (NILSSON 1968). In the highest dose group, leukaemia was not detected among the spontaneously dead mice. This was the case in each of the other series, however, the highest frequency being in the 0.4 μCi group. The existence of a clear optimum of carcinomas, osteosarcomas and leukaemia for the doses 1.6, 0.8 and 0.4 μCi/g, respectively, seems to indicate a strong dependency on dose and on the topographic location of the tissue involved. The radiation delivered from a certain dose of radiostrontium to the mucous membranes close to bone is much less than to the osseous tissues and to the bone marrow. Thus, doses inducing carcinoma may generally be too destructive for induction of osteosarcomas at a maximum rate, and even more so for induction of leukaemia. Only doses with an effect not more destructive than allowing recovery and a certain degree of regeneration will promote an optimal frequency of neoplasia. It was also shown in an earlier investigation (NILSSON 1967) that female mice injected with an optimal osteosarcoma dose had a significantly reduced number of osteosarcomas when an increased excretion (30 %) of ^{90}Sr was brought about by lactation. At the same time, however, there was a significant increase of leukaemia compared with non-lactating mice.

Relation between dose and latency time. As previously demonstrated in this investigation, there was a correlation between dose and latency time not only for the first occurrence of neoplastic changes but also for the appearance of the destructive and regenerative phases and non-neoplastic proliferations (Figs 9 and 10). This clearly indicates that the latency time is a function of dose and time.

In this relation, the total accumulated dose seems to be a more decisive factor in tumour genesis than the initial dose rate (NILSSON et coll. 1967, VAN PUTTEN 1961).

Rate of tumour development. After becoming established as a stage of development, the fibroblastic non-neoplastic proliferations were more rapidly transformed into intramedullary fibroblastic osteosarcomas and to overt neoplasms within the higher dose groups (Table 4). There was thus a significantly more rapid development of the fibroblastic tumours in the 1.6 than in the 0.8 and 0.4 μCi groups. The reason for this might be a combination of factors, such as the occurrence of more selectively altered, radioresistant cell clones after higher doses, the growth of which may not be suppressed since the immunologic capacity and resistance of the host are diminished.

The growth rate of osteoblastic buds was not enhanced by dose (Table 4). These tumour 'buds' from the histologic point of view are more highly differentiated than the fibroblastic ones and, consequently, might not be influenced in the same degree by irradiation. The reason for the general reduction of the mean induction time with increasing dose seems to be primarily the earlier occurrence of tumour cell clones and not a generally increasing growth rate.

Relation between dose and tumour multiplicity. It is seen from Table 5 that the multicentric genesis of tumours is a function of time and dose, since with increasing survival there is a perpetual rise in the number of tumours in each group of animals.

Considering the relation between tumours of macroscopic and microscopic extent, it is of interest to note that the latter predominated strongly in the 0.8 μCi group. Many microscopic tumours develop with a superoptimal, not too destructive, dose but the survival time for the mice is too short for their transition into macroscopic entities. The induction time with a suboptimal dose might be too long for the development of an appreciable number of macroscopic tumours.

Dose-related histologic variations in bone tissue. When comparing homologous bones between different dose groups, the degree and extent of histologic changes have been found to be strongly dose-dependent. Such differences are however also obvious when comparing heterologous bones inside each group. This variation seems to be related to the fact that the radiation dose absorbed is determined by many factors such as the size, geometry and metabolic rate of the bones. This may explain the more serious effect on the femur than on the lumbar vertebrae and why the latter are more damaged than the thoracic vertebrae.

In order to transform the various radiation doses into terms of their mutual biological effect, the nucleated chondrocytes in the epiphyseal plate of the distal

femur were counted (Fig. 5). These cells were expected to provide a better reference than the osteoblasts to the dose relations in this part of the bone. Only in the 0.4 μCi group were there signs of recovery and a temporary regeneration. In the two other groups, and particularly in the 1.6 μCi group, there was a continuously enhanced cellular depletion with increasing dose. The osteoblastic and osteoclastic populations in the distal femur showed more variations (Figs 6 and 7). There was thus a continuously decreasing number of cells in the 1.6 μCi group. In the group given 0.8 μCi, which is the optimal osteosarcoma dose, the osteoblastic population was forced into a state of instability characterized by a diphasic fluctuation. The recovery observed after 60 days might be characterized as an abortive regeneration, which may indicate its origine from a cell population so heavily damaged that it could only survive a limited number of divisions. The second regeneration, which started after about 180 days, appeared to give birth to scattered, focal, non-neoplastic proliferations (Figs 2 and 3) which, however, successively were transformed into tumours. This might indicate that cells originating from this second regeneration were selected, possibly quite radioresistant, but defective, and potentially malignant cell elements. The destructive effect of the highest dose will not allow any appreciable degree of regeneration in the distal femur, as mirrored by the low incidence of tumours in the latter. The 0.4 μCi dose, on the other hand, may not cause damage sufficient to induce the instability necessary in the osteoblastic population to create optimal conditions for tumour development. Of particular interest is however that the changes most prominent in the 0.4 μCi group were located in the reticular cells of the bone marrow to a much higher degree than in the other groups. These cells give rise predominantly to the fibroblastic type of osteosarcomas (Fig. 4). Some observations made in the 0.4 μCi group as well have shown advanced destruction and necrosis in the bone marrow, including the reticular cells. This might be an expression of circulatory disturbances inside the bone marrow. A lesser radioresistance of the reticular cells compared with that of the osteoblastic elements might also explain why the osteoblastic elements more often proliferate in the higher dose groups.

Relation between dose and tumour location. The importance of dose for the tumour location is illustrated in Table 7. There is a remarkable shift from a significant predominance of tumours in the long bones, particularly the femur, in the 0.8 and the 0.4 μCi groups to a clear under-representation in the 1.6 μCi series. The number of tumours in the latter group is thus only 28 % of that in the 0.8 group. In the pelvic bones and the spine there are instead 20 % and 39 % more tumours in the 1.6 μCi than in the 0.8 μCi group. It should also be pointed out that with increasing dose there are more tumours located in such

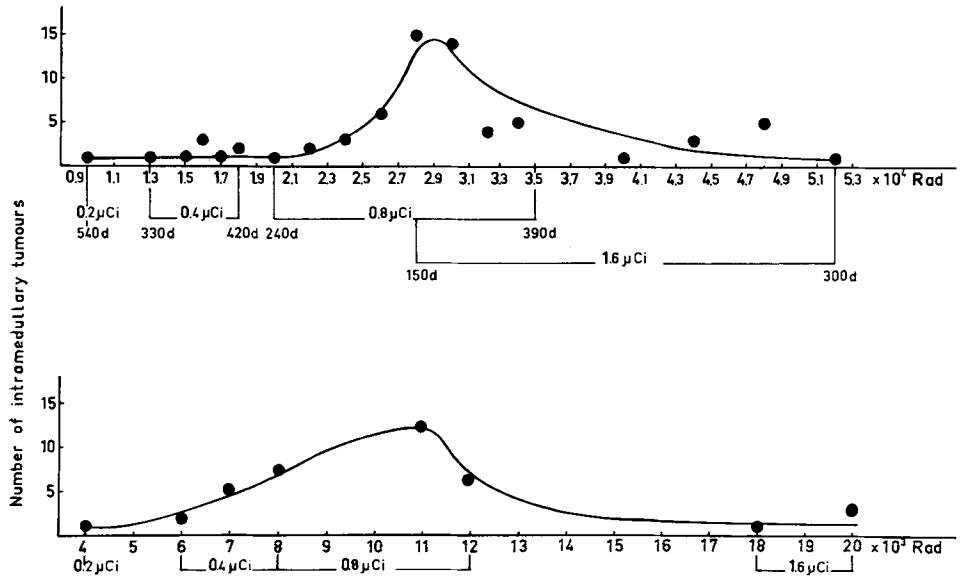

Fig. 11. Occurrence of intramedullary osteosarcomas in relation to the total accumulated dose in the different dose groups. *Upper curve:* dose to the distal and proximal epiphyseal parts of the femur. *Lower curve:* dose to the midshaft of the femur.

small bones as the thoracic and coccygeal vertebrae. The same applies to the location of tumours in the head. This may be connected with the previously mentioned differences in the radiation doses absorbed in various parts of the skeleton. A comparison of the 0.4 and 0.8 µCi groups reveals a fairly constant pattern of tumour location.

The tumour sites inside particular bones also reveal a changed pattern with decreasing dose. About 25 % of the tumors in the long bones of the 1.6 and 0.8 µCi groups were located in the diaphysis. In the 0.4 µCi series, 73 % were located there.

Relation between total accumulated dose in various bones and tumour frequency. It seemed of interest to investigate the influence of the total accumulated dose upon the tumour rate in the various dose groups. The femur was chosen as model for this purpose. The doses absorbed in the distal epiphysis, the diaphysis and the proximal epiphysis of the femur were calculated. The mice, 75 days old when injected, and weighing 21 to 22 g, were given 0.8 µCi ^{90}Sr/g body weight, and the weight of the femur and its various parts were determined. According to the investigation of PARMLEY et coll. (1962) the absorbed energy

in the whole femur was 38 %. The mean energy of ^{90}Sr—^{90}Y is 1.13 MeV per disintegration. On the basis of these data and the retention curves for the femur, an approximative radiation dose to the various parts of the femur can be calculated. The doses in Fig. 11 are given merely as reference values and refer only to microscopic intramedullarily situated osteosarcomas. The doses in Fig. 11 (upper curve) relate to osteosarcoma buds located only in the proximal and distal parts of the femur, and those in Fig. 11 (lower curve) only to buds located in the central part of the diaphysis. There is a range between 9 500 and 52 000 rad, particularly between 27 000 and 31 000 rad, in which most tumours occur. This optimum can be assigned almost solely to the 0.8 μCi group, the dose in the 1.6 μCi group being too high for an optimal tumour induction in the femur.

In the diaphysis of the femur most tumours appeared around 10 000 to 12 000 rad. A possible explanation of this might be that the magnitude of the dose that induces irreversible changes leading to tumour formation is in fact much smaller than indicated by these figures and the figures for the distal and proximal parts. Tumours have exceptionally been observed after only 4 500 rad, and around 9 000 rad in the distal part. On the assumption that a minimum time, and thus a minimum accumulated dose, is necessary for induction of histologic changes successively leading to formation of tumours it seems probable that the dose delivered after this time does not have much importance. The dose is constantly increasing during the time it takes for a tumour to develop. This 'surplus dose' will be higher at certain sites, like the distal metaphysis of the femur, since the dose rate there is much higher than in the diaphysis. In some cases this 'surplus' seems to have some effect in promoting the rate of development of fibroblastic osteosarcoma, at least in the highest dose group. No such effect was observed for the osteoblastic type, however. On the other hand, it must be borne in mind that a supraoptimal dose might have a depressing effect initially on tumour induction and later also on its further development.

It may be of interest to note that the frequency of diaphyseal tumours in relation to the total number in the femur was 36 %. The dose delivered was 35 % of that in the distal and proximal epiphysis. As mentioned earlier, most tumours in the 0.8 μCi group occurred in the femur and in the 1.6 μCi group in the spine, particularly the lumbar vertebrae. It would have been of interest to compare the doses delivered to these bones but no such measurements have however been made. A rough approximation from retention curves of femur and lumbar vertebrae, and also from calculations made by MARSHALL & FINKEL (1959), reveals an estimated dose to the lumbar vertebrae of about 50 % of that delivered to the femur. This seems to be the explanation of the optimal tumour frequency in the spine for the highest dose level.

Tumour types. The percentage of fibroblastic tumours was enhanced with decreasing dose. This observation is however contradictory to that of STRELTSOVA (1959), who reported an increasing number of osteoblastic (osteosclerotic) tumours with decreasing dose in rats. In the 0.4 μCi series, this type of tumour had a tendency to be overrepresented in the diaphysis of the long bones (69 %), where the radiation dose was only about 30 % of that delivered to the metaphyseal parts of the bone. The mean tumour induction time, on the other hand, was about 160 days longer than in the 1.6 μCi group.

Some cases of angiosarcoma have been found in all the dose groups, but only one chondroblastic osteosarcoma was detected in the 1.6 μCi group. The percentage distribution of angiosarcomas indicates a tendency to increase with decreasing dose.

SUMMARY

The dose dependency was investigated in four groups of male CBA mice injected intraperitoneally with, respectively, 1.6, 0.8, 0.4 and 0.2 μCi of ^{90}Sr/g bodyweight. A group of 95 animals was used as controls. Five mice from each group were sacrificed at certain intervals. It was shown pathologically and by conventional histologic methods that not only latency time and tumour frequency but also type, multicentricity, location and, to some extent, the growth rate of osteosarcomas were related to the dose. The pathologic changes preceding tumour development, and their relation to the dose given, are discussed.

ZUSAMMENFASSUNG

Die Dosisabhängigkeit wurde in vier Gruppen von männlichen CBA-Mäusen, die intraperitoneal mit bzw 1,6 μCi, 0,8 μCi, 0,4 μCi und 0,2 μCi von ^{90}Sr/g Körpergewicht injiziert wurden, studiert. Eine Gruppe von 95 Tieren wurde als Kontrolle benutzt. Fünf Tiere aus jeder Gruppe wurden bei verschiedenen Intervallen getötet. Pathologisch und histologisch wurde gezeigt, dass nicht nur die Latenzzeit und Frequenz der Tumoren sondern auch Art, Multizentrisität, Lage und, im gewissen Masse, Zuwachsrate von Osteosarcomen von der Dosis abhängig sind. Pathologische Veränderungen bevor Beginn der Tumorentwicklung im Verhältnis zur gegebenen Dosis werden diskutiert.

RÉSUMÉ

L'auteur a étudié l'influence de la dose sur quatre groupes de souris mâles CBA auxquelles était injecté par voie intrapéritonéale respectivement 1,6 μCi, 0,8 μCi, 0,4 μCi et 0,2 μCi de ^{90}Sr/g de poids corporel; un groupe de 95 animaux a servi de témoin. Cinq souris de chaque groupe ont été sacrifiées à certains intervalles. Les méthodes anatomo-pathologiques et histologiques habituelles ont montré que non seulement le temps de latence et la fréquence des tumeurs, mais aussi leur type, leur caractère multicentrique, leur siège et dans une certaine mesure la vitesse de croissance des ostéosarcomes sont liés à la dose. Les modifications anatomo-pathologiques qui précèdent le début du développement de la tumeur et leur relation avec la dose administrée sont etudiées.

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