Acta Oncologica 28 (1989) Fasc. 1

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RADIOSTRONTIUM-INDUCED ONCOGENESIS AND THE ROLE OF IMMUNOSUPPRESSION

I. Influence of ^{90}Sr dose, adult thymectomy and antilymphocyteglobulin treatment on the development of neoplastic and preneoplastic lesions in the skeleton of CBA mice

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Abstract

Ionizing irradiation by incorporated strontium-90 exerts two major effects: it induces tumours (mainly osteosarcomas and lymphoreticular tumours) and depresses the immune system. The interrelation between these functions, i.e. the significance of decreased immunological responsiveness in the oncogenic process, remains unclear. The influence of the ^{90}Sr dose and the role of immune modulation on the tumour yield, were investigated in young adult CBA mice. The animals were exposed to different single doses of ^{90}Sr and, in addition, some groups were subjected to long-term unspecific immune suppression by adult thymectomy (ATx) and/or prolonged antilymphocyteglobulin (ALG) treatment. The present paper (part I) reports on the effects of the treatments on bone tumour responses as reflected by incidence, multiplicity, latency time, histologic characteristics and growth behaviour. The histogenesis of osteosarcomas, as evidenced morphologically by preneoplastic and early neoplastic growth, is illustrated and discussed. The results demonstrate a positive dose–response relationship for osteosarcomas, in which the relative incidences of the various osteosarcoma subtypes were differentially affected. Thus, well-differentiated tumours were gradually replaced by less differentiated types as the dose decreased. A correlation was also observed between the incidence of osteosarcomas and that of assumed preneoplastic lesions in the same bones and sites. Immune suppression by ATx and/or ALG did not distinctly alter the neoplastic or preneoplastic responses at any dose-level of ^{90}Sr.

Key words: Carcinogenesis; ^{90}Sr, immunosuppression, mice, osteosarcoma, preneoplasia.

A multiple-step process for the pathogenesis of neoplasia has become a basic assumption in cancer research, including radiation carcinogenesis (1, 2). The present study was initiated to improve our understanding of the oncogenic process induced by incorporated strontium-90, with special reference to the role of the host's immunological responsiveness. ^{90}Sr is well-known as a potent carcinogen, inducing osteosarcomas and lympho-reticular tumours in many species; however, much less is known about its ability to impair the function of the immune system (3–6). The present study was designed primarily to determine whether protracted unspecific immunosuppressive treatment influences tumour development in ^{90}Sr-contaminated animals.

The observation that preimmunization of mice with syngeneic ^{90}Sr-induced bone tumour cells prevents tumour formation upon subsequent challenge suggests that these tumours are antigenic and that anti-tumour immunity is protective (7, 8). Moreover, unspecific stimulation of the immune system by BCG has been shown to decrease the incidence and to delay the appearance of ^{90}Sr-induced tumours (9, 10). If the immune system identifies and eradicates early malignant cells (11, 12), it can be assumed that depression of the immune system, e.g. by irradiation, would render animals more susceptible to manifest malignant growth.

Bone tumour induction by exposure to ^{90}Sr has been investigated in mice with regard to several parameters, such as dose and tumor incidence, histologic types, latency time, growth behaviour and anatomical predilection

Accepted for publication 14 April 1988.

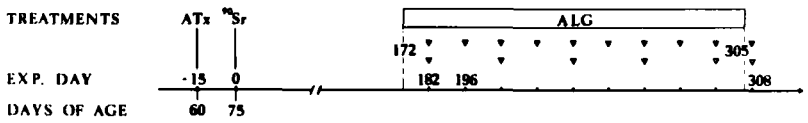


Fig. 1. Scheduling of experimental procedures. Adult thymectomy (ATx). Exposure to radiostrontium by intraperitoneal injection (^{90}Sr). Weekly intraperitoneal injections of antilymphocyte-globulin (ALG) days 172 to 305 after ^{90}Sr. Arrow heads indicate time points for monitoring immune competence (not reported in this paper) and analysis of the neoplastic responses of B and C mice in series 1 (▼) and series 2 (▽).

(13–20). Also the influence of endogenous biological factors, like sex, age and hormones (21–24) and viral interaction (25–28) has been studied. Little attention, however, has been paid to the role of immunodepression by ^{90}Sr, and few studies have concerned the effect of additional long-term impairment of the immune system. A permissive role of the immune system during different phases of tumour development and spread has been continuously debated during the recent decade, but still remains to be critically examined, particularly concerning long-term immunosuppression. In the present investigation the tumour yield was studied in mice exposed to different single doses of ^{90}Sr and simultaneously subjected or not to severe unspecific immunosuppression by adult thymectomy (ATx) and/or long-term treatment with antilymphocyte-globulin (ALG).

The present paper is restricted to preneoplastic and neoplastic lesions in bone and associated hard tissues, and the data are discussed in relation to treatments employed and immunosuppressive effects obtained.

A later paper (Bierke & Nilsson, in preparation) will deal with lympho-reticular tumours and extraskeletal neoplastic lesions and will also include a summarizing discussion of the experiments.

Material and Methods

Animals and experimental procedures. Inbred male CBA/SU mice, supplied from the Department of Genetics, Stockholm University, were used (Origin: CBA/Carter to CBA/Harwell to CBA/Stockholm University, 1956). The animals were divided into 4 main series and exposed to different doses of ^{90}Sr (range: 1.85–29.6 kBq/g body weight) by intraperitoneal injection at the age of 75±3 days. Each dose series was subdivided into groups subjected to ATx and/or ALG treatment in combinations as depicted in Table 1. The treatment schedule is illustrated in Fig. 1. In dose series 2 the animals were of specific pathogen-free (SPF) quality, while they derived from a conventional disease-free stock in the other dose series. Mice in experimental series A (see Table 1) were allowed to live out their full life-span or killed when moribund. They were then used for determinations of incidence, multiplicity, latency, histologic type, location and metastases of induced tumours. Mice in the parallel running experimental series B and C were used when screening for preneoplastic and early neoplastic bone lesions during the ALG treatment period (i.e. bone tumour latency or early expectancy time). However, they were primarily intended for use in a survey of immune competence, the results of which will be reported elsewhere (see Fig. 1).

Isolator-maintained SPF mice—Series 2. The CBA mouse strain was made SPF by hysterectomy and cross-fostering at Anticimex Breeding Laboratories (new name: A-LAB). Males were raised SPF and maintained in this state by isolation in high pressure, plastic film animal-isolators (Metall + Plastic) and through sterile technique management. Thereby these animals were not influenced by opportunistic infections. The mice were kept in Macrolon II cages with wood shavings as bedding and free access to sterilized tap water and an autoclaved pelleted diet, R-3 Feed for Rats and Mice, Astra-Ewos (after sterilization it corresponds in nutritional content to Standard Feed for Rats and Mice).

Conventionally maintained mice—Series 1, 3 and 4. The mice were housed in conventional animal rooms in Macrolon III cages with wood shavings as bedding. They were given free access to a commercial pelleted diet (Standard Feed for Rats and Mice, Astra-Ewos) and water.

Air temperature in the animal rooms was 22–24°C, relative humidity 50–65%, and the light–dark cycle 12/12 h (7 p.m.–7 a.m.).

Owing to the great number of animals and procedures, deliveries were made and the experiments started on six consecutive occasions. On arrival, the mice were randomly assigned to the predetermined treatments and marked individually by ear clipping. A complete set of A-coded mice was always maintained together with a complete set of similarly treated B or C coded mice in the same cage. Thus the influence of possible unknown factors related to the individual cage milieu was kept equal. An exception was made for groups (I) and VI, which were kept separately in order to exclude the possibility of secondary ^{90}Sr-contamination.

Surgery. Thymectomy was performed at 60±3 days of age, as described elsewhere (4).

Radionuclide. Carrier-free strontium-90 nitrate in 1-N nitric acid (supplied by Amersham International, Amersham, England) was diluted in saline to activities ranging between 203 and 3 108 kBq per ml to allow intraperitoneal (i.p) administration of 1.85, 7.4, 14.8 or 29.6 kBq ^{90}Sr per g

Table 1

Experimental protocol. (A) Animals for analysis of tumour yield and survival time. (B) Animals for analysis of mitogen responsiveness and WBC counts (not reported in this paper) and preneoplastic or early neoplastic responses, days 182–308. (C) Animals for analysis of RES phagocytic function (not reported in this paper), and preneoplastic or early neoplastic responses, days 182–308. ATx = adult thymectomy, ALG = antilymphocyteglobulin

^{90}Sr dose series	Group code	Treatments		Exp. series A	B	C
				No. of mice		
1. 29.6 kBq (0.8 μCi)/ g body weight	(I)	Untreated control		100	50	50
	I	Untreated cagemate control		100	50	50
	II	^{90}Sr	Caged	100	50	50
	III	^{90}Sr+ATx	together [a]	100	50	50
	IV	^{90}Sr+ATx+ALG		100	50	50
	V	^{90}Sr+ALG		100	50	50
	VI	ALG		100		
2. 14.8 kBq (0.4 μCi)/ g body weight	I	Untreated control		40	12	12
	II	^{90}Sr		40	12	12
	IV	^{90}Sr+ATx+ALG	Isolator maintained [b]	40	12	12
	VII	ATx+ALG		40	12	12
3. 7.4 kBq (0.2 μCi)/ g body weight	I	Untreated control [c]		30		
	II	^{90}Sr		50		
	III	^{90}Sr+ATx		50		
	IV	^{90}Sr+ATx+ALG		50		
4. 1.85 kBq (0.05 μCi)/ g body weight	I	Untreated control [c]				
	II	^{90}Sr		50		
	III	^{90}Sr+ATx		50		
	IV	^{90}+ATx+ALG		50		

[a] The broken line enframes animals caged together in numbers of 10, with 2 representatives of each treatment (I–V) randomly precoded A or B, alternatively A or C. This arrangement was aimed to ensure equality with regard to extraneous influences.

[b] To minimize the risk of infection by normally harmless microbes as a consequence of immunosuppressive treatment, the animals of series 2 were raised SPF and housed in plastic-film isolators under sterile technique management.

[c] This group served as control group in dose series 3 and 4.

body weight in volumes of 200 to 300 μl, depending on the series.

ALG. Freeze-dried, horse-raised, anti-mouse lymphocyteglobulin (obtained from TNO, Rijswijk, the Netherlands) was dissolved in saline to reach a concentration of 30 mg/ml. The ALG treatment was started 172 days after exposure to ^{90}Sr, i.e. prior to bone tumour expectancy. The mice in series 1, 3 and 4 were given 6 mg ALG as a weekly injection intraperitoneally for 8 weeks, followed by 3 mg ALG per week for 12 more weeks. The dose was reduced after the occurrence of some unexpected deaths following the injection. The isolator-maintained mice in series 2 were given 6 mg ALG per week i.p. during the corresponding 20-week period.

Autopsy procedures and histopathology. All mice, spontaneously dead or killed by an overdose of ether anesthesia, were submitted to dorso-ventral radiography before autopsy in order to detect and localize intraosseous or discrete hard tissue lesions. For this purpose we used a Type AEG-50 x-ray Tube. The carcasses and selected organs were routinely weighed. Tissues histologically examined were: lung, heart, thymus, liver, spleen, adrenal gland, kidney, testis, lymph nodes (axillary, brachial, inguino-femoral and mesenteric), small intestine with Peyer's patch, skull, vertebrae (cervical, thoracic, lumbar and sacral), sternum, ilium, ischium, femura, tibiae, humeri and radii. Any gross abnormalities or lesions indicated on the radiograms were also sampled. The tissues were fixed in Stieve's fluid, and hard tissues decalcified in 20% formic acid before trimming and embedding in paraplast. Conventional histologic methods were used, and the sections were stained with haematoxylin and eosin (HE), by the van Gieson's method and with special stains when required.

Table 2

Crude bone tumour incidences and survival times of CBA mice subjected to adult thymectomy (ATx) and treated with ^{90}Sr and antilymphocyteglobulin (ALG), as explained in the text and in Table 1. OS = osteosarcoma/s, Obl = osteoblastic, Fbl = fibroblastic

^{90}Sr dose series	Group	Treatment	No. of mice examined (%)	No. of mice with OS (%)	No. of mice Obl-OS (%)	No. of mice Fbl-OS (%)	No. of mice other OS
1	A(I)	Control	98	1 (1)	1 (1)	0	0
	A I	Control, cagemate	100	1 (1)	1 (1)	0	0
	A II	^{90}Sr	99	92 (93)	85 (86)	45 (45)	7 (7)
	A III	^{90}SrATx	100	96 (96)	91 (91)	45 (45)	11 (11)
	A IV	^{90}SrATxALG	99	92 (93)	85 (86)	50 (50)	10 (10)
	A V	^{90}SrALG	97	89 (92)	79 (81)	37 (38)	9 (9)
	A VI	ALG	100	0	0	0	0
2	A I	Control	37	0	0	0	0
	A II	^{90}Sr	39	30 (77)	15 (38)	22 (56)	6 (15)
	A IV	^{90}SrATxALG	40	24 (60)	13 (32)	16 (40)	7 (18)
	A VII	ATxALG	40	0	0	0	0
3	A I	Control	30	0	0	0	0
	A II	^{90}Sr	50	12 (24)	3 (6)	8 (16)	3 (6)
	A III	^{90}SrATx	50	12 (24)	2 (4)	8 (16)	2 (4)
	A IV	^{90}SrATxALG	50	8 (16)	4 (8)	3 (6)	2 (4)
4	A I	Control	30	0	0	0	0
	A II	^{90}Sr	49	3 (6)	1 (2)	2 (4)	0
	A III	^{90}SrATx	49	0	0	0	0
	A IV	^{90}SrATxALG	50	0	0	0	0

[a] ±Standard error. [b] Mean number irrespective of type.

The neoplastic lesions were histologically classified in accordance with the system adopted by EULEP, the European Late Effects Project Group (29). A neoplastic clone in bone tissue, i.e. an early neoplastic lesion developing along the endosteum or in the bone marrow, is here defined as an initial group of preosteoblast-like or fusiform to reticular mesenchymal type cells with phenotypic signs of malignancy. The term microtumour is congruent with intra-osseous bone tumour, and the term macrotumour is used when a bone tumour clearly extends outside the limits of the normal bone contour.

Statistics. The bone tumour responses are expressed as crude tumour incidences in tables and figures, and analyzed for significance by Students's t-test.

Actuarial tumour appearance, implying consideration of competing causes of death, was calculated in accordance with a method and formula reported by Kellerer & Chmelevsky (30) and discussed by Gimeno et al. (31).

Results

Changes in the skeleton predominated among the postmortem observations and included regressive, regenerative and neoplastic lesions in bone and haematopoietic tissues. The results presented here are restricted to tumours in bone and associated tissues.

Animal survival. Mice injected with ^{90}Sr died sooner than untreated control mice and the survival rate was correlated with the activity administrated. Concomitant treatment with ATx or ALG or both did not markedly influence survival time in any dose-series (Fig. 2). The mean survival rates for all mice and of mice developing osteosarcoma for each group are given in Table 2. The survival time for mice with osteosarcoma characteristically exceeded that for the entire treatment group which included mice which developed other forms of radiation-induced diseases.

Radiography. Most bone tumours were readily detected on x-ray film. Lesions in very early stages of development were commonly localized by discrete deviation in the density of the diseased bone or bone marrow, or lysis of the bone tissue.

Osteosarcoma rate and multiplicity. Almost all bone tumours appearing after exposure to ^{90}Sr were osteosarcomas. The cumulative incidences of mice with tumour(s) at end of their life-span are illustrated in relation to time after ^{90}Sr-exposure in Fig. 3. The tumour responses are further characterized by treatment group in Table 2 as the crude number of mice with osteosarcoma(s), or osteosarcoma(s) of a particular type. The total numbers of osteosarcomas and of osteoblastic and fibroblastic subtypes are tabulated, as are the mean numbers of osteosarcomas per mouse and of osteosarcomas per mouse with tumour.

Table 2 cont.

Total No. of OS	Obl-OS of total (rel%)	Fbl-OS of total (rel%)	All mice		Mice with osteosarcoma	
			Mean survival (days)[a]	No. of OS per mouse[b]	Mean survival(days)[a]	No. of OS per mouse[b]
1	(100)	(0)	694±14	0.01	764	1.00
2	(100)	(0)	662±14	0.02	696	2.00
229	(77)	(20)	270±4	3.02	274±4	3.25
318	(76)	(21)	277±4	3.18	281±4	3.31
297	(70)	(27)	280±5	3.00	288±5	3.23
262	(77)	(20)	271±7	2.70	274±3	2.94
0	–	–	696±16	0	–	–
0	–	–	744±20	0	–	–
71	(28)	(63)	370±16	1.82	413±10	2.37
61	(29)	(54)	356±18	1.52	434±8	2.54
0	–	–	697±34	0	–	–
0	–	–	715±23	0	–	–
15	(27)	(53)	490±24	0.30	546±26	1.25
15	(13)	(73)	475±24	0.30	587±21	1.25
9	(44)	(33)	511±25	0.18	659±31	1.12
0	–	–	715±23	0	–	–
3	(33)	(67)	617±23	0.06	760±126	1.00
0	–	–	605±23	0	–	–
0	–	–	643±17	0	–	–

With regard to the multiple occurrence of tumours or pre-tumour lesions, and for comparison, the data are also expressed per 100 individuals (Figs 4 and 5). The dose dependency of osteosarcoma incidence and tumour multiplicity and their interrelationship are presented in Figs 5a and c. Further immune suppression did not influence this pattern, nor did it influence tumour growth, as judged by the size distribution of tumours (Fig. 5b). The relative incidences of mice with multiple tumours are further illustrated by histograms (Fig. 6).

Actuarial osteosarcoma appearance. Corrections were made taking competing causes of death into account to improve the basis for comparison of effects between groups. The actuarial appearance represents the probability of an animal developing osteosarcoma after adjustment for intercurrent deaths, here based on calculation up to the level of 10 surviving mice (31). As seen in Table 3, the differences in crude tumour responses within the respective dose series decreased when the actuarial method was applied.

Osteosarcoma subtypes. A variety of osteosarcomas with different histologic features developed in each of the ^{90}Sr dose series. Among the radiation exposed A-coded mice a total of 1350 osteosarcomas were observed. The relative contributions of the various subtypes were: osteoblastic 69%; fibroblastic 27%; anaplastic/pleomorphic 2.3%; mixed 1.4%; osteoclastic, eburnizing, chondroblastic and epithelioid taken together 0.7%. The ^{90}Sr dose influenced the relative occurrence of subtypes while the excessive immunosuppressive treatments did not. Thus well-differentiated osteoblastic osteosarcomas were by far the most common tumours at the highest dose level but their representation decreased drastically in favour of fibroblastic and other less differentiated subtypes at lower doses (Table 2, Figs 4, 5a and c). Conversely, the relative contribution of fibroblastic tumours, particularly those with a telangiectatic pattern, declined markedly at the highest dose levels of ^{90}Sr.

Osteosarcoma sites. The distribution pattern of induced bone tumours was very similar for each dose level, and deviations were mainly restricted to relative incidence. Distribution and frequency were not notably influenced by the immunosuppressive treatments employed (see Table 2). Thus at the highest dose level (Series 1) an average of 48% of the tumours occurred in the long bones, especially in the distal femur, 38% in the vertebral column, especially in the lumbar vertebrae, 9% in the skull, especially in the jaws, and 5% in the pelvic bones. In dose series 2 and 3 the corresponding percentages were 71; 22; 2; 3 and 47; 33; 2; 18 respectively. In dose series 4 the 3 osteosarcomas observed were all sited in the vertebral column.

Osteosarcoma metastases. Bone tumour metastasis was observed in 3% of the mice dying with osteosarcoma (15/460 mice, all groups pooled) and were seeded in lung (11/15), liver (2/15) or kidney (2/15). No more than one

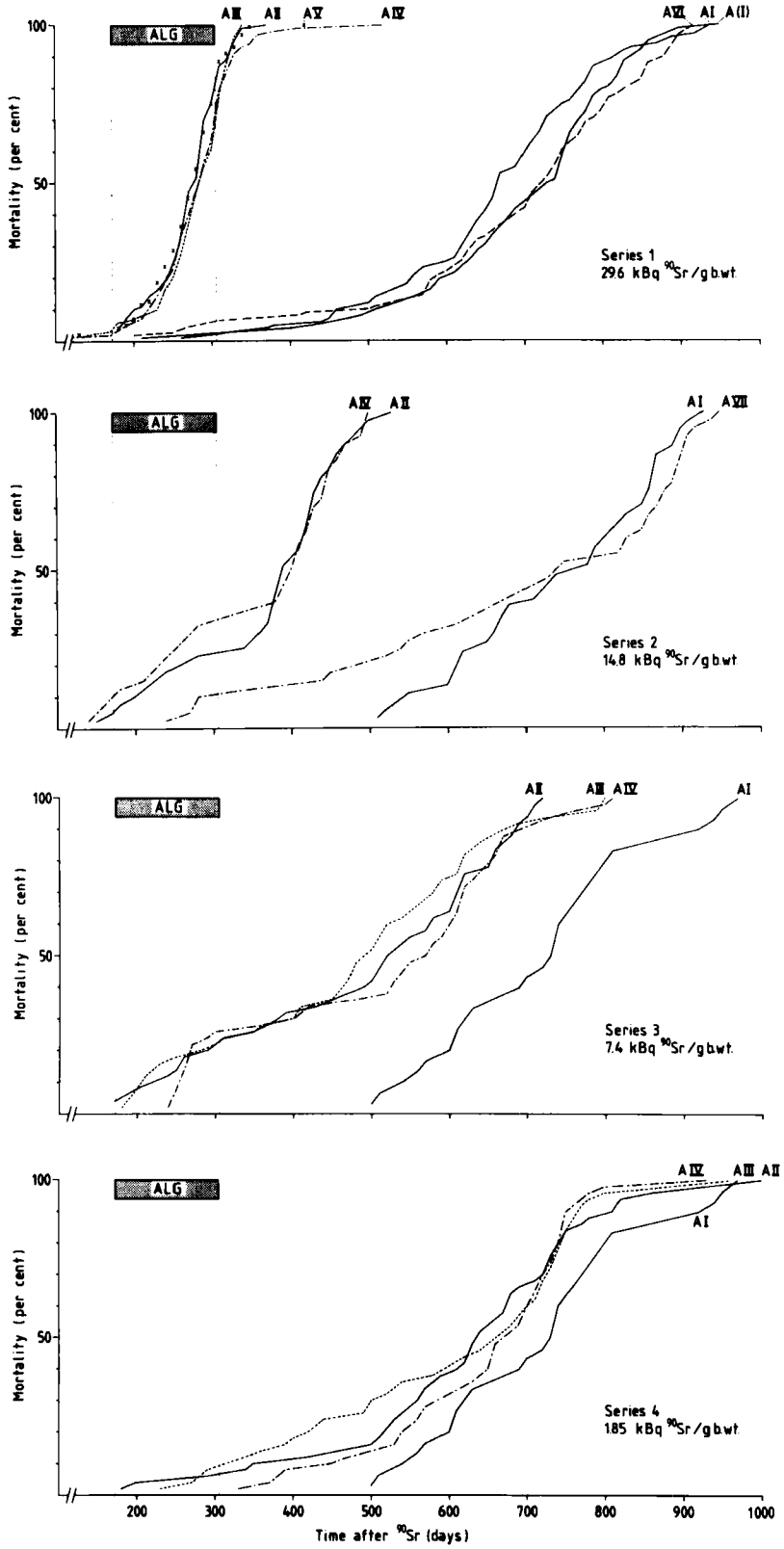


Fig. 2. Mortality (%) in relation to time after ^{90}Sr injection, registered at 10-day intervals. ALG = weekly injection of anti-lymphocyteglobulin, days 172–305. Group codes are explained in Table 1 and in the text to Fig. 3. For the sake of clarity the A III curve in series 1 is merely indicated by small cross symbols.

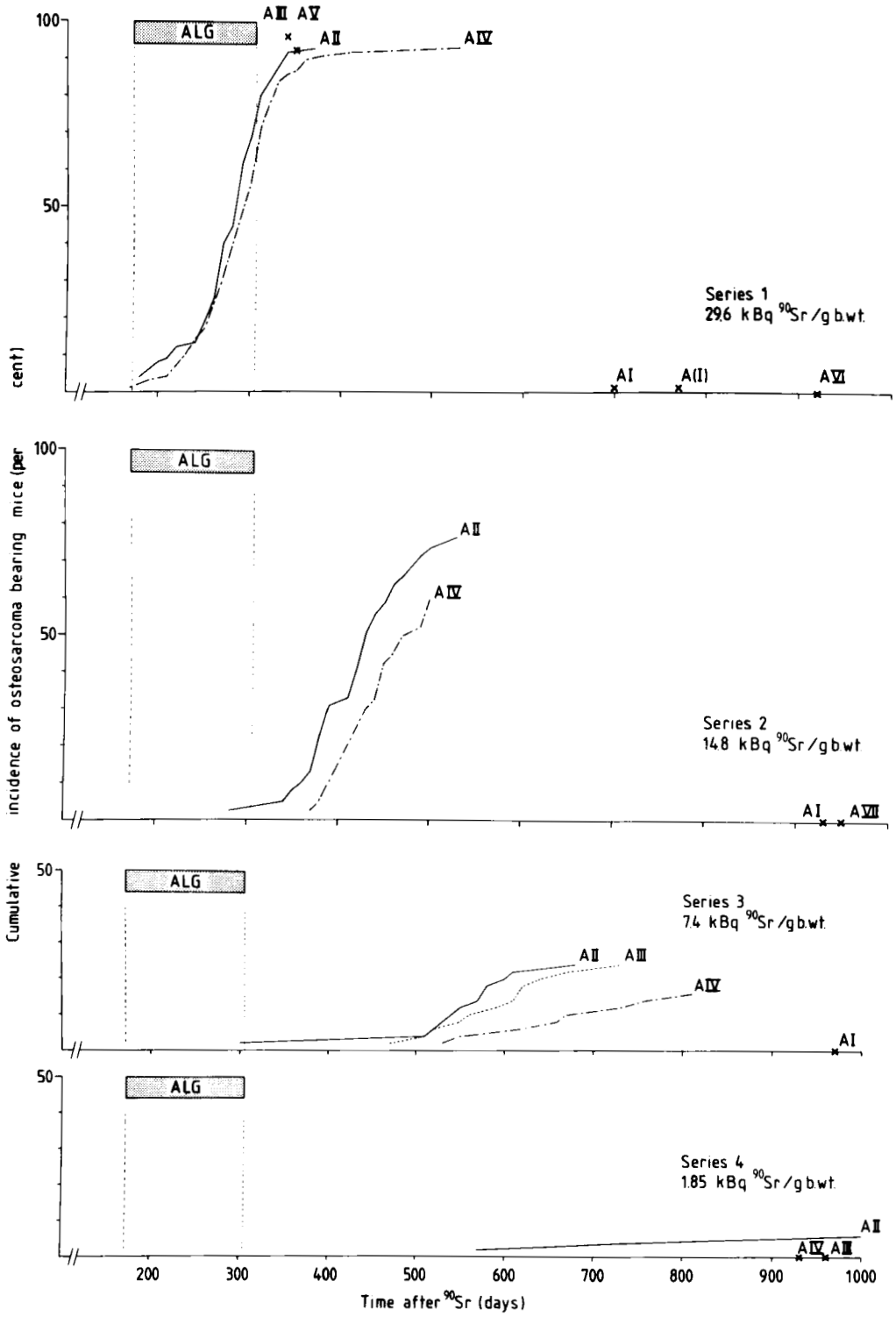


Fig. 3. Cumulative incidence of osteosarcoma-bearing mice (%) registered at 10-day intervals. ALG = weekly injection of anti-lymphocyteglobulin, days 172–305. A (I) = untreated control, A I = untreated cagemate control, A II = ^{90}Sr, A III = ^{90}Sr+ATx, A IV = ^{90}Sr+ATx+ALG, A V = ^{90}Sr+ALG, A VI = ALG, A VII = ATx+ALG. Crosses (×) indicate terminal points of omitted curves.

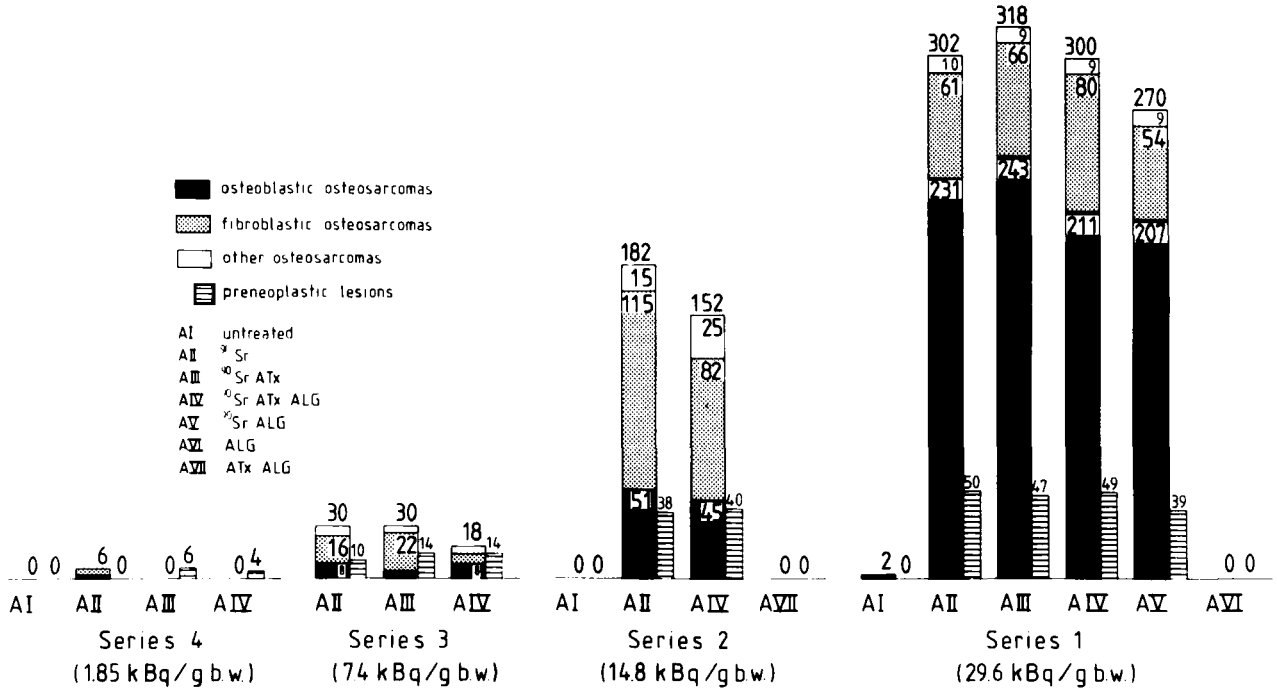


Fig. 4. Histogram showing the total number of osteosarcomas and preneoplastic lesions in the skeleton of A-coded mice (expressed per 100 animals) after different single injections of ^{90}Sr with or without simultaneous immunosuppression by ATx and/or ALG. Treatments and number of mice examined per group (AI–AVII) are given in Tables 1 and 2. Black portion of bars represents osteoblastic osteosarcomas, grey portion fibroblastic osteosarcomas and white portion other osteosarcoma subtypes (pooled). Striped bars represent preneoplastic lesions.

Table 3

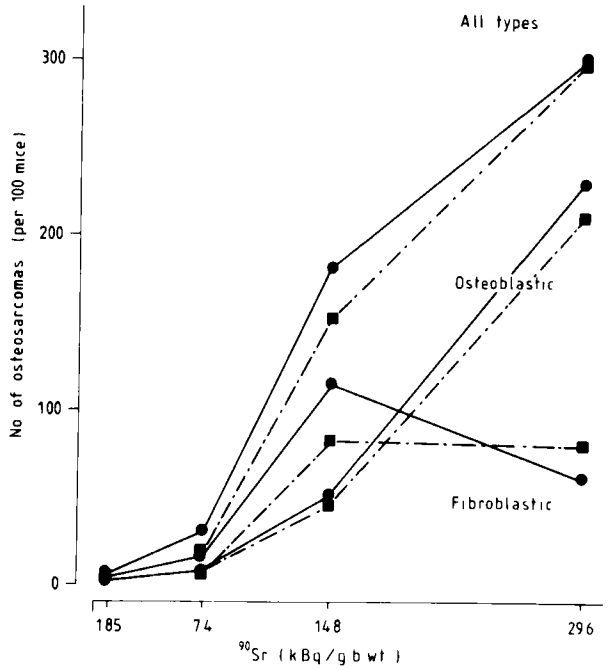
Comparison between crude incidence of mice with osteosarcoma and corresponding actuarial appearance ± standard error, i.e. probability of an animal developing a tumour

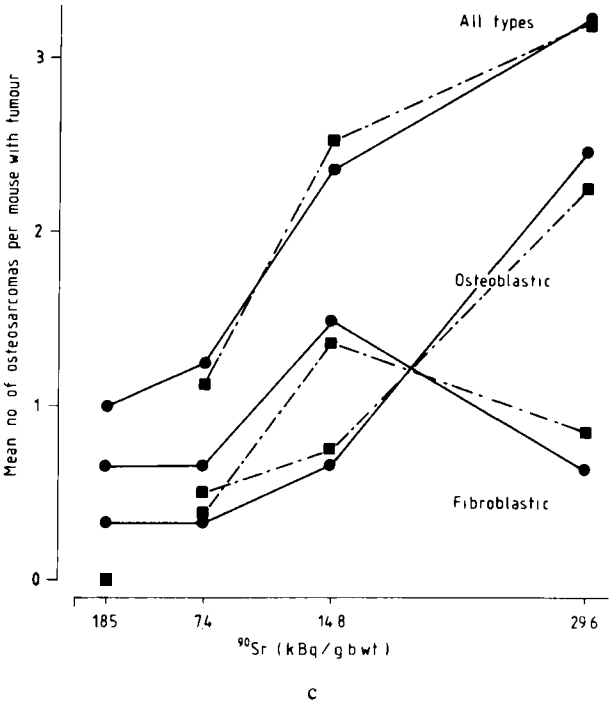
Series	Group	Crude %		Actuarial appearance	Mean time until actuarial appearance of tumours
1	AII	93[a]	83[b]	0.901±0.029[c]	275±5 days
	AIII	96	86	0.906±0.028	
	AIV	93	83	0.901±0.029	287±5 days
	AV	92	82	0.897±0.031	
2	AII	77	51	0.692±0.079	414±10 days
	AIV	60	35	0.696±0.086	436±8 days
3	AII	24	22	0.386±0.089	567±27 days
	AIII	24	16	0.381±0.106	
	AIV	16	8	0.191±0.090	732±30 days
4	AII	3	2	0.104±0.074	961±70 days
	AIV	0	0	0	>926 days
Control	AI	2	2	0.021±0.015	738±33 days

[a] Incidence when all mice had lived their full life-span.
[b] Incidence when 10 mice were still alive.
[c] Actuarial incidence based on tumour appearance up to the level of 10 surviving mice.

target organ was ever affected by metastatic growth. Notably, the spleen was never involved. The histologic types of the primary tumours were anaplastic/pleomorphic (7/15), osteoblastic (4/15), fibroblastic (3/15) or mixed (1/15). No relation between treatment and dissemination was observed.

Osteosarcoma pathogenesis as reflected histologically. The light microscopic screening of skeleton bones dis-





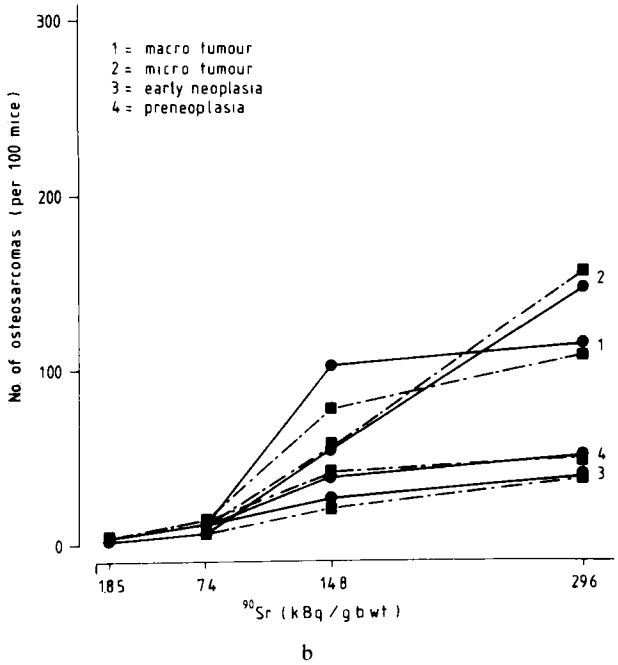


Fig. 5a, b and c. Bone tumour responses in A-coded mice exposed to ^{90}Sr at 4 dose-levels, with or without simultaneous ATx+ALG treatment, ^{90}Sr (●—●), ^{90}Sr+ATx+ALG (■---■). a) total number of osteosarcomas (all types) and the respective contribution of osteoblastic and fibroblastic subtypes, expressed per 100 animals. b) distribution of osteosarcomas (all types) according to size (macro-, micro-, or early neoplasia) and frequency of preneoplastic lesions, expressed per 100 animals and c) mean number of osteosarcomas in osteosarcoma-bearing mice. The number of animals investigated at each point is given in Table 2.

played osteosarcomas at virtually every stage of development, i.e. dysplasia and cell atypia (preneoplasia); clones of cells with malignant appearance (early neoplasia); malignant growth confined to bone or to the marrow cavity (microtumour); and overt tumour growth (macrotumour). Incidences in relation to treatments are shown in Fig. 5b.

The preneoplastic lesions were generally formed by proliferation and swelling of cells belonging to the stromal framework of the bone marrow, thus giving the image of a cell-rich meshwork with minor condensed areas and single aberrant nuclei (Figs 10, 11). In other cases proliferation of morphologically heterogeneous osteoblast- or preosteoblast-like cells along existing bone surfaces in the marrow cavity dominated the picture (Fig. 8). Concomitant radiogenic depletion of haematopoietic cells regularly made these proliferative reactions conspicuous. Characteristic spheroidal, blood-filled spaces of various size and continuous with disrupted marrow sinusoids were often

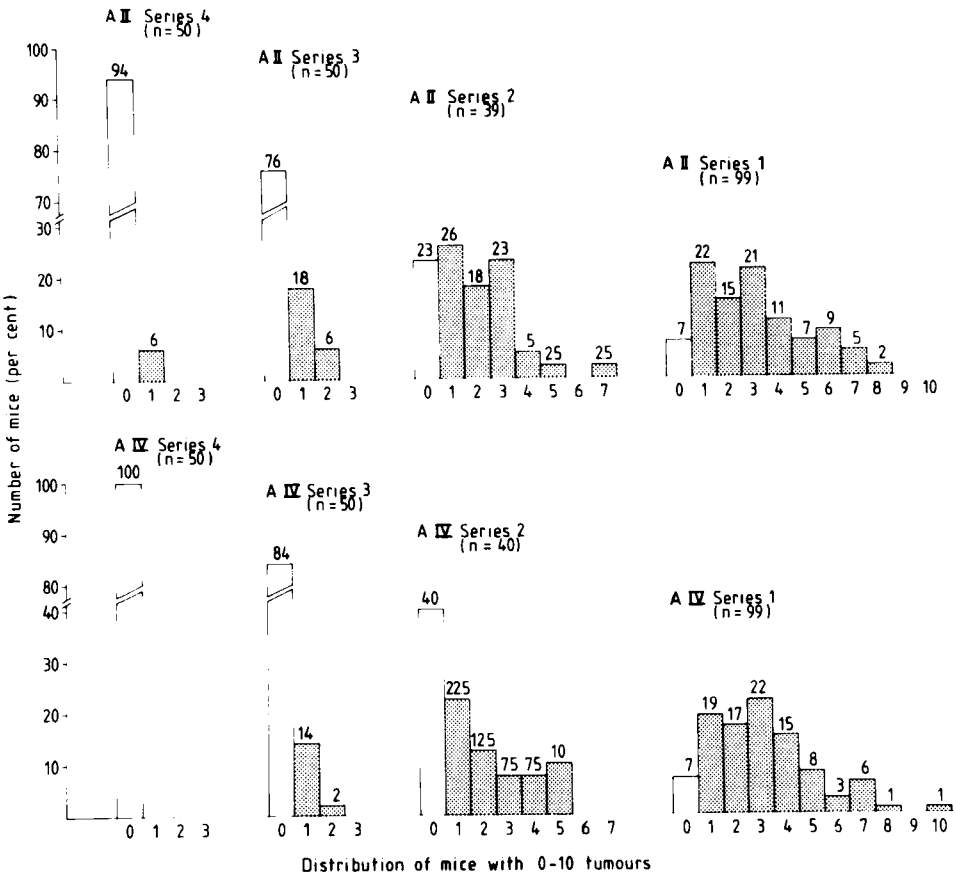


Fig. 6. Multiplicity of induced osteosarcomas after exposure to different single doses of ^{90}Sr with or without simultaneous ATx+ ALG treatment (groups A II and A IV in dose series 1, 2, 3, and 4). Bars represent the percentage of mice with indicated number of osteosarcomas (0–10).

concurrent with proliferative lesions, indicating previous radiogenic damage to the bone marrow (Fig. 13). Close correlations between preneoplastic proliferations and osteosarcomas, as regards frequency and anatomic location, were revealed in all dose series. Data from Series 1 are given in Table 4.

Early neoplastic lesions evidently arose frequently within preneoplastic areas (Figs 9, 12, 14). Those of 'stromal cell' origin emerged by focal proliferation of fusiform or, more rarely, osteoblast-like malignant cells. Already at this early stage of development they occasionally proved capable of forming primitive bone structures but generally did not express this ability before contacting nearby preformed bone. The other 'endosteal variant' of early neoplasia was characterized by proliferation of pleomorphic and heterochromatic osteoblast-like cells accumulating on endosteal surfaces or encompassing bone trabeculae and actively producing osteoid or immature bone.

The implication of these observations is that 'osteosarcoma candidate cells', i.e. bone progenitor cells, are to be found not only within the endosteal cell layer but also, and seemingly more often, within the reticular stroma of the bone marrow, particularly when engaged in regeneration and repair processes. In addition, tumour development occasionally seemed to have started within cortical bone or possibly, however less likely, in the periosteum.

Support for the concept of an evolution from preneoplasia to neoplasia in radiation carcinogenesis was given by the B and C mice autopsied from day 182 to 238. At this stage the preneoplastic lesions predominated over tumours in the tubular bones, i.e. an inverse proportional distribution compared to the situation in survivors (A-mice).

Other tumours in bone and associated tissues. Chondroma was observed in 5 non-irradiated old mice: 4 in distal femora and one in a mandibula. Osteoma was observed in one ^{90}Sr-exposed mouse and in 3 untreated controls. In contrast to the osteosarcomas, the osteoma in the irradiated mouse was sited in the flat bones of the skull and growing expansively into the cranial cavity. Ameloblastoma was observed in 2 irradiated mice. This finding is interesting since the osteosarcomas of the jaws regularly concerned—and presumably originated—in the periodontal membrane. Only 2 haemangiosarcomas were

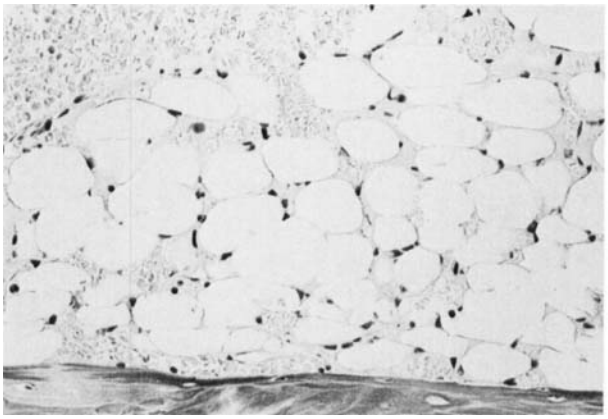


Fig. 7. Radiogenic bone marrow hypoplasia and disrupted sinusoids with haemorrhage (upper left). No signs of regeneration or repair. Van Gieson; ×280

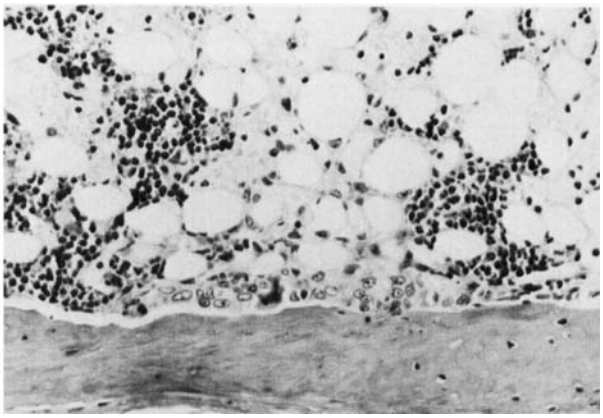


Fig. 8. Reoccurrence of haematopoietic cells, slight increase of stromal cells, and focal proliferation of immature endosteal cells (endosteal preneoplastic lesion). Van Gieson; ×280.

Table 4

Total number of osteosarcomas and preneoplastic bone marrow stromal cell proliferations and their respective distributions (%) in skeletal bones, of A-coded mice of series 1 (see Table 1). nd = not done because the anatomical conditions made microscopic screening doubtful

Group code	A (I)	A I	A II	A III	A IV	A V	A VI
Treatments	Control	Contr. cage-mate	^{90}Sr – –	^{90}Sr ATx –	^{90}Sr ATx ALG	^{90}Sr – ALG	– – ALG
No. of mice	98	100	99	100	99	97	100
Total No. of osteosarcomas and preneopl. lesions	1; 0	2; 0	299; 50	318; 47	297; 49	262; 38	0; 0
Percentage in							
Skull							
Upper jaw	0; nd	0; nd	5.3; nd	6.3; nd	5.0; nd	5.7; nd	0; nd
Lower jaw	0; nd	0; nd	2.7; nd	2.8; nd	2.3; nd	1.9; nd	0; nd
Other bones[a]	0; nd	50; nd	1.3; nd	1.3; nd	1.7; nd	0.4; nd	0; nd
Vertebrae							
Cervical[a]	0; 0	0; 0	0.3; 2.0	0.3; 2.1	1.0; 0	1.1; 0	0; 0
Thoracal	0; 0	50; 0	10.3; 36.0	10.4; 44.7	6.4; 36.7	6.5; 32.4	0; 0
Lumbar	0; 0	0; 0	23.9; 44.0	23.3; 36.2	18.4; 38.8	25.2; 27.0	0; 0
Sacral	0; 0	0; 0	5.0; 0	3.8; 2.1	5.4; 2.0	8.4; 5.4	0; 0
Coccygeal[a]	0; 0	0; 0	0.3; 0	0.6; 0	0.3; 0	0.4; 0	0; 0
Sternum	0; 0	0; 0	0.7; 0	0; 0	0; 0	0; 0	0; 0
Scapulae[a]	0; nd	0; nd	0; nd	0; nd	0; nd	0.4; nd	0; nd
Humeri[a]	0; 0	0; 0	6.3; 4.0	5.0; 0	6.4; 4.1	7.3; 2.7	0; 0
Radii/ulnae[a]	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0
Pelvic bones[a]	0; nd	0; nd	5.6; nd	6.0; nd	5.7; nd	4.6; nd	0; nd
Femora	100; 0	0; 0	28.6; 14.0	30.8; 12.8	34.8; 18.4	29.0; 27.0	0; 0
Tibiae[a]	0; 0	0; 0	9.6; 0	10.1; 2.1	12.7; 0	9.2; 5.4	0; 0
Phalanges, ribs[a]	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0

[a] The data represent minimum values since microscopy was restricted to situations when radiography indicated or suggested a lesion in bone or bone marrow.

diagnosed in bone and bone marrow, but fibroblastic osteosarcomas, with different degrees of telangiectatic features, were common and often reminiscent of angiosarcomas. Lympho-reticular tumours, especially malignant lymphomas, predominated among 'other tumours in bone' and were fairly evenly distributed within each dose series.

Summary of observed bone tumour responses

Untreated controls developed osteosarcoma in 2 cases (≤1% per group), both of which occurred in old individuals.

Mice treated only with ATx/ALG did not develop osteosarcoma.

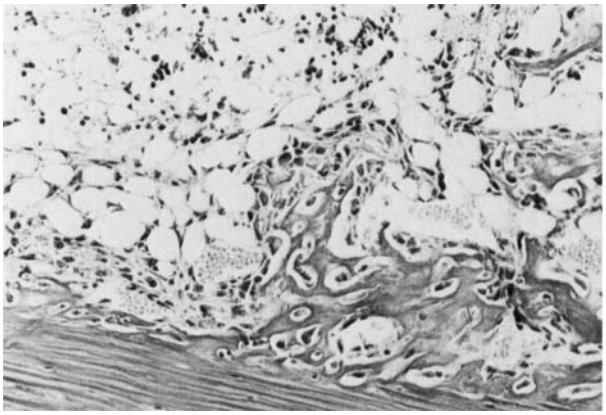


Fig. 9. Early neoplastic lesion of endosteal origin consisting of bone-forming cells of osteoblastic appearance. Van Gieson; ×210.

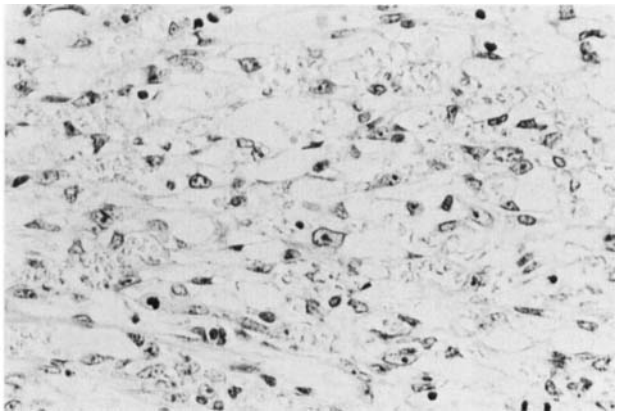


Fig. 10. Bone marrow devoid of haematopoietic cells showing reactive proliferation and swelling of stromal cells, some of which display atypical nuclei. Van Gieson; ×450.

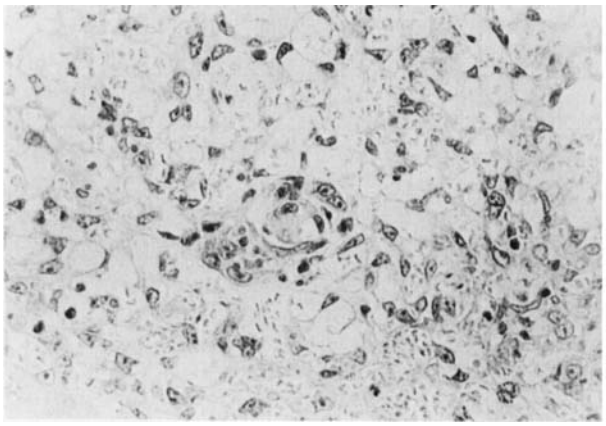


Fig. 11. Same as Fig. 10 plus area of stromal cell condensation (stromal preneoplastic lesion). Van Gieson; ×450.

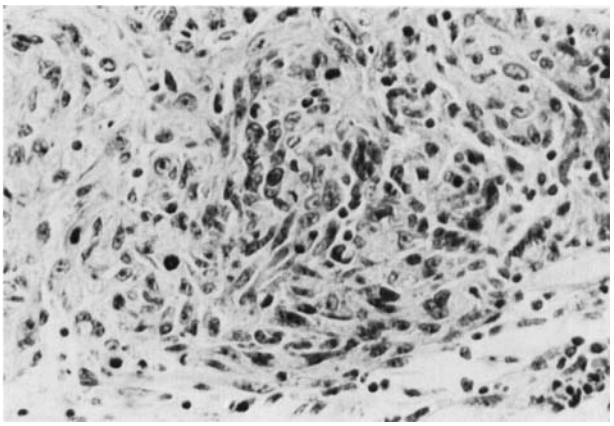


Fig. 12. Early neoplastic lesion in bone marrow consisting of undifferentiated, plump and elongated cells. Van Gieson; ×450.

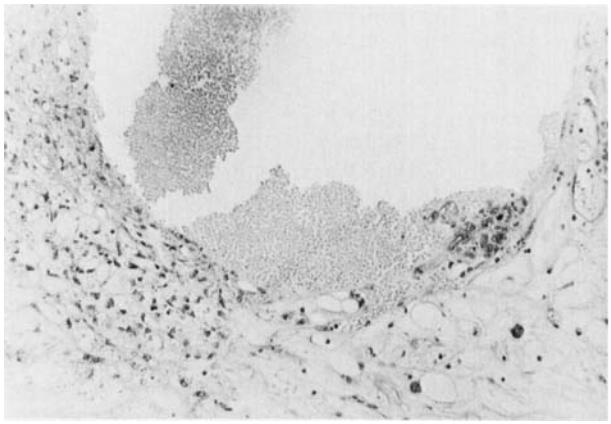


Fig. 13. Bone marrow showing stromal regeneration and repair plus blood lake with intruding cluster of pleomorphic cells with rounded nuclei and abundant cytoplasma. Van Gieson; ×160.

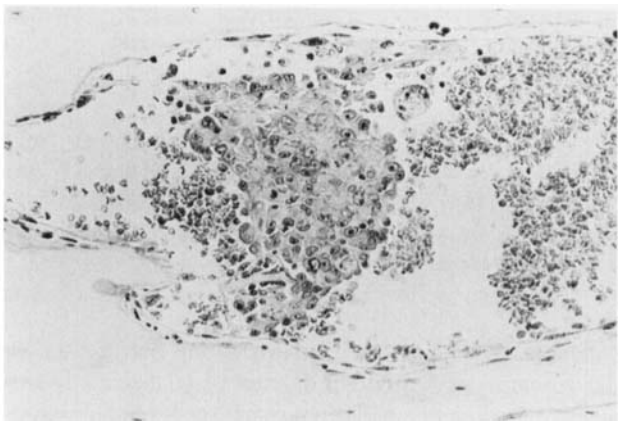


Fig. 14. Early neoplastic lesion of epithelioid appearance in bone marrow cavity. H. and E.; ×280.

In ^{90}Sr-exposed groups the bulk of the osteosarcomas appeared after 180–370 days (Series 1), 340–500 days (Series 2), 510–680 days (Series 3) and 560–990 days (Series 4) respectively, depending on dose.

Although the majority of osteosarcomas were sited in the femur, tibia or humerus and in the thoracic-, lumbar- or sacral vertebrae, they were also common in jaws and pelvic bones.

Osteosarcoma development was characteristically preceded by reactive proliferation of atypic bone marrow stromal cells (preneoplasia).

The incidence of preneoplastic bone lesions and osteosarcomas increased while latency times decreased in response to an increase in the ^{90}Sr dose.

The multiplicity of osteosarcomas, as expressed by the mean number of tumours per tumour-bearing mouse, increased with the ^{90}Sr dose. At the extreme, 10 tumours, each in a different bone, occurred in one mouse.

The proportional representation of each of the various osteosarcoma subtypes, and the relative proportion of mice bearing each subtype depended greatly on the ^{90}Sr dose. Osteoblastic osteosarcomas were the most common type at the highest dose-level. At lower doses fibroblastic osteosarcomas and other less well differentiated subtypes (pleomorphic-, anaplastic-, etc.) prevailed.

Osteosarcoma metastases were rare (3%); consequently, their incidence could not be related to other factors.

No significant effects on susceptibility to osteosarcomas or on osteosarcoma characteristics were induced by the ATx/ALG treatment in ^{90}Sr-exposed mice.

^{90}Sr-induced malignant bone tumours appeared to originate in bone precursor cells located in the stromal network of the bone marrow about as often as they originated in the endosteal cell layer. In rare instances, they originated in congruent cells occupying porosities in compact bone.

Discussion

The process by which radiostrontium induces bone tumour formation was studied from 2 perspectives: one concerning the dose-response relationship and its possible dependence on suppressed immune functions, and the other relating to histopathology of pretumourous and early stages of osteosarcoma development.

Tumour and host interaction. Tumour development can be described as a continuous struggle between certain abnormal cells and the host which continues until rejection, remission or, ultimately, death of the host. The existence of humoral and cell-mediated immune reactions, capable of specific tumour cell destruction, is well-established. Thus antibodies plus complement, K-cells with bound antibodies, activated T-cells, NK-cells and macrophages all play a vital role in the lysis of tumour cells. The potency of each of these effector mechanisms in preventing tumour development has, however, been difficult to determine but is assumed to vary widely. The most relevant approach to this problem seems to be comparison between the incidence of neoplasia in intact animals and in animals with suppressed immune reactions. ATx/ALG treatment suppresses responses normally mediated by T-cells, such as transplant rejection reactions and the formation of thymus-dependent antibodies. Our previous and on-going experiments indicate that ATx/ALG also suppresses these reactions in ^{90}Sr-exposed mice (3, 4). ^{90}Sr itself concomitantly exerts a suppressive influence on the whole haematopoietic system, which notably leads to the depletion of bone-marrow dependent natural killer (NK) cells (unpublished observation).

The hypothesis that the immune system is of potential importance in determining the course of radiation carcinogenesis presumes that tumour cells possess structures that can serve as rejection targets for the immune system. For ^{90}Sr-induced osteocarcomas, indicators of immunogenic properties have been proposed (7–9). Tumours which develop in ^{90}Sr-exposed animals may do so a) as a direct consequence of reduced immunologic responsiveness, i.e. a breakdown of the immune system by irradiation, leaving initiated cells free to multiply, or b) owing to a state of continuous tumour microevolution, implying escape from immune rejection, brought about by a combination of antigenic drift and the selection pressure exercised by the immune system. The immune system would constantly be one step behind and eventually fail. A third possibility would be c) immunologic blocking of antigens, leaving the tumour cells in a protected state, again free to proliferate. In other words, a tumour-induced immune reaction may in fact enhance rather than retard tumour growth, as has been suggested, for instance in chemical carcinogenesis (32, 33). All of these tentative pathways for radiogenic tumour development seem worthwhile to challenge by systematic modulation of the immune capacity.

Animal survival in relation to treatment. The mortality curves (Fig. 2) indicate that the injected amount of ^{90}Sr is the only variable affecting life-span, reducing it in a dose-dependent manner. No clear influence of ATx/ALG treatment was discernible among ^{90}Sr-exposed mice, but when used alone an earlier onset of mortality was observed (A VII, Ser. 2). In some cases fatal internal bleeding followed repeated ALG injection.

Tumour response in relation to ^{90}Sr dose. The bone tumour responses obtained in the present study are in good agreement with previous data from the same mouse strain (16, 18, 19). The amounts of ^{90}Sr employed here clearly demonstrate the positive relationship between dose and total tumour incidence, induction time, number of tumour-bearing mice and tumours per mouse. The close resemblance between osteosarcoma curves and mortality curves in the higher dose series reflects the predominance of this tumour type among fatal diseases. With a decrease in dose other factors gradually replace osteosarcomas as the cause of death, and survival times

increase accordingly. The investigation shows that the dose also exerts a clear influence on tumour histology. Thus an optimal tumourigenic dose yields a high proportion of well differentiated osteoblastic osteosarcomas, while lower doses induce relatively more of less-differentiated subtypes. The mechanism by which this dose-related response occurs is not well understood but could well involve a dose-related suppression of the immune system or a dose-determined variation in antigenic expression of the different tumour subtypes. Synchronous radiation-induced tissue regression with its associated impairment of blood supply and nutrition might also influence the development of specific subtypes, e.g. by escape of immune control. The influence of ^{90}Sr dose on the location of induced osteosarcomas within and between bones was, in the current dose range, restricted to variation in relative tumour incidence. Higher, more damaging doses substantially alter this pattern, owing to the extensive cell killing, including the death of arising neoplastic clones and the relocation of the radionuclide (16, 19).

The relationship between dose and the frequency of osteosarcomas of various size as well as preneoplastic lesions appears to be close to linear, with the exception of the macro-tumours (Fig. 5b). The macro-tumour curve levels off when approaching the incidence of 100 tumours/100 mice. The explanation for flattening off is obviously that a macro-tumour relatively soon terminates the animal's life, stopping any further transition from micro- to macro-tumours.

Tumour response in ^{90}Sr-exposed immunocompromised mice. Protracted nonspecific immune suppression, resulting from ATx or ALG or both, did not significantly influence the bone tumour panorama induced by ^{90}Sr. This was true regardless as to whether the ^{90}Sr dose used was highly, moderately or poorly tumourigenic. The tumour parameters investigated were latency time, incidence, multiplicity, location, histologic type, growth potential and metastatic behaviour. The only difference observed in these parameters was a slight reduction and delay in tumour appearance in the ATx/ALG-treated group in Series 3 (AIV versus AII). A similar tendency could be traced in Series 2 and 4, when evaluations were restricted to the crude data. These observations, which to date lack an explanation, cannot be ignored, particularly since a similar decreasing tumour incidence was observed after ATx treatment in a previous experiment (unpublished observation). One can speculate, however, that in certain situations ATx/ALG creates a deficiency in or disturbed balance between various T-cell subsets, leading to enhanced anti-tumour reaction, and thereby delaying as well as reducing tumour incidence.

Dose–response relationship from actuarial tumour incidence. Attempts to estimate the dose–response relationships based on the actuarial appearance of tumours, as shown in Table 3, involve several difficulties. First, it must be determined whether or not the radiation dose, to which the bones were exposed, was directly proportional to the injected amounts of ^{90}Sr. Walinder et al. (34) found that retention of activity in the mouse humerus following injection with 14.8 kBq ^{90}Sr/g body weight was similar to that previously found after injection of 3.7 kBq/g body weight. Thus, in the following calculations the skeletal dose was considered to be proportional to the amount of ^{90}Sr injected.

The parameters n and k in the exponential expression given below have been estimated on the basis of the figures for the actuarial appearence of bone tumours in Table 3 by using the square method;

$$\hat{I}(D)-\hat{I}(0) = k \times D^n$$

$\hat{I}$ (D) is the actuarial appearance of bone tumours at a given 'dose' D; $\hat{I}$ (0) is the corresponding value from the control animals, and D is the 'dose' expressed as the amount of ^{90}Sr injected per g body weight. The values of n were 0.88 ± 0.10 for group AII and 1.19 ± 0.46 for group AIV. Accordingly, the deviations from linearity in the dose–response relationships were not significant. The correlation coefficients were 0.986 and 0.931 respectively.

The actuarial incidence, however, represents the probability for tumour induction, i.e. it cannot surpass unity, implying that high tumour frequencies distort the relationship at lower doses. Unfortunately, the numbers of tumours in Series 3 and 4 were too small for analyses. A rough estimate may be made, however, by pooling the data from groups II and IV in each of the Series 2, 3 and 4. Pooling should be justifiable, since the actuarial appearances are not significantly different within each series. Hence, the value obtained for n is 2.11 ± 0.47. This figure is higher than those obtained for all dose series, although it is obviously still not significantly different from unity. Thus the specific question as to the shape of the dose-response curve must remain unanswered.

Interdependency of tumours. The number of tumours per animal has been analysed to determine whether the variable follows a Poisson-distribution. A χ^2 analysis testing the goodness-of-fit between the observed numbers of mice with 0, 1, 2, ... tumours per animal and the corresponding expected Poisson-distribution was not significant. However, the ratio between the observed and expected number of tumours per animal did not demonstrate any correlation with the numerical tumour burden of the animals. Thus, there is no significant evidence that established tumours enhanced subsequent tumour formation.

Preneoplasia and early neoplasia. Since the cellular origin of osteosarcomas is still being debated, an important goal here was to make a microscopic study of bone tumour morphogenesis with emphasis on the earliest detectable stages and preneoplastic lesions. The results revealed that overt osteosarcomas are indeed often mixtures of different cell types (i.e. osteoblastic, fibroblastic, chondroblastic and osteoclastic) which suggests that they

are either genotypically diverse and multiclonal or that stimulating and/or inhibiting factors in the micro-milieu determine the ultimate differentiation and thus the tumour morphology. At the very early stages of development such morphologic characteristics were not easily discernible; instead the cells often had immature mesenchymal cell traits and were not yet capable of bone formation. Since the sites of these early malignant clones proved to correspond with the locations of the typically preceding hyper- and dysplastic stromal cell proliferations, we suggest that many bone tumours develop from such preneoplastic lesions and from pluripotent cells. Accordingly, bone tumours may arise at any site where such mesenchymal precursor cells are present; this not only applies to the bone marrow cavity but also to the resorption cavities in compact bone and, theoretically, even to extraskeletal tissues.

Concluding remarks. The difficulties and limitations of the present experimental model system have become increasingly obvious during the course of this investigation. Still, it represents one of the few studies performed in which the role of the immune system in radiation carcinogenesis has been challenged in the in vivo induction situation. The results with regard to tumour responses, although incomplete, contradict the view that the immune system plays a regulatory role in radiation carcinogenesis. Instead, it appears as if ^{90}Sr-induced bone tumours are weakly immunogenic or nonimmunogenic in the host of origin, or, alternatively, that they regularly modulate their antigens to evade immune defences. The evaluation of other tumour responses and a summarizing discussion will follow in part II of this report.

ACKNOWLEDGEMENTS

This work was carried out as part of the programme established by the European Late Effects Project Group (EULEP). B.-M. Svedenstål and A. Broomé-Karlsson offered experimental assistance while M. Ahlbom, U. Hammarström and I. Tränck prepared microscope slides. Professor G. Walinder helped with statistical calculations. The work was begun at the Swedish National Defence Research Institute (FOA), Sundbyberg, where most of the experiments were performed, and supported in part by a grant from the Swedish Cancer Society (Project No. 790-B76-02XB).

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REFERENCES

1. Casarett GW. Pathogenesis of radionuclide induced tumours. In: Radionuclide carcinogenesis. AEC symposium series, 1973; 29: 1.
2. Fry RJM, Ley RD, Grube D, Staffeldt E. Studies on the multi-stage nature of radiation carcinogenesis. In: Hecker E, Fusenig NE, Kunz W, Marks F, Thielmann HW, eds. Cocarcinogenesis and biological effects of tumour promoters. New York: Raven Press, 1982: 155–65.
3. Bierke P. Influence of ^{90}Sr, adult thymectomy and antilymphocyteglobulin on haematopoietic tissues and peripheral blood leucocytes in CBA mice. Acta Radiol Oncology 1986; 25: 147.
4. Bierke P, Gidlund M. Influence of ^{90}Sr, adult thymectomy and antilymphocyteglobuline on T-cells in mouse peripheral blood. Acta Radiol Oncology 1984; 23: 61.
5. Sjernswärd J. Immunosuppression by carcinogens. Antibiotica Chemother 1969; 15: 213.
6. UNSCEAR Report. United Nations Scientific Committee on the effects of atomic radiation. Sources and effects of ionizing radiation. Report to the General Assembly. New York, 1977: 622.
7. Moore M, Williams DE. Studies on the antigenicity of radiation induced murine osteosarcomata. Br J Cancer 1972; 26: 90.
8. Nilsson A, Eriksson KH. Antigenicity of radiostrontium-induced osteosarcomas. Radiat Res 1972; 52: 395.
9. Nilsson A, Revesz L, Stjernswärd J. Suppression of strontium-90-induced development of bone tumours by infection with Bacillus Calmette–Guérin (BCG). Radiat Res 1965; 26: 378.
10. Shvedov VL, Ganchenkova NP. Vliianie vaktsinatsii BTsZh na chastotu razvitiia u krys osteosarkom, indutsirovannykh 90-Sr (Effect of BCG vaccination on the incidence of the development of 90-Sr induced osteosarcomas in rats). Eksp Onkol 1986; 8: 58.
11. Burnet FM. Immunological surveillance. Pergamon Press, Oxford 1970.
12. Thomas L. In cellular and humoral aspects of hypersensitive states. Lawrence HS, ed. London: Cassel 1953: 529.
13. Andersson WAD, Zander G, Kuzma JF. Carcinogenic effects of ^{45}Ca and ^{90}Sr on bones of CF1 mice. Arch Path 1956; 62: 262.
14. Finkel MP, Bergstrand PJ, Biskis BO. The latent period, incidence and growth of ^{90}Sr induced osteosarcomas in CF1 and CBA mice. Radiology 1961; 77: 269.
15. Finkel MP, Biskis BO. The induction of malignant bone tumours in mice by radioisotopes. Acta Univ Int Cancer 1959; 15: 99.
16. Nilsson A. Strontium 90 induced bone and bone-marrow changes. An experimental study with special reference to the histogenesis and histopathology of osteosarcomas (Thesis). Medical Div Res Inst of Natl Defence, Sundbyberg, Dept of Pharmacol, Royal Vet Coll, Stockholm, 1962.
17. Nilsson A. Early development of transplanted ^{90}Sr-induced osteosarcoma buds. Acta Radiol Ther Phys Biol 1966; 4: 7.
18. Nilsson A. Dose-dependent carcinogenic effect of radiostrontium. Radiation-induced cancer. Vienna: International Atomic Energy Agency, 1969: 173–82.
19. Nilsson A. Pathologic effects of different doses of radiostrontium in mice. Dose effect relationship in ^{90}Sr-induced bone tumours. Acta Radiol Ther Phys Biol 1970; 9: 155.
20. Nilsson A, Sundelin P, Sjödén I. Ultrastructure of ^{90}Sr-induced osteosarcomas and early phases of their development. Acta Radiol Ther Phys Biol 1974; 13: 107.
21. Nilsson A. Influence of biological parameters on the induction of ^{90}Sr-induced osteosarcomas. In: Bone and bone seeking radionuclides. Physiology, dosimetry and effects. Rotterdam: EULEP Symposium, EUR, 1981: 111.
22. Nilsson A, Bierke P, Walinder G, Broomé-Karlsson A. Age and dose related carcinogenicity of ^{90}Sr. Acta Radiol Oncol 1980; 19: 223.
23. Nilsson A, Broomé-Karlsson A. Influence of steroid hormones on the carcinogenicity of ^{90}Sr. Acta Radiol Ther Phys Biol 1976; 15: 417.
24. Nilsson A, Rönnbäck C. Influence of oestrogenic hormones on carcinogenesis and toxicity of radiostrontium. Acta Radiol Ther Phys Biol 1973; 12: 209.
25. Finkel MP, Biskis BO. Osteosarcomas induced in mice by

FBJ virus and ^{90}Strontium. In: Delayed effects of bone-seeking radionuclides. Mays CW et al, eds. Salt Lake City: University of Utah Press, 1969: 417–35.
26. Finkel MP, Biskis BO, Reilly CA Jr. Influence of FBJ osteosarcoma virus given before or after Strontium-90 upon the induction of bone cancer in mice. IV^{e} Congrès International de Radiobiologie et de Physico-chimie des Rayonnements, Livre des Résumés, 1970: 72.
27. Finkel MP, Biskis BO, Reilly CA Jr. Interaction of FBJ osteosarcoma virus with ^{90}Sr and with ^{90}Sr osteosarcomas. In: Clark RL,ed. Oncology, Vol 1, Cellular and molecular mechanism of carcinogenesis. Chicago: Year Book Medical Publishers, 1971: 422–34.
28. Finkel MP, Reilly CA Jr, Biskis BO. Pathogenesis of radiation and virus-induced bone tumours. In: Grundmann E, ed. Malignant bone tumors. Heidelberg: Springer-Verlag, 1976: 92–103.
29. European Late Effects Project Group, Committee on Standardization. Gössner W, Hollander CF, Maisin JR, Nilsson A, eds. EULEP pathology atlas. Munich 1981.
30. Kellerer AM, Chmelevsky D. Analysis of tumour rates and incidences—A survey of concepts and methods. In: Broerse JJ, Gerber GB, eds. Proc European seminar on neutron carcinogenesis. Luxembourg: EUR 8084 EN 1982: 209–31.
31. Gimeno EJ, Walinder G, Feinstein RE, Rehbinder C. Effects of high ^{131}I doses to the thyroid gland on tumorigenicity of ^{90}Sr and ^{90}Y in mice. Acta Radiol Oncol 1985; 25: 261.
32. Prehn RT. The immune reaction as a stimulator of tumor growth. Science 1972; 176: 170.
33. Prehn RT, Prehn LM. The autoimmune nature of cancer. Cancer Res 1987; 47: 927.
34. Walinder G, Feinstein RE, Gimeno EJ. Effects of high ^{131}I doses on the bone uptake and retention of ^{90}Sr and ^{90}Y. Acta Radiol Oncol 1986; 25: 255.
35. Walinder G, Müller WA. Concentration of ^{90}Sr and ^{90}Y in various organs in the female mouse. Sundbyberg: FOA report C 40021-A3, 1975