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INFLUENCE OF OESTROGENIC HORMONES ON CARCINOGENESIS AND TOXICITY OF RADIOSTRONTIUM

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Oestrogenic hormones given after sublethal irradiations bring about a striking increase of mortality rate in mice (THOMPSON et coll. 1965, TREADWELL et coll. 1943). Given before irradiation, however, they are said to be protective (ROOKS & DORFMAN 1961). In the intact animal (DOUGHERTY 1952, KAPPAS & PALMER 1963) these hormones cause acute thymic involution, which, seemingly, severely impairs the immunologic capacity (THOMPSON et coll. 1966). On the other lymphatic tissues their effects are minimal, variable, and appear to relate to the species investigated (DOUGHERTY 1952, KAPPAS & PALMER 1963).

Oestrogenic hormones also regularly cause a marked formation of new endosteal bone in mice (GARDENER 1936, 1946, URIST et coll. 1950, SIMMONS 1962). The proposed mechanism involves an increased formation of osteoblasts by transformation of reticular cells and primitive connective tissue cells from the bone marrow (SIMMONS 1962). There are also observations (MILLER et coll. 1943) that oestrogen accelerates the development of osteosarcomas in strains of mice with spontaneous bone tumours. In a number of soft tissues such as the

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Table 1

Experiment I. Day 0: Start of experiment. All mice 75±3 days old. Oestrogen was given as Estradurin

Group of mice	No. of mice	Day 0: oestrogen mg s.c.	Day 7: μCi ^{90}Sr/g body weight i.p.	Day 30: oestrogen mg s.c.	Day 60: oestrogen mg s.c.	Day 67: μCi ^{90}Sr/g body weight i.p.
A (males)	143	1.0	0.8	0.5	0.25	—
B "	118	—	0.8	—	—	—
C "	68	1.0	—	0.5	0.25	—
D "	50*	1.0	—	0.5	0.25	0.8
E (females)	50*	0.25	0.4	0.25	0.25	—
F "	100*	—	0.4	—	—	—

* One was lost during the course of the experiment.

mammary gland, testes and lymphoid tissues, pituitary and uterus oestrogens also exert a carcinogenic action in mice (GARDNER 1957).

Radiostrontium is a potent carcinogen. Given in doses which induce bone tumours it initially exerts a strongly suppressive effect on the bone cells. Later there is an increased activity and proliferation which ultimately leads to osteosarcoma induction (NILSSON 1962, 1970). It might be anticipated — since carcinogenesis requires cells capable of proliferation — that factors such as oestrogen, which stimulates these cell lines and their precursors to proliferation, will increase the carcinogenicity of ^{90}Sr.

The present investigation will primarily deal with the influence of oestrogen on the skeleton of ^{90}Sr-treated mice. Some combined effects on mortality and blood and blood-forming tissues are recorded but not systematically investigated.

Material and Methods

The material was divided into two main experiments. Male CBA mice (groups A, B, C and D) and female mice (groups E and F), all 75 ± 3 days old, were used. The mice, ten in each cage, were fed during the experiment on a standard diet ad libitum and kept in the same room under similar environmental conditions.

Experiment I. Six groups of mice were given $^{90}Sr(NO_3)_2$ intraperitoneally and in some cases also oestrogen of long duration (Estradurin, Leo) subcutaneously as recorded in Table 1. The mice were inspected twice a day during the whole experimental period. Before autopsy films were exposed of all dead mice.

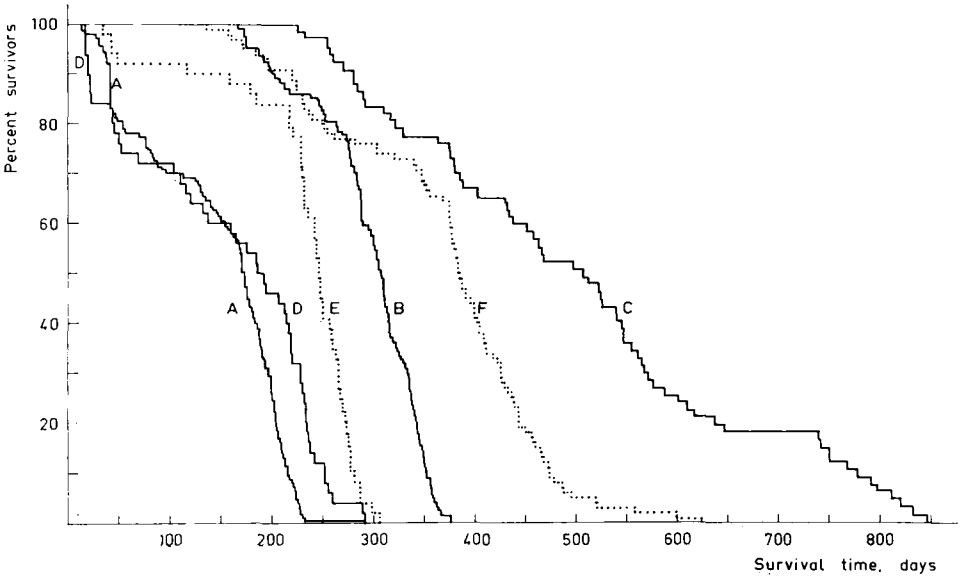

Fig. 1. Survival time in days of different groups of mice after treatment. For explanation of different group designation see Table 1.

The diagnosis of bone tumours in this experiment is based entirely upon roentgenologic and post mortem findings, and thus the number of tumours recorded is a minimum. Histologic classification of the bone tumours found was not performed, which means that tumours classified here as bone tumours are not necessarily true bone tumours. Leukaemia and neoplasia in other tissues were, however, histologically verified. Routinely the head was cut longitudinally through its midline and fixed. The histologic methods applied to this material are identical with those used in experiment II.

Experiment II. These experiments were performed in order to investigate the histologic changes preceding tumour development and for classification of the tumours induced. The experimental procedures are recorded in Table 2. Only male mice were used. The mice were killed by cervical dislocation at different intervals as shown in Table 2. Before sacrifice the mice were anaesthetized with Mebumal intraperitoneally. Films were exposed for diagnostic purposes and blood samples were obtained with a Pasteur pipette from the retro-orbital veins. The carcases were weighed, as well as the thymus, spleen and adrenal glands. These organs and both femora, tibiae, humeri, sternum, pelvic bones and the spine were fixed in Stieve's fluid. The head was cut longitudinally through its midline

Table 2

Experiment II. Day 0: Start of experiment. All mice 75±3 days old. Oestrogen was given as Estradurin

Group number	No. of mice	Treatment			
		Day 0: oestrogen mg s.c.	Day 7: μCi ^{90}Sr/g body weight i.p.	Day 30: oestrogen mg s.c.	Day 60: oestrogen mg s.c.
1	58	1.0	0.8	0.5	0.25
2	14	1.0	—	0.5	0.25
3	25	—	0.8	—	—
4	10	—	—	—	—

and also fixed in Stieve's fluid. The bones were decalcified in 20 % formic acid. Ordinary histologic techniques were used and all sections were stained according to the van Gieson method and with haematoxylin and eosin. Selected sections were also stained with PAS — orange G (head with tumours of the pituitary), azure-eosinate, azan according to Heidenhain, and Foot and Foot's silver method. Mice spontaneously dead before sacrifice were autopsied and handled in the same way.

Definition of tumour classification. Bone tumours, when bone formation occurs, are called osteosarcomas. These can be subdivided into fibroblastic, chondroblastic, osteoblastic or osteoclastic types depending upon whether the collagenous, cartilaginous, osseous or osteoclastic components predominates. In addition pleomorphic, mixed and anaplastic types can be distinguished, depending upon whether a strongly pleomorphic cell type, equal parts of at least three cell components or a low differentiated tissue predominate. This subdivision has been recommended by the Committee of Pathology of the European Late Effects Project Group (EULEP) (1971).

Results

Experiment I

Survival time. From Fig. 1 it is seen that mice given oestrogen + ^{90}Sr had a significantly shorter survival than mice given only ^{90}Sr. The mean survival times are recorded in Table 3. During the first 3 months after the start of the experiment 45 mice (31.5 %) died in group A. The mortality was concentrated to 3 consecutive periods of 2 to 3 weeks' duration following approximately 11 to 14

Table 2 *(cont.)*

No. of mice killed; day after injection of ^{90}Sr and day after 1st injection of oestrogen (within brackets)										Number of mice dead before sacrifice
7 (14)	38 (45)	60 (67)	73 (80)	83 (90)	113 (120)	121 (128)	143 (150)	173 (180)	203 (210)	
2	2	5	2	5	5	2	5	5	5	20
2	2	—	2	4	—	2	2	—	—	0
—	—	5	—	—	5	—	—	5	5	5
—	—	2	—	3	—	2	—	—	3	0

days after the preceding treatment with either ^{90}Sr or oestrogen. The highest death rate was seen after the second injection of oestrogen, when 25 mice (17.5 %) died. In group D a similar pattern of mortality was observed after the ^{90}Sr administration in spite of the fact that ^{90}Sr was given seven days after the last hormone injection on day 60. Within 2 months 14 mice (28 %) died. Also in group E some mice died very early as compared with mice treated with only ^{90}Sr or oestrogen.

Causes of death. Since there was a high initial death rate in mice given the combined treatment, the material was divided into two parts: mice dying before (Table 4) and after the appearance of the first bone tumour (Table 5). Negative sections (Table 4) without gross anatomic or histologic changes which could unambiguously explain the cause of early death were numerous in group A and also in group D. Possible explanations will be discussed later. It is also seen that haemorrhage (usually haemothorax or haemocoelia) was a frequent cause of early death both in group A (27 %) and in group D (40 %).

In group A the first death in haemorrhage appeared 77 days after injection of ^{90}Sr or 17 days after the last oestrogen injection. Between days 77 and 129, 12 cases occurred. In group D all cases were found between days 17 and 120 after the injection or ^{90}Sr. In this group all oestrogen injections were given before administration of ^{90}Sr. In group E inanition in combination with a more or less insufficient haematopoiesis predominated.

From Table 5 is evident that, in all groups except C, bone tumours predominated as cause of death. In group E, however, many cases of bone tumours were complicated by a more or less severe pyometra, which in some cases made it difficult to settle the exact cause of death. In group F a high death rate in

Table 3

Survival time, frequency of neoplasia and induction time

	Group of mice
	A
Number of mice	143
Mean survival, all mice, days	147.4±4.4
Number of mice dead before first bone tumour	45
Mean survival, mice dead before first bone tumour, days	54.0±3.9
Occurrence of first bone tumour, day after ^{90}Sr	127
Mean induction time, days	194.1±2.6
Number of mice with bone tumours	96
Number of mice without bone tumours	2
Total number of bone tumours (macroscopic within brackets)	414 (196)
Number of bone tumours/mouse	414/98 = 4.2
Number of bone tumours/mouse, whole material	414/143 = 2.9
Occurrence of first leukaemia, day after ^{90}Sr	76
Mean induction time, days	131.7±8.6
Number of mice with leukaemia	9
Percentage of leukaemia, whole material	6.3
Eosinophilic adenoma, pituitary	—
Mean induction time, pituitary adenoma, days	—
Other tumours	—
Mean induction time, other tumours, days	—

leukaemia was also noted. In group C, besides a high frequency of peritonitis, there was also a high frequency of leukaemia (20.6 %) and other malignant neoplasm (10.3 %), such as carcinomas of the liver, leiomyosarcomas and fibrosarcomas in the peritoneal cavity. Seven eosinophilic adenomas of the pituitary were also observed, out of which five (7.4 %) were the cause of death. In this context a case of periarteritis nodosa of an abdominal vessel should also be mentioned, although not causing the death of the animal.

Frequency of bone tumours. The number of bone tumours per mouse, calculated from the whole material (intramedullary + overt tumours) in experiment I or only from overt tumours, was approximately a factor 2 greater in groups A and D compared to group B (Table 3, Fig. 2) and in group E in relation to group F. The induction time for these tumours was also significantly shorter ($p < 0.001$) in all the groups treated with oestrogen and ^{90}Sr as compared to mice treated with ^{90}Sr alone. No bone tumours appeared in group C treated

Table 3 *(cont.)*

B	C	D	E	F
118	68	50	49	99
293.4±2.9	502.1±21.2	157.9±12.3	229.3±9.5	368.1±10.4
5	0	20	6	5
173.2±1.3	—	58.3±9.8	73.7±20.8	158.4±6.5
189	—	159	179	184
316.1±1.1	—	224.4±5.8	251.0±4.1	379.3±9.7
99	0	29	42	75
14	68	0	1	19
264 (134)	—	133 (58)	175 (110)	186 (119)
264/113 = 2.3	—	133/29 = 4.6	175/43 = 4.1	186/94 = 2.0
264/118 = 2.2	—	133/49 = 2.7	175/49 = 3.6	186/99 = 1.9
168	256	42	250	170
209.8±14.9	371.9±32.4	122.4±35.0	262±4.9	223.9±8.2
9	14	5	4	17
7.6	20.6	10.2	8.2	17.2
—	7	—	—	—
—	620±65	—	—	—
—	7	—	—	—
—	486±67	—	—	—

Table 4

Causes of death in mice dying before the appearance of the first bone tumour

Group of mice	No. of dead mice	Cause of death				
		Haemorrhage	Leukaemia	Inanition*	Neg. autopsy	Not stated**
A	45	12	1	—	15	17
B	5	—	3	—	2	—
C	0	—	—	—	—	—
D	20	8	3	4	5	—
E	6	—	—	5	1	—
F	5	—	2	3	—	—

* These cases were combined with bone marrow hypoplasia.

** On account of cadaverous changes and cannibalism.

Table 5

Causes of death in mice dying after appearance of first bone tumour in each group

Group of mice	No. of mice	Cause of death							
		Bone tumours	Leukaemia	Not stated**	Inanition	Peritonitis	Haemorrhage	Neoplasia	Other causes
A	98	74	8	4	5	—	7	—	—
B	113	88	6	14	2	1	—	—	2
C	68	—	14	9	1	31	1	12	—
D	29	26	2	1	—	—	—	—	—
E	43	29*	4	2	1	1	—	—	6***
F	94	74	15	3	—	—	—	—	2

* In many cases pyometra also occurred.
** On account of cadaverous changes and cannibalism.
*** Pyometra.

only with oestrogen in spite of a survival time approximately 3.3 times longer than for mice treated with both ^{90}Sr and oestrogen. The occurrence of osteosarcomas is extremely rare in these mice.

Site of tumours in the skeleton. With respect to the anatomic distribution of the tumours there were only insignificant differences when oestrogen + ^{90}Sr treated mice were compared to those given ^{90}Sr alone, except for a lower frequency of tumours in the head in the former group.

Incidence of leukaemia. The frequency and latency time for leukaemia are recorded in Table 3. The leukaemia cases were subdivided into those starting as thymic lymphomas and those originating as bone marrow lymphomas, the percentage distribution of which is shown in Fig. 3. The spontaneous incidence of leukaemia in this strain is in the order of 0.5 to 1.0 per cent.

Experiment II

Histology. Serial sections were performed in order to investigate presumptive differences in histology between mice treated with oestrogen, ^{90}Sr or oestrogen + ^{90}Sr. A classification of bone tumours was also made (Table 6).

Mice treated with oestrogen. In general the histology was in good agreement with that of previous observations (GARDNER 1946, URIST et coll. 1950). The endosteal bone apposition is most obvious in the metaphysis of the distal femur, proximal tibiae and proximal humerus. It was less marked in the sternum and intermediary in the pelvic bones and vertebrae. On day 14 the trabeculae in the femoral metaphysis was somewhat longer and broader than normally. At certain

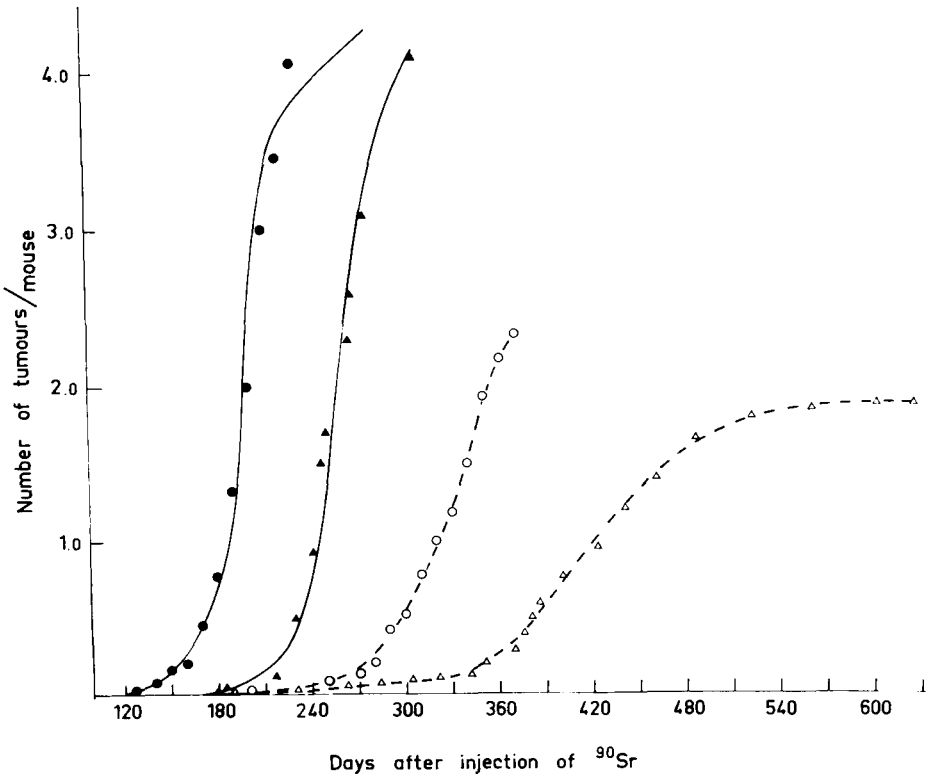

Fig. 2. Number of tumours per mouse in relation to time after injection of ^{90}Sr. ● group A, ○ group B, ▲ group E, △ group F. For explanation of different group designation see Table 1.

Table 6

Bone tumour classification, serially killed mice, experiment II

Treatment	No. of tumours	Type of osteosarcoma, per cent				
		Osteoblastic	Fibroblastic	Osteoclastic	Mixed type	Pleomorphic
^{90}Sr+oestrogen	247	61.1	14.9	5.3	17.4	1.2
^{90}Sr	9	66.7	33.3	—	—	—

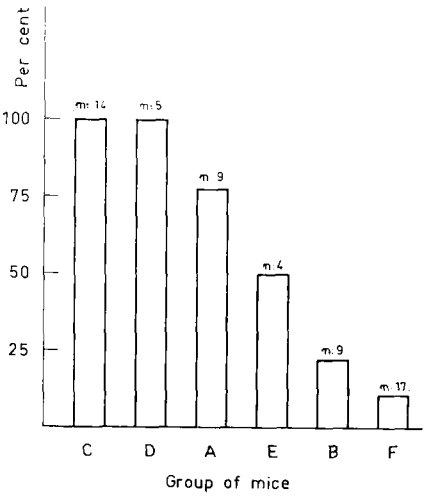

Fig. 3. Percentage distribution of thymic lymphomas in relation to non thymic (bone marrow) lymphomas.

places small clones of enlarged osteoblasts were detected. The number of osteoclasts seems to have diminished. In the sternum the bone formation was much less significant. On day 45 about half of the femur from the distal metaphysis to the middle of the diaphysis was more or less filled with newly formed bone (Fig. 4). The osteoblasts were still very active but were not so large as previously (Fig. 5). Osteoclasts were still seen. In the sternum the production of new bone occurred and the osteoblasts were numerous. Osteoclasts were somewhat reduced in number.

From day 67 to 150 after oestrogen injection the whole medullary cavity of the femur successively became more or less obliterated with new bone (Fig. 6). The osteoblasts were small and quite inactive. Osteoclasts were few.

Mice treated with ^{90}Sr. The histology does not differ from that of earlier descriptions (NILSSON 1962, 1970).

Mice treated with oestrogen + ^{90}Sr. On day 14 after oestrogen injection the formation of bone in the femur was about the same as among mice treated only with oestrogen, but in addition there was a very marked formation of fibres and fusiform cell elements between and along the bone spiculae and endosteal linings. Numerous enlarged osteoblasts were seen. Osteoclasts were also numerous and many of them were located in typical Howship's lacunae. In the sternum there was a very marked increase of active osteoblasts and osteoclasts and in

Fig. 4

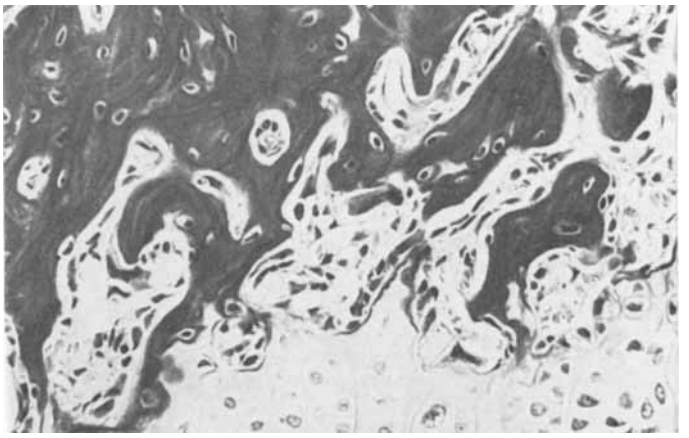

Fig. 5

Fig. 4. Femur, male mouse, 45 days after first injection of oestrogenic hormone. Heavy formation of new bone filling up marrow cavity at distal end of the bone. Van Gieson approx. × 7.

Fig. 5. Magnification of distal metaphyseal part of the bone in Fig. 4. Oestoblastic activity and newly formed bone. Van Gieson × 200.

some cases numerous mitoses were seen. On day 45 the histologic examination of the femur differed remarkably from that in mice treated only with oestrogen (Fig. 7). The occurrence of newly formed bone was very much less, and the area between bone spiculae was filled with a quite cell-deficient fibrous tissue (Fig. 8). The predominating cell type in this tissue was fibroblast like, fusiform cell elements intermingled with reticular cells, osteoclasts and macrophages. Practically no typical osteoblasts were seen along the endosteal linings of the bone. At some

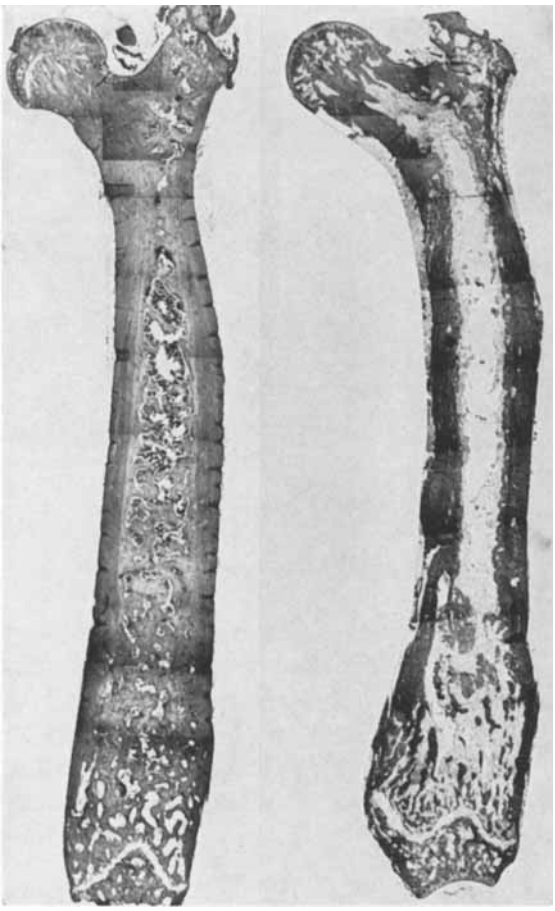

Fig. 6. Femur, male mouse, 121 days after first injection of oestrogen. Almost complete occlusion of the marrow cavity by newly formed bone. Van Gieson approx. × 7.

Fig. 7. Femur, male mouse, 45 days after first injection of oestrogen and 38 days after injection of ^{90}Sr. Compared with the bone in Fig. 4 the formation of new bone is poor. Almost complete aplasia of the marrow. Van Gieson approx. × 7.

Fig. 6 Fig. 7

places there was an obvious osteoclastic activity. In the sternum there was a great increase of very active osteoblasts, and the formation of bone was much greater than among mice given oestrogen alone. Numerous osteoclasts were also seen. From day 67 to 150 after the first oestrogen injection the difference between the two groups is even more accentuated. In the femur the 'compactization' decreased with time since the newly formed 'oestrogenic' bone was broken down and to a great extent replaced by the formation of a fibrous tissue (Fig. 9). In the sternum, on the other hand, the bone formation inside the medullary cavity was more intense than in the group treated with oestrogen alone. No replacement

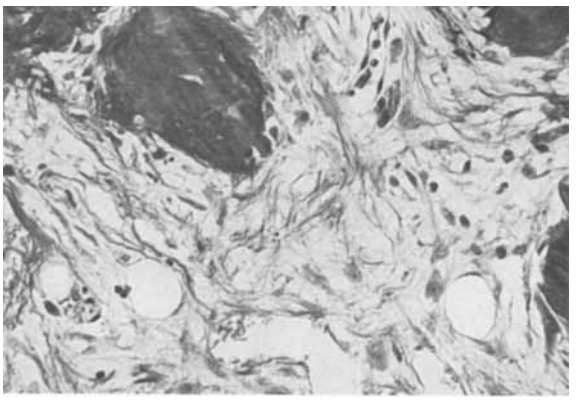

Fig. 8. Magnification of Fig. 7. Destruction of newly formed bone and formation of fuchsinophilic and argyrophilic fibres. Some macrophages and fusiform cell elements but no normal osteoblasts are seen. Aplastic fatty marrow. Van Gieson × 175.

of 'oestrogenic' bone by a fibrous tissue component was observed in the sternum of mice treated with oestrogen + ^{90}Sr in contrast to that in the femur.

It is inside these fibrous, originally quite cell-deficient tissues that small islands with morphologic characteristics of malignancy appeared. These buds usually have an appearance of greater histologic activity and variation than was seen among mice treated with ^{90}Sr alone. Of particular interest was the frequent occurrence of more or less pure osteoclastic osteosarcoma buds (Fig. 10). Of importance also is the fact that the first microscopic osteosarcomas in the oestrogen + ^{90}Sr treated mice were detected histologically already on day 121 after the ^{90}Sr injection in both femur and spine, whereas the first microscopic tumours among mice treated with ^{90}Sr alone did not appear until after 173 days.

Tumour frequency and histologic classification. In the oestrogen + ^{90}Sr treated mice in experiment II altogether 247 osteosarcomas were found, of which 98 were macroscopically detectable. For the whole material, 58 mice, there were 4.2 tumours/mouse. This figure increases to 5.6 when only the 46 tumour-bearing mice were taken into account. In the ^{90}Sr group only 9 tumours were found, giving a mean of 0.4 tumours/mouse for 25 mice.

The classification of tumours is recorded in Table 6. In the group treated with oestrogen and ^{90}Sr osteoblastic tumours predominated, followed by fibroblastic and mixed type, osteoclastic (Fig. 11) and pleomorphic type osteosarcomas. With reservation for the small number of tumours occurring among mice given only ^{90}Sr there was good agreement with earlier observations (Nilsson 1962).

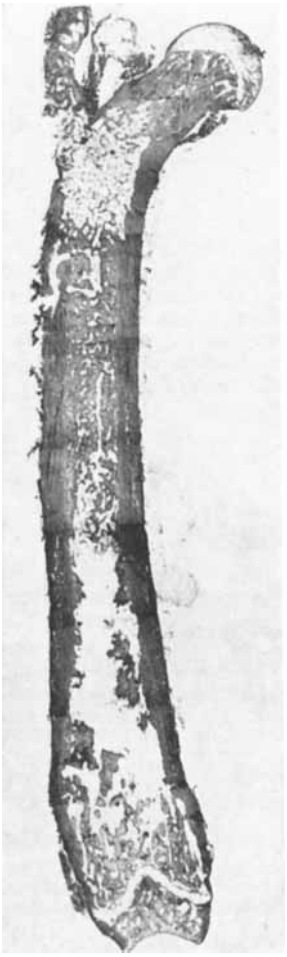

Fig. 9

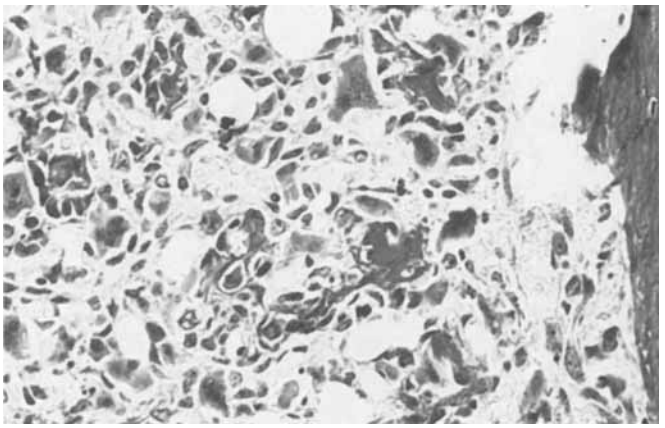

Fig. 10

Fig. 9. Femur, male mouse, 121 days after first injection of oestrogen and 114 days after injection of ^{90}Sr. In the diaphysis remnants of newly formed 'oestrogenic' bone. Distally most of the 'oestrogenic' bone destroyed and devitalized. In the proximal part small growing 'osteosarcoma bud' destroying preformed compact bone. Van Gieson approx. × 7.

Fig. 10. Magnification of proximal 'osteosarcoma bud' with numerous osteoclast like and fusiform cell elements. Van Gieson × 200.

Blood and haematopoietic tissues. The bone marrow depletion in the oestrogen + ^{90}Sr group was initially more severe than among the other groups, particularly in the femur. On account of the earlier mentioned heavy formation of fibrous tissue of the marrow cavities in the femur, marrow regeneration was also strongly impaired. Great differences were also seen in the sternal marrow, varying from an almost complete restoration of cellularity among the ^{90}Sr treated mice to a quantitatively strong impairment on account of heavy bone formation in the oestrogen + ^{90}Sr treated mice (Fig. 12). In the mice treated with oestrogen

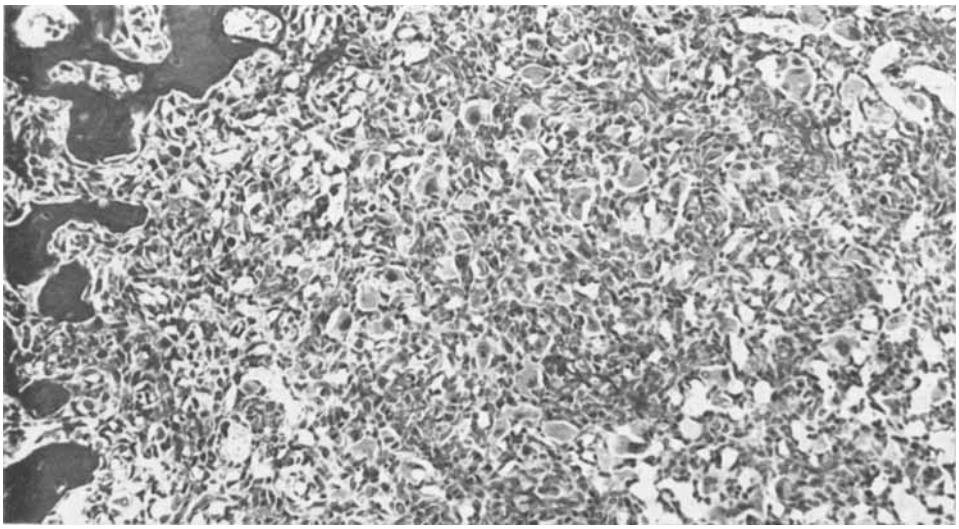

Fig. 11. Osteoclastic osteosarcoma, lumbar spine, male mouse 182 days after combined oestrogen and ^{90}Sr treatment. Van Gieson × 140.

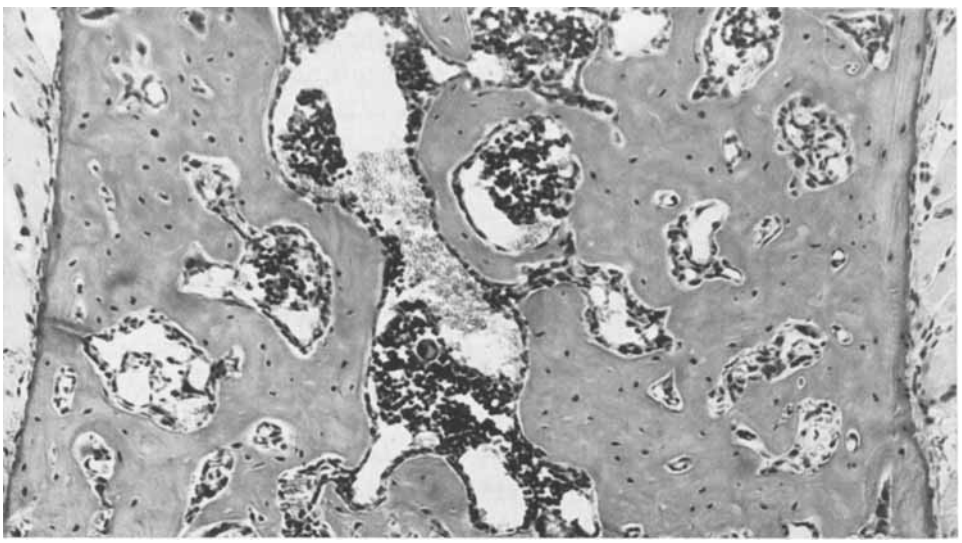

Fig. 12. Sternal vertebrae, 67 days after first oestrogen and 60 days after ^{90}Sr injection. Marrow cavity occupied by newly formed bone. Van Gieson × 140.

Table 7

Blood data and weights of spleen and thymus of mice treated with ^{90}Sr and oestrogen + ^{90}Sr

Day after ^{90}Sr	Mice, serially killed			
	^{90}Sr			
	60	113	173	203
Leukocytes	1972 ± 264	2156 ± 162	2212 ± 171	2384 ± 186
Haemoglobin (g/100 ml blood)	—	14.02 ± 0.97	12.64 ± 0.55	12.88 ± 0.32
Erythrocytes 10^6	8.59 ± 0.21	7.64 ± 0.22	6.55 ± 0.30	6.55 ± 0.40
Weight of spleen mg	102.14 ± 3.02	90.06 ± 6.71	62.00 ± 2.67	72.70 ± 1.47
Weight of thymus mg	31.88 ± 1.46	21.32 ± 2.26	11.60 ± 2.47	14.56 ± 0.50
Thrombocytes	—	300 000 ± 25 700	298 000 ± 31 100	477 000 ± 84 900

* Two cases of unilateral lymphomas

alone there appeared to be a slightly diminished cellularity of the bone marrow as compared to control animals.

There was also a general tendency to bleeding in the mice in experiment II. Out of the 20 mice dying spontaneously 3 died of haemothorax and haemocoelia. Small bleedings were also observed in all mice killed on days 60 and 83 and in 4 out of 5 on day 113. Thrombocyte counts were only slightly depressed and may not be related to the haemorrhages observed (Table 7). The weight of the thymus among oestrogen + ^{90}Sr treated mice was significantly diminished and that of the spleen increased ($0.02 > p > 0.01$) up to day 113 as compared with mice given only ^{90}Sr (Table 7). The increased weight of the spleen was due largely to an increased extramedullary haematopoiesis of all types of cells. The number of leucocytes in the oestrogen + ^{90}Sr group was much more reduced than in the ^{90}Sr group; up to day 113 the difference was significant ($p < 0.001$) (Table 7). In the group given oestrogen + 0.4 μCi ^{90}Sr/g the weight of the spleen increased to 5 to 10 times normal weight. In many of these cases an enormous proliferation of myeloblastic elements and granulocytes took place in a late stage in the spleen and in the liver, adrenals, lymph glands and bone marrow. The megakaryocytopoiesis also was usually quite prominent, but the erythropoiesis was successively reduced.

In experiment II two cases of unilateral lymphoma were detected among oestrogen + ^{90}Sr treated mice.

Table 7 *(cont.)*

Oestrogen + ^{90}Sr					
60	83	113	143	173	203
956±120	1208±142	968±54	1280±124	1544±245	1800±305
–	–	13.48±0.18	12.9±0.30	12.36±1.01	12.24±0.22
7.90±0.35	7.48±0.19	7.62±0.27	7.39±0.15	6.62±0.56	6.81±0.29
142.28±13.40	112.1±2.08	116.5±7.87	101.56±11.10	96.56±19.4	110.46±8.48
7.64±1.39	5.66±0.75	9.48±3.09	18.36±7.10*	6.56±1.71	13.60±2.19
–	206 800± 9 920	151 000± 25 300	265 600± 18 950	348 400± 32 200	362 600± 27 300

Discussion

The combination of ^{90}Sr and oestrogenic hormones has revealed a highly potentiated effect in mice as compared with either treatment alone. A highly significant reduction of the mean survival time was thus noted. Despite this the bone tumour rate was significantly enhanced. The tumour induction time was also significantly shortened and the histologic appearance of the tumour modified.

As evidenced by histologic observation elsewhere (NILSSON 1962, 1970) it has been found that ^{90}Sr brings about a severe suppression of the osteoblastic cell population. This depletion is later followed by an increased cellular activity and proliferations at circumscribed areas along the endosteal linings or more diffusely in the bone marrow cavity. The start of this proliferation is dependent upon dose, but it usually commences within 5 to 6 months after optimal doses of ^{90}Sr. These cells are, however, functionally defective and morphologically atypical, fusiform elements which may undergo neoplasia. Oestrogenic hormones induce an intense formation of new bone. When combined with ^{90}Sr, this bone apposition is, however, largely inhibited (Fig. 7). The osteoblasts are replaced by functionally defective fusiform cells producing an abundance of fibrous tissue instead of bone. Numerous osteoclasts attacking the 'oestrogenic' bone also appear. These histologic events appear very early, are more intense and more widespread than after ^{90}Sr alone. The reason for this may be related to the stimulating effect of oestrogen on the cell compartment, as shown by SIMMONS (1962). He states that the primary effect of these hormones on the skeleton is to stimulate the onset of the modulations of the undifferentiated marrow cells to form osteoblasts. Such osteogenic potentials of reticular cells leading to a rapid transformation into

preosteoblasts and osteoblasts come into effect, however, only when the cells are approached by bony surfaces growing out from the endosteum. This means that ^{90}Sr irradiates not only a much more numerous cell population, but also a very alert, constantly stimulated cell population. Both these factors might be of importance for the increased tumour frequency found. The tumour frequency, however, was the same whether ^{90}Sr was given during or after the last oestrogen injection. This seems to indicate that the overpopulation is a most decisive factor, since the greater the population irradiated, the greater the chances for malignant clones to develop. Intimately associated with this enhanced development and earlier appearance of malignant clones, micro- and macroscopic tumours are probably also immunological factors. This is indicated by a threefold prolonged skin allograft survival and an impaired primary haemaglutination response when oestrogens are combined with external irradiation (THOMPSON et coll. 1966), and by the fact that ^{90}Sr-induced bone tumours are antigenic (NILSSON et coll. 1972).

The occurrence of osteoclastic and mixed osteosarcomas, which are extremely rare when ^{90}Sr alone is given, might be related to the fact that osteoclasts were numerous already in early stages preceding tumour development.

The relation of irradiation to the increased tumour frequency is not clear. From Table 3 and Fig. 2 it is seen that the tumour incidence in group E (female mice which were given oestrogen and 0.4 μCi ^{90}Sr/g body weight) was almost a factor of two (1.8) greater than in group B (male mice given only ^{90}Sr, 0.8 μCi/g body weight). Also the tumour induction time in the former group was significantly shorter ($p < 0.001$). With reservations for possible sex differences in the rate of tumours these facts seem to indicate that irradiation does not play a decisive role in the enhanced occurrence of tumours in the groups with combined treatment. This relation is, however, studied in a separate investigation and will be discussed elsewhere.

In many cases pathology was practically negative. As THOMPSON et coll. (1965) have stated, earlier anaemia seems to be of little importance for the early mortality. Despite the conspicuous destruction of the marrow found in this investigation, the spleen seems to be able to compensate for the erythropoietic insufficiency of the marrow. A factor of consequence for the enhanced early mortality might, however, as previously suggested by THOMPSON et coll. (1965), be related to the strong impairment of the myeloid and lymphatic tissues (Table 7), which makes them unable to achieve levels necessary to maintain survival. This is particularly true since ^{90}Sr doses of the same size as used in this investigation have been shown to induce a maximum depression of leucocyte counts around 16 days after the injection of the nuclide (NILSSON 1962). At this time the neutrophils are almost eliminated and the lymphocytes strongly

reduced. In combination with oestrogenic hormones this effect (Table 7) is even more severe. This deficiency and the fact that oestrogenic hormones exert a strong suppression on the immunologic responsiveness (THOMPSON & RUSSE 1965, THOMPSON et coll. 1966) might suggest the possibility of peracute infection without visible pathologic lesion. A general immunologic suppression, although not tested, might also have occurred in this investigation as judged from the severe damage to the thymus, spleen and bone marrow.

The reason for the variation of the leukaemia incidence between the different groups (Table 3) cannot be satisfactorily explained and will be further investigated, as well as the relation between the treatment and frequency of thymic and non-thymic lymphomas.

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SUMMARY

Groups of male and female CBA mice were treated with oestrogenic hormone alone, ^{90}Sr alone or with oestrogen $+^{90}$ Sr. Among the males given both oestrogen and ^{90}Sr there was a mean number of 4.2 bone tumours/mouse with a mean induction time of 194.1 ± 2.6 days as compared with 2.3 among those given only ^{90}Sr. The mean induction time for the latter was 316.1 ± 1.1 days. The female mice treated with ^{90}Sr+oestrogen developed 4.1 bone tumours/mouse and those given ^{90}Sr alone 2.0. The induction times were 251.0 ± 4.1 and 379.3 ± 9.7 days, respectively. No bone tumours were found in the group of mice treated with only oestrogen. Osteoclastic and mixed osteosarcomas were frequent in combination treatment groups in contrast to the result when ^{90}Sr was given alone.

ZUSAMMENFASSUNG

Gruppen von männlichen und weiblichen CBA-Mäusen wurden mit östrogenem Hormon alleine, ^{90}Sr alleine oder mit Östrogen+^{90}Sr behandelt. Bei den Männchen, denen sowohl Östrogen als auch ^{90}Sr gegeben war, fand sich eine durchschnittliche Anzahl von 4.2 Knochentumoren/Maus mit einer mittleren Induktionszeit von $194,1 \pm 2,6$ Tagen verglichen mit 2,3 bei denen, die nur ^{90}Sr erhalten hatten. Die mittlere Induktionszeit für letztere betrug $316,1 \pm 1,1$ Tage. Die mit ^{90}Sr+Östrogen behandelten Weibchen entwickelten 4,1 Knochentumoren/Maus und die nur mit ^{90}Sr behandelten Weibchen 2,0. Die Induktionszeiten betrugen $251,0 \pm 4,1$ bzw. $379,3 + 9,7$ Tage. Bei der Gruppe von Mäusen, die nur mit Östrogen behandelt worden war, fanden sich keine Knochentumoren. Osteoklastische und gemischte Osteosarcome waren bei den kombiniert behandelten Gruppen im Gegensatz zu den nur mit ^{90}Sr behandelten Gruppen häufig.

RÉSUMÉ

Des groupes de souris CBA mâles et femelles ont été traités par l'hormone oestrogénique seule, par le ^{90}Sr seul ou par l'association oestrogène+^{90}Sr. Parmi les mâles qui avaient reçu les oestrogènes et le ^{90}Sr, il y avait un nombre moyen de 4,2 tumeurs osseuses par souris avec un temps moyen d'induction de $194,1 \pm 2,6$ jours, comparé à 2,3 tumeurs par souris pour

celles qui avaient reçu seulement le ^{90}Sr. Le temps moyen d'induction pour ce dernier groupe était de $316{,}1 \pm 1{,}1$ jours. Les souris femelles traitées par ^{90}Sr+oestrogène ont eu 4,1 tumeurs osseuses par souris et celles qui n'avaient reçu que ^{90}Sr ont eu 2,0 tumeurs par souris. Les temps d'induction étaient respectivement de $251{,}0 \pm 4{,}1$ et $379{,}3 \pm 9{,}7$ jours. Les souris traitées seulement par l'oestrogène n'ont pas présenté de tumeur osseuse. Les ostéosarcomes ostéoclastiques et les ostéosarcomes mixtes ont été fréquents dans les groupes traités par l'association oestrogène $+^{90}Sr$, à la différence des résultats donnés par le ^{90}Sr seul.

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