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Sr^{90}-INDUCED OSTEOSARCOMAS

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Appreciation of the place of radioactive substances in the formation of bone tumours dates from the 1929 paper by *Martland* and *Humphries* (39) "Osteogenic sarcoma in dial painters using luminous paint" and their demonstration of the aetiological association between radium and malignant skeletal tumours. Since their contribution, work in this field has been intensive and has demonstrated that several radioactive substances have a carcinogenic effect. The major interest has centred about radiostrontium, primarily because of the increased risks of exposure to this substance arising from military and civil uses of atomic energy. Numerous reports have been published on the induction of tumours in various species (for mice see references 2, 13, 17, 19, 20, 34, 50; for rats see references 3, 29, 30, 34, 35, 36, 48; for rabbits see references 9, 28, 34, 41, 42, 47; and for dogs see references 1, 16, 18, 37). The incidence of tumour formation and the induction time in relationship to the manner of exposure and the amount of strontium administered has been touched upon in several reports (3, 16, 17, 19, 41, 42). Another problem which has been taken up is the effects of a single or divided dose of strontium upon the ultimate tumour incidence (16, 21). Descriptions of the sites of tumour formation can also be found (2, 9, 15, 19, 29, 30, 34, 37, 41, 48). The optimal dose for tumour induction in mice and dogs has also been calculated (18, 20).

On the whole, existing histological descriptions are more or less limited to labelling the Sr^{90}-induced tumours as osteosarcomas without going into detail (2, 3, 19, 20, 29, 30, 37, 41, 42, 47). Tumours are generally multiple (9, 17, 30, 37).

This report covers part of an investigation into the histogenesis and dynamics of Sr^{90}-induced tumours in mice. The in-

duction time for tumour formation and the sites of tumour formation will also be dealt with since these aspects contribute to an understanding of the genesis of these tumours. Detailed histological studies afford the only means of ascertaining the tissue in which the tumours arise. As a corollary to this it is obviously necessary to study the histological appearance of the sometimes multiple tumours in a particular animal and to study the differences in the incidence of metastasis and the rate of growth of tumours of different histological appearance. It is also essential to know whether tumours of different histological type can develop independently of one another and whether the type of tumour is a function of its site. Furthermore, there is the question of a possible association between survival time and type of tumour.

MATERIAL

All experiments were commenced when male mice derived from brother-sister matings within the CBA strain reached 75 to 85 days of age. Each of a group of 200 mice were given 0.67 μC carrier-free Sr^{90} $(NO_3)_2$ per g. bodyweight intraperitoneally. Another group of 125 mice was kept as a control of the natural incidence of tumours in the CBA strain. The Sr^{90} solution was calibrated against a standard purchased from Amersham, England, and there calibrated with an accuracy of $\pm$ 2 per cent. The accuracy of our own calibration has been estimated as $\pm$ 4 per cent. All the mice were kept under uniform conditions and precautions were taken to prevent coprophagy. The mice were fed a standard mouse diet (25) which contains 2.5 g. Ca per 100 g. ashed feed.

METHODS

The mice were killed when tumours were detected. Moribund animals were killed even if no tumours were evident upon inspection. The precise survival time could not be calculated for this reason; on the other hand, fresh material for histological examination was obtained. The animals in the Sr^{90} group were anaesthetised with an intraperitoneal injection of 0.15—0.30 ml. 0.6 per cent Mebumal ®[1])

[1]) The formula of "Mebumal" is as follows:

5-ethyl-5(methylbutyl)-malonylcarbamid(pentobarbitone)		1.8 g
Pentobarbitone sodium		4.0 „
Urethane		25.0 „
Spir. conc.		15.0 „
Glycerin		12.5 „
Aq. steril.	ad	100 ml.

solution and examined roentgenologically before being killed. Dorsoventral roentgenograms were made of the mice fastened by tape to Osray or Structurix film. The roentgen films were developed immediately and used as a guide for locating tumours in the mice which were then killed and autopsied. Slices about 4 mm. thick were taken through the tumours along their greatest diameter and then fixed in Stieve's fluid (46) and in some cases also in 10 per cent neutral formalin, absolute alcohol, Carnoy's fluid, Helly's fluid, or absolute acetone. If necessary, the tissue slices were decalcified in 20 per cent formic acid under reduced pressure. Conventional histological methods were used — dehydration in alcohol and methyl benzoate, embedding in paraffin, and sectioning on a Leitz sledge microtome set at 5 μ. Ehrlich's haematoxylin-eosin (46) and van Gieson were used routinely and other stains on particular sections — Foot and Foot's silver method (46), Heidenhain's azan (46), Lillie's azure-eosinate (32), and PAS according to Hotchkiss (44). Tests for alkaline phosphatase activity were carried out with Fredricsson's cobalt method (44) and with Gomori's diazo method (22) on tissue decalcified by Greep's method (44) in buffered formic acid at pH 4.9. As a control, sections were boiled in distilled water for 10 minutes to inactivate phosphatase. To demonstrate the presence of RNA, untreated sections and sections treated with ribonuclease were stained with toluidine blue by Brachet's method (4). Basophilic material which was removed by ribonuclease treatment was considered to be RNA. The presence of glycogen was tested by comparing sections stained by the PAS method with and without exposure to diastase.

Microradiography was carried out by Engström's method (11) on material fixed in absolute alcohol and embedded in methyl-methacrylate by the method of Newman et al. (40). The sections were ground by hand until they were 50 to 130 μ thick. A Macklett tungsten-beryllium tube and a maximum resolution plate (developer D 158) were used. Electron microscopy (Siemens El-microscope at 80 kV) was also carried out on tissue from a non-boneforming, subcutaneously transplanted tumour in the eleventh passage. The tissue was fixed in Palade's (43) buffered osmium tetroxide solution.

The volume relationships between bone and tumour cells or between stroma and tumour cells in histological sections were studied by means of the method described by *Chalkley* (6) and utilized by *Lagerstedt* (31) and *Engfeldt* (10). Five thin glass threads were glued to the diaphragm of the ocular. The principle of the method is simply to record the cell types which happen to lie under the tips of the threads in random microscopic fields. For the purpose of this study, 25 "hits" on the appropriate cell type has been taken as an unit and the basis for the statistical calculations. For each histological section 12 units, i. e. 300 hits, were counted. The mean values, standard deviation, and standard error have been calculated for each tumour group and the results tabulated.

Ten tumours of the predominantly boneforming osteoblastic type and ten of the predominantly non-boneforming fibroblastic type were

chosen at random for determinations of mitotic activity, i. e. the number of mitotic configurations per 1000 cells counted in each tumour. As recorded here, mitosis includes all stages from disappearance of the nuclear membrane to division into daughter cells. Counts were made at a magnification of 1250 ×. To facilitate counting cross-hairs were mounted in the ocular to give 4 equal sectors in the microscopic field. The number of cells per field has ranged between 50 and 100. In each instance counts were made in the most cellular regions of the section.

Serum alkaline phosphatase activity was determined for a group of normal male mice, 75 days old, and for each of the 10 mice in the groups which had received transplants of osteoblastic or fibroblastic tumours respectively. The tumours were transplanted subcutaneously into the neck when the animals were 65 days old. Blood samples were taken with a Pasteur pipette from the medial canthus of the eye. The non-boneforming fibroblastic tumours grew very rapidly and blood samples were taken 12 days after transplantation when the tumours were generally about 2.5 cm. in diameter. Blood samples were taken from the mice which had received the boneforming osteoblastic tumours when the transplants had attained the same size as in the previous group, 53 days after transplantation. The method described by *King & Armstrong* (5) was used and phosphatase activity expressed in King-Armstrong units.[1])

RESULTS

Tumour incidence. Of the 200 mice given Sr^{90}, 179 or 89.5 per cent developed tumours. There were 391 primary tumour sites in the tumour-bearing mice (Table I). Ten mice (5 per cent) developed lymphatic leukaemia, in 2 animals (one per cent) in combination with osteosarcoma. Of the 11 animals (5.5 per cent) which did not develop tumours, 2 (one per cent) died of acute purulent bronchopneumonia. The remaining 9 animals (4.5 per cent) died of inanition and anaemia. It can be seen from Table I that tumour formation reached its peak between 241 and 270 days after the administration of Sr^{90} when about 41 per cent of the tumours and of the tumour-bearing mice were detected. No tumours were detected in the control mice during the observation period of 462 days.

Induction time. The first tumour appeared 181 days after Sr^{90} injection and the last at 462 days. The mean induction time for this material was 266 days. It can be pointed out, however, that in another experiment not included in this report and in which

[1]) King-Armstrong Unit = 1 mg phenol derived from 5/1000 M disodiumphenylphosphate in 15 minutes at 37° C in bicarbonate-carbonate buffer pH 10.

Table I. Tumour distribution in relationship to time after injection of 200 mice with Sr^{90}. Each mouse received 0.67 μC/g. bodyweight.

Days after injection of Sr^{90}	Mice with osteosarcomas		Total number of primary osteosarcomas		Mean number of tumours per mouse	Number of mice with leukaemia	Number of mice with leukaemia and osteosarcomas	Number of mice dying of other causes	Number of mice with metastases
	number	per cent of mice with induced tumours	number	per cent of induced tumours					
151—180	0	0	0	0	—	2	—	1	—
181—210	18	10.06	27	6.91	1.50	1	—	4	2
211—240	36	20.11	64	16.38	1.78	2	2	—	1
241—270	74	41.34	160	40.96	2.16	3	—	—	1
271—300	38	21.23	111	28.42	2.92	—	—	3	1
301—330	10	5.59	22	5.63	2.20	—	—	3	1
331—360	2	1.12	5	1.28	—	—	—	—	—
361—450	—	—	—	—	—	—	—	—	—
451—480	1	0.56	2	0.51	—	—	—	—	1
Total	179	100	391	100	2.18	8	2	11	7

Table II. Site of 391 tumours in 179 tumour-bearing mice out of 200 injected with Sr^{90}

Site of tumours involving a single bone	Days after injection of Sr^{90}							Total	Number of tumours involving more than one bone
	181—210	211—240	241—270	271—300	301—330	331—360	451—480		
	n=18	n=36	n=74	n=38	n=10	n=2	n=1		
Vertebral column									
C 7		1						1	
Th 4			1					1	
Th 6		1						1	
Th 7			1	1				2	
Th 8		1						1	1a)
Th 10		1						1	
Th 12				1				1	
Th 13		1	3	2				6	
Total		4	5	4				13	1
L 1		1	6	3				10	
L 2			4	1	1			6	
L 3	1	4	4					9	8b)
L 4	2	2	3	1				8	
L 5	1	1	4	4	1			11	5c)
L 6	3	3	7	11				24	
Total	7	11	28	20	2			68	8
S 1	2	6	11	4	2			25	
S 2			2					2	8d)
S 3			1				1	2	
S 4		1		1				2	
Total	2	7	14	5	2		1	31	8
Cocc. 1	1	1	2					4	3e)
Cocc. 3				1				1	
Total	1	1	2	1				5	3
Femur, dist. metaph.	6	10	32	18	6	1	1	74	
Femur, prox. metaph.		1	2	4	3			10	7f)
Femur, diaphysis			2	2				4	
Femur, entire		2	9	5				16	1g)
Total	6	13	45	29	9	1	1	104	
Tibia, prox. metaph.	5	5	22	30	2			64	
Tibia, diaphysis				1				1	
Tibia, entire			1					1	
Total	5	5	23	31	2			66	

Table II (continued).

Site of tumours involving a single bone	Days after injection of Sr^{90}					
	181—210	211—240	241—270	271—300	301—330	331—
	n=18	n=36	n=74	n=38	n=10	n=
Humerus			2	4		
Ilium	2	6	12	6	2	1
Ischium			2	1	1	
Pubis			1			
Total	2	6	15	7	3	1
VI:th rib		1				
XIII:th rib			1			
Maxilla			1			
Sphenoid bone					1	
Scapula			1	1		
Occipital condyle				1		
Mandible			1			
Total		1	4	2	1	
Total number of tumours in a single bone	23	49	138	103	19	2
Total number of tumours in more than one bone	4	15	22	8	3	3

a. Tumour in thoracic vertebrae only.
b. Tumours in lumbar vertebrae only.
c. Tumours in lumbar and sacral vertebrae.
d. Tumours in sacral vertebrae only.
e. Tumours in coccygeal vertebrae only.
f. Tumours in femur and tibia.
g. Tumours in femur, tibia, sacral and lumbar vertebrae and ilium.
h. Tumours in humer
i. Tumours in more t
j. Tumours in ilium, vertebrae.
k. Tumours in ribs o
l. Tumour involving bones.

Sr^{90}-treated animals were killed at regular i histologically-detectable tumour appeared at

Tumour multiplicity. Tumours with dimen 2 mm. were generally easy to detect on the ro at autopsy. The mean number of tumours pe tire tumour-bearing material was 2.2 and th value of 2.9 was attained between 271 and 3(From Fig. 1 it can be seen that 69 mice (39 tumour and 110 mice (61 per cent) had 2 or m

ty-one mice (51 per cent) had 2 or 3 tumours and 19 mice (10 per cent) had 4 or more tumours.

Site within the skeleton. Of the 391 tumours detected, 336 were limited to a particular bone (Table II). In 55 instances it was impossible to decide whether several individual tumours had fused or whether a single tumour had extended into adjacent bones (Fig. 2). Forty-five of the tumours included in Fig. 2 were located in the long bones, vertebrae, pelvis, skull, or ribs. In 10 instances, 2 tumours in the humerus-scapula region and 8 tumours involving various combination of the pelvis and adjacent bones, it proved impossible to ascertain the type of bone in which the tumours originated. Of the total number of tumours, 381 could be referred to one or other of the bone types listed — 183 (48 per cent) to the long bones, 143 (37.6 per cent) to the vertebrae, 44 (11.6 per cent) to the pelvis, 5 (1.3 per cent) to the skull, 4 (1.1 per cent) to the ribs, and 2 (0.5 per cent) to the scapula.

The particular bone involved could be established for 356 of the tumours (Table II). The femur was the most frequent site (about 31 per cent) followed by the tibia and lumbar vertebrae (about 20 per cent each) and then the pelvis and sacral vertebrae (about 10 per cent each). The skull, cervical and thoracic vertebrae, the ribs, and the bones of the fore limbs provided only about 9 per cent of the tumour sites and the coccygeal vertebrae about one per cent.

Site in the particular bones. In the femur about 71 per cent of the tumours originated in the distal portion. Practically all the tumours in the tibia (97 per cent) originated from the proximal region. Of the tumours in the pelvis, about 86 per cent arose in the ilium, particularly near the acetabulum and in the tuber coxae. Of the lumbar vertebrae, L6 was the most common site of tumour formation (about 35 per cent). Eighty per cent of the tumours in the sacral vertebrae originated from S1.

Seven mice (4 per cent) with a total of 23 skeletal tumours — 19 boneforming and 4 non-boneforming — also had tumours in the internal organs, most often in the lungs and liver (Fig. 28) but also in the kidney, adrenal, mediastinum, or peritoneum. Tumours were not observed in the lymph nodes.

Macroscopical observations.

Because of the minuteness of mouse bones, it was often difficult to ascertain the exact site of each tumour in each bone.

The macroscopical description is of necessity limited to changes in the larger long bones. It is evident from Table II that most tumours in the long bones were situated in either the proximal or distal regions and few in the diaphysis. At their largest the tumours reached a diameter of 2 to 3 cm. The smaller tumours were distinctly limited to one end of the bone and usually occupied some portion of the medullary cavity. A few tumours filled out the entire medullary cavity but did not break through the cortex.

On macroscopical and roentgenological grounds a division can be made into predominantly boneforming and predominantly non-boneforming tumours. The predominantly non-boneforming tumours consisted of a firm, sometimes partially necrotic greyish tissue with a yellowish or pinkish tinge. In the larger tumours, necrosis and haemorrhage could be fairly extensive and fragments of pre-formed bone could be observed. In predominantly boneforming tumours the bony tissue was usually located centrally and surrounded by greyish-white tissue. The tumour bone usually had a yellowish tinge and could be fragile or hard, even extremely hard.

The osteoblastic tumours were readily detected at autopsy and, because of their density, on the roentgenograms (Fig. 3). The fibroblastic tumours were more difficult to see, especially if they did not happen to produce a break in contours. Most of these tumours were in fact first detected at autopsy. Various degrees of rarification and destruction up to complete osteolysis of cortical bone could be observed.

HISTOLOGICAL OBSERVATIONS

I. *Predominantly non-boneforming, fibroblastic osteosarcoma.*

These tumours were characteristically strongly pleomorphic (Figs. 5—8). The cytoplasm of the tumour cells was usually predominantly basophilic and often contained numerous small vacuoles (Fig. 9), especially evident in phase-contrast. Only few cells contained small amounts of glycogen or PAS-positive material. The nuclei were of the leptochromatic type but had a distinct membrane and contained nucleoli which usually stained basophilic. Giant cells of widely varying shapes were regularly encountered but their number and size could vary considerably from tumour to tumour (Figs. 10—12). These giant forms usually

lay in groups, were uni- or multinucleated, and had an eosinophilic and often vacuolated cytoplasm. Their nuclei were densely chromatic and contained usually eosinophilic nucleoli.

These tumours lacked a general pattern other than that afforded by the strands of cells and fibres coursing in all directions and planes (Figs. 7, 18). Collagen fibres and argyrophilic and weakly PAS-positive fibres were abundant in some regions and sparse in others. In places, practically every cell was surrounded by argyrophilic fibres (Fig. 13). There were also some small groups of argyrophilic fibres arranged circularly or elliptically in tufts (Fig. 14) around tumour cells which in turn were separated by fine argyrophilic fibres. The tumours were richly vascular, sometimes with large sinusoids. The central parts of the tumours were often necrotic or haemorrhagic or both, sometimes with neutrophil infiltration. Few signs of bone or cartilage formation were observed but the tumours regularly contained small or large fragments of pre-formed bone, sometimes necrotic. The fibrillary pattern, pre-formed cartilage and cementing lines in pre-formed bone gave a positive PAS reaction. Only a few places showed signs of alkaline phosphatase activity.

Electron microscopy. The cells were either fusiform, circular, or oval. The centrally situated, circular or irregularly shaped nucleus occupied the greater part of the cell (Figs. 30, 31). The nuclear membrane was double and readily visible. The nucleoli, usually multiple, were very dense and often lay in the centre of the nucleus or in a row along the long axis of the nucleus. The endoplasmic reticulum was very well developed and close to its ramifications were numerous "Palade granules" (Figs. 30—32). The canaliculi were greatly dilatated, almost vesicular, and were relatively immense in some places (Figs. 30—32). The vesicular forms were filled with a relatively strongly contrasted, finely granular substance of unknown nature. Most cells contained a large number of mitochondria (Figs. 30, 31). The christae mitochondriales were distinct (Fig. 32). Some cells contained cytoplasmic vacuoles of varying size (Fig. 30). A large number of granules, not particularly electron dense, were uniformly distributed throughout the cytoplasm.

II. *Predominantly boneforming osteoblastic osteosarcoma.*

Bone formation varied greatly both between different tumours and between different regions of a particular tumour. Cartilage

formation, on the other hand, occurred only rarely and then only to a small extent (Fig. 15). All degrees from the formation of fine bone spicules to practically complete eburnation were encountered (Figs. 19—21): Ossification was generally most pronounced in the central portions with a gradual decline in the degree of mineralisation and osteogenesis towards the periphery (Fig. 22). The peripheral non-ossified zone varied in width from a few mm. to a very broad, deeply lobulated tissue mass enclosing a small and poorly developed bony nucleus. In the most heavily ossified tumours (Fig. 19), the bone formed a coarse network of trabeculae with a mature appearance. In silver-stained sections, however, the fibres in these trabeculae were coarse and irregular, characteristics of immature "coarse-fibred" bone. In tumours with less accentuated bone formation, there were often radial or irregular strands of osteoid tissue with varying degree of mineralisation and varying degrees of collagen formation. The collagen fibres could form tight networks or whorls or lack a discernible pattern.

Pre-formed bone was encountered to a greater extent in these tumours than in those of the fibroblastic type. Structural details were often remarkably well preserved. In some of the smaller tumours, in fact, tumour tissue occupied the entire medullary cavity and burrowed through the cortex and periosteum at one point and then welled out into the surrounding tissues.

Argyrophilic fibres were sparse in the spaces enclosed by the bony trabeculae but abundant towards the periphery of the tumours where bone formation was less evident.

The bony trabeculae in the central parts of the tumours were lined by one or more layers of fusiform or oval osteoblasts (Fig. 23). These cells had a generally basophilic cytoplasm and, unlike the cells in the fibroblastic tumours, displayed a weakly eosinophilic juxtanuclear zone (Fig. 24). Little glycogen was present but the amount was somewhat greater than in the fibroblastic tumours. The nuclei were hyperchromatic making it difficult to detect the basophilic nucleoli, if any were present. The cells lying in lacunae in the bone were larger than normal osteocytes and had dense, hyperchromatic nuclei. Giant cells of irregular morphology but usually with several hyperchromatic nuclei and an eosinophilic cytoplasm were irregularly present, usually in conjunction with small erosions along the surfaces of trabeculae. Towards the periphery, where bone formation was in an early

stage, the tumour cells were larger and their nuclei more leptochromatic than in the heavily ossified central portions. Cell borders were not as evident and the cytoplasm less basophilic than in the case of the more centrally situated cells. A distinct juxtanuclear zone was generally absent. The basophilic nucleoli usually 2 or 3 in number, were distinct. In this type of tumour as well, the cytoplasmic basophilia could be referred to a high RNA content.

The tumours were highly vascular, especially peripherally were some blood vessels had a sinusoidal appearance.

Alkaline phosphatase activity was great in these tumours (Fig. 25). Newly formed bone and osteoid tissue were strongly PAS-positive while the fibrillar pattern gave only a weak reaction.

Table III. Volume relationships between bone:tumour cells and stroma:tumour cells. "Stroma" here refers to non-bony formed extracellular elements as seen in sections stained with Heidenhain's azan and Foot and Foot. Chalkley's point sampling method.

Osteosarcomas	Number of tumours	Mean ratio					
		Bone: tumour cells	± SE	± SD	Stroma: tumour cells	± SE	± SD
Fibroblastic	9	0.06	0.016	0.17	0.70	0.036	0.37
Osteoblastic, moderate bone formation	4	0.27	0.032	0.22	0.30	0.036	0.25
Osteoblastic, intense bone formation	5	1.09	0.082	0.64	0.19	0.022	0.14
Osteoblastic, very intense bone formation	5	1.92	0.105	0.81	0.15	0.034	0.26

The microradiographical studies demonstrated that there was practically no mineralisation in the fibroblastic tumours. Mineralisation within the osteoblastic tumours varied greatly but was generally most intense in the coarse, newly formed bony trabeculae lying close to preformed bone (Fig. 26) in the central parts of the tumours and declined towards the periphery. In places there was a suggestion of a primitive Haversian system. The degree of mineralisation of the trabeculae decreased from

the centre towards the periphery of the tumours, often with great variations from trabecula to trabecula. In the pre-formed and often heavily mineralised bone there were often deep and irregular defects produced by osteolysis.

The volume measurements obtained with Chalkley's point sampling method are listed in Table III. These measurements were made on tumours representing the fibroblastic type and the osteoblastic type with moderate, heavy, or very heavy bone formation. Each tumour in the entire material had been classified in this way and representatives for each type were selected at random.

The classification made on the basis of the histological appearance agreed fairly closely with the more objective method in respect of bone formation. It can also be seen from Table III that an argyrophilic stroma was formed in inverse relationship to the amount of bone formation. The relative amount of stroma in the fibroblastic tumours, in which little or no bone could be found, was great and then declines as one moves along the scale to the heavily ossified tumours. The variations about the mean values reflect the wide variations in the histological structure within the particular tumours.

The osteoblastic type predominated. Out of 391 tumours, 324 (83 %) were osteoblastic and 67 (17 %) were fibroblastic. The distribution of tumour types in the various bones is shown below.

Site	Fibroblastic type		Osteoblastic type	
	no.	%	no.	%
Femur	33	49	71	22
Tibia	13	19	53	16
Humerus	4	6	2	0.6
Pelvis	9	13	25	8
Vertebrae	3	5	115	36
Other bones	0	0	8	3
Tumours involving 2 or more bones	5	8	50	15
Total	67	100	324	100

III. *Confluent tumours representing different types of osteosarcoma.*

For only a few of the 55 tumours which involved 2 or more bones could a distinction be made between secondary tumour infiltration and the bone or bones with the primary tumour. By

serial sectioning of 4 tumours it could be shown that these either fibroblastic or osteoblastic tumours were actually confluent tumours with several points of origin in adjacent bones (Fig. 17). There were also instances of fibroblastic and osteoblastic tumours developing separately but concomitantly in a single bone (Fig. 16). The histological appearance of the tumours otherwise accorded with the description given above for the respective tumour types.

Tumour multiplicity and tumour type.

Osteosarcomas of different histological type can be encountered in different sites in animals with multiple tumours. Of 57 mice with multiple osteosarcomas, 15 or 26.3 per cent had tumours of both types. These 15 mice had a total of 45 tumours, 22 osteoblastic and 23 fibroblastic. The entire series of 57 mice had a total of 144 tumours or 2.5 tumours per mouse, 107 (74.3 per cent) osteoblastic and 37 (25.7 per cent) fibroblastic.

The histological appearance of extraskeletal tumours.

Since all these extraskeletal tumours had a histological appearance which agreed so closely with the appearance of the skeletal tumours in the respective animals they have been considered to be metastases. All these metastases were of the osteoblastic type. Spread was apparently haematogenous; small tumour emboli were encountered in many blood vessels. Metastases were often multiple and ranged in size from the microscopical up to 1 cm. in diameter. The small metastases could be strongly boneforming (Fig. 27) but sometimes consisted of cells resembling those in the most cellular regions of the parent tumour. The larger the metastases, the more they resembled the parent tumours in cell type, bone and stroma formation, and general architecture. Some metastases, however, contained a cartilaginous component which was much more extensive than the usually sparse occurrence of this tissue in the parent tumour (Fig. 29).

Mitotic index.

The mitotic index was significantly greater ($P < 0.01$) for the fibroblastic tumours than for the osteoblastic (Table IV). The magnitude of the standard deviations reflects the great variations in the mitotic index between tumours within each group. Among the osteoblastic tumours the greatest number of mitoses

Table IV. Number of mitoses in different types of tumours.

Tumour	Mean number of mitoses per 100 tumour cells	SD	SE	t-test
Fibroblastic (n = 10)	16.00	± 6.68	± 2.12	t = 3.1305
Osteoblastic (n = 10)	8.80	± 2.82	± 0.89	0.01 > p > 0.001

encountered per 1000 cells counted was 15 and the least was 5. Corresponding values within the fibroblastic group were 30 and 8 respectively.

Serum alkaline phosphatase activity.

Blood serum from the normal mice had an alkaline phosphatase activity of 10.13 ± 0.46 King-Armstrong Units. Mice bearing subcutaneous transplants from fibroblastic tumours had a serum activity of 4.07 ± 0.03 units and those bearing osteoblastic transplants 84.10 ± 6.86. At one extreme, then, there was a highly significant decrease ($P < 0.001$) and at the other a highly significant increase ($P < 0.001$) in serum alkaline phosphatase activity compared with the normal. The error of the method was ± 3 per cent.

DISCUSSION

The incidence of tumours at the dose level of Sr^{90} used here agreed closely with the results reported by *Finkel et al.* (19, 20). Furthermore, the induction time corresponds with that reported by *Finkel et al.* (18), *Krayevskiy et al.* (29), *Litvinov* (35, 36), and *Lisco et al.* (34) among others.

All primary tumours, apart from the few examples of lymphatic leukaemia, were found in the skeleton. Somewhat fewer tumours were observed in the bones of the extremities and skull and somewhat more in the vertebrae and pelvis than recorded by, among others, *Finkel* (15) and *Lisco et al.* (34). *Kuzma et al.* (30) did not observe tumours in the vertebrae of rats after the injection of Sr^{89} but found them in this site in rats given Ca^{45}. The large number of tumours in the vertebrae of animals given Pu^{239} has been attributed by *Lisco* (34) to the greater affinity of Pu for collagen than for bone tissue. If the number of tumours in the long bones and those in the vertebrae, pelvis, skull, ribs and scapula are expressed as a ratio, the results for the present series

would be 1.28, 4.16, 36.6, 45.75, and 91.5. The corresponding ratios for the animals given Sr^{89} in the series of *Finkel et al.* (19) are 2.4, 13.8, 8.2, and 263; the scapula was not mentioned. For animals given Sr^{90}, *Finkel et al.* (15) report ratios of 1.8, 12.2, 19.0, and 65.3 (scapula not mentioned) and *Lisco et al.* (34) ratios of 2.8, 10.1 and 61.1 for the ribs. These differences can probably be attributed to differences between strains of mice, different species, and the potential range of biological variation. This assumption is supported by the results *Finkel et al.* (14) obtained in experiments with CBA mice.

The cells of the fibroblastic and predominantly non-bone-forming osteosarcomas would seem to represent a low degree of differentiation of an osteoblastic component. This assumption is supported by the results of serial transplantation of osteoblastic osteosarcomas. After several passages the tumours gradually assumed an appearance which resembled that of the fibroblastic type; the tumour cells came to resemble the fibroblastic type and lost the ability to form bone and display alkaline phosphatase activity. There are also the observations of *Pritchard* (45) on the derivation of osteoblasts from fusiform cells with characteristics practically identical with those of fibroblasts and with little or no alkaline phosphatase activity. The cells in the osteoblastic tumours, especially in regions where bone formation was heavy, meet the criteria for highly active osteoblasts. The explanation for the wide range of histological appearances in these tumours with the presence of bone, collagen tissue and sometimes even cartilage may lie in the fact, as *Weinemann & Sicher* (49) have pointed out, that bone is surrounded by and encloses mesenchymal cells which can develop into cartilage and bone as well as collagen tissue. The cytoplasmic changes seen with electron microscopy, dilatation of the canaliculi and vesicle formation, seem to be the result of accumulation of large amounts of a relatively electron-dense, finely granular substance. This substance is most abundant and the dilatation of the canaliculi is most evident in cells containing large numbers of mitochondria; formation of the unidentified finely granular substance would seem to be associated with a high level of metabolic activity in the cells.

Mitotic index.

The mitotic activity in the fibroblastic osteosarcomas was significantly greater than in the osteoblastic tumours. The fibroblastic tumours grew much more rapidly when transplanted

and were apparently less mature. A predominantly boneforming tumour was transplanted in 22 passages; the mitotic index increased from 8 per 1000 cells for the parent tumour to 22.5 ± 2.84 per 1000 cells for the series as a whole.

Alkaline phosphatase activity.

The histochemical observations as well as the changes in serum activity levels accord with previously recorded results. According to *Gutman* (24), this enzyme is produced by boneforming cells in bone and in cartilage undergoing calcification. An increase in the serum activity level commonly accompanies osteosarcomas (26, 27) but decreases are uncommon. Decreases in serum activity levels can be observed in conjunction with surgical hypoparathyroidism or the cachexia of neoplastic disease provided that tumours do not occur in the liver or skeleton. Alkaline phosphatase activity has also been demonstrated histochemically in osteosarcomas by *Gomori* (23). The abnormally low level of serum alkaline phosphatase activity in the mice receiving subcutaneous transplants of fibroblastic tumours can probably be explained by the effects of their rapid growth on the nutritional state of the host animals.

The tumour types described here have, at their extremes, great differences in their histological appearance. Without going into details of the transplantation experiments which were done alongside the studies reported here, it can be mentioned that isologous transplantation was easily performed by the subcutaneous, intraperitoneal, or intravenous injection of tissue from both fibroblastic and osteoblastic tumours. The fibroblastic transplants grew very rapidly and by 14 to 21 days could reach a diameter of 2.5 to 3 cm. Transplants of this size often rupture, with fatal haemorrhage. Osteoblastic transplants grew much more slowly and required 2 or 3 months to reach the same size as the fibroblastic transplants. This difference in growth rate corresponded to differences in the mitotic indices. Neither the primary fibroblastic tumours nor their transplants metastasised. The rapid growth of these tumours undoubtedly shortened the life of the mice to such an extent that metastases did not have time to form. The osteoblastic tumours, on the other hand, could metastasise. Tumours of this type with an especially pronounced tendency to form bone were highly differentiated and grew slowly. In many transplantation experiments with this type of tumour,

however, multiple metastases often developed 2 or 3 months after the transplants had been placed subcutaneously in the neck. The mice survived a relatively long time. No direct comparison of survival times can be made for mice in the primary series with tumours of the fibroblastic or osteoblastic types since these animals were killed. Transplantation of a tumour inevitably led to death of the recipient mouse.

There is no universally accepted system of classification for tumours of the type we are dealing with here. Even the epithet "osteogenic" has been the subject of much controversy. The Bone Sarcoma Registry of the American College of Surgeons (12), *MacDonald & Budd* (38) and *Weinemann & Sicher* (49) define "osteogenic" as implying origin from bone while others use the term to signify the property of forming bone or osteoid. Here, to comply with modern viewpoints on the subject (8, 33), the phrase "osteogenic sarcoma" has been avoided. Bone tumours are usually very heterogenous and are made up of collagenous, cartilaginous, and osseous components and, in the event bone formation occurs, can be called osteosarcomas. These in turn can be subdivided into fibroblastic, chondroblastic, or osteoblastic types depending upon whether the collagenous, cartilaginous, or osseous component predominates. Fibrosarcomas are purely fibrous connective tissue tumours, usually with some degree of collagen formation, and chondrosarcomas are purely cartilaginous tumours. Tumours of these latter two types were not encountered in the Sr^{90}-treated mice. On macroscopical, roentgenological, and histological grounds, then, the Sr^{90} tumours in mice, with the exception of the examples of lymphatic leukaemia, were divided into two groups.

I. Poorly differentiated, predominantly non-boneforming fibroblastic osteosarcoma.

II. Predominantly boneforming osteoblastic osteosarcoma.

The site of tumour formation would seem to have some bearing on the type of tumour which develops. Of the 67 fibroblastic tumours, no fewer than 53 originated in the long bones and the rest in the vertebrae or pelvis. This means that only 2 per cent of all tumours in the vertebrae but 29 per cent of all those in the long bones were of fibroblastic type.

Multicentricity. Many of the mice in this experimental series had several primary tumours in different sites, an observation

which agrees with previous reports (9, 17, 30, 37). The occurrence of both fibroblastic and osteoblastic tumours in a particular mouse has also been described (35, 48). In all instances these tumours have been considered to be primary and an expression of multicentric tumour induction. The accumulation of such a strong carcinogenic agent as Sr^{90} in the skeleton as a whole affords a reasonable background for multicentric tumour formation. Furthermore, not only fibroblastic and osteoblastic tumours but also within these general types tumours of widely different histological appearance can develop in one animal to give additional support to the assumption that the tumours are genuinely multicentric. Even within particular bones or adjacent bones it is possible to find clearly delimited tumours of fibroblastic and osteoblastic type. The results of the transplantation experiments also imply that these tumour types are independent entities. Neither in the primary series nor in the transplantation experiments did the fibroblastic tumours metastasise. The osteoblastic tumours, on the other hand, when they did metastasise regularly resulted in boneforming metastases of much the same histological appearance as the parent tumour. Even when cell suspensions of the fibroblastic type were injected intraperitoneally or intravenously, the resulting tumours were always of the fibroblastic type. Against this background, it is scarcely likely that metastases from one type of tumour can develop as tumours of the other type which, as opposed to primary multicentricity, could be an explanation for the concomitant occurrence of both tumour types. Serial transplantation of osteoblastic tumours did eventually result in some loss of boneforming properties but this occurred only after a large number of passages and can have little bearing on the observations made on the primary series.

There is another observation which supports the assumption that these tumours have a multicentric origin — metastases had a pronounced affinity for soft tissue. Many mice in the primary series had multiple skeletal tumours but no metastases in the soft tissues. Two mice actually had 6 skeletal tumours each but no soft tissue metastases. In the transplantation experiments, on the other hand, skeletal metastases were obtained only in mice with a large number of soft tissue metastases. There is, of course, the reservation that metastasis from primary tumours and from subcutaneous tumour transplants may follow somewhat different courses.

REFERENCES

1. *Andersen, A. C. and Goldman, M.:* Pathologic sequelae in beagles following continuous feeding of Sr^{90} at a toxic level. Univ. Calif. Davies, Fourth Annual Progress Report, UCD 104, 1961, 92.
2. *Anderson, W. A. D., Zander, G. and Kuzma, J. F.:* Carcinogenic effects of Ca^{45} and Sr^{89} on bones of CFl mice. Arch. Path. 1956, *62*, 262.
3. *Anderson, W. A. D., Zander, G. and Kuzma, J. F.:* A study of toxic doses of Sr^{90} in the adult rat. Arch. Path. 1956, *62*, 433.
4. *Brachet, J.:* The use of basic dyes and ribonuclease for the cytochemical detection of ribonucleic acid. Quart. J. micr. Sci. 1953, *94*, 1.
5. *Carr, J. and Reimer, M.:* Standard methods of clinical chemistry. Acad. Press 1953, *1*, 75.
6. *Chalkley, H. W.:* Method for the quantitative morphologic analysis of tissues. J. Nat. Cancer Inst. 1943—44, *4*, 47.
7. *Coventry, M. B. and Dahlin, D. C.:* Osteogenic sarcoma. A critical analysis of 430 cases. B. Bone Surg. 1957, *39 A*, 741.
8. *Dahlin, D. C.:* Bone tumours. Charles C. Thomas, Springfield, 1957.
9. *Downie, E. D., MacPherson, S., Ramsden, E. N., Sissons, H. A. and Vaughan, J.:* The effect of daily feeding of Sr^{90} to rabbits. Brit. J. Cancer 1959, *13*, 408.
10. *Engfeldt, B.:* Studies on parathyroidal function in relation to hormonal influences and dietetic conditions. Acta Endocrin. suppl. VI., 1951.
11. *Engström, A., Björnerstedt, R., Clemedson, C.-J. and Nelson, A.:* Bone and radiostrontium. Almqvist och Wiksell, Stockholm, 1957.
12. *Ewing, J.:* A review of the classification of bone tumours. Surg. Gyn. Obst. 1939, *68*, 971.
13. *Finkel, M. P.:* Late effects of internally deposited radioisotopes in laboratory animals. Radiation Research suppl. 1, 1959, 265.
14. *Finkel, M. P., Bergstrand, P. J. and Biskis, B. O.:* The latent period, incidence and growth of Sr^{90} induced osteosarcomas in CFl and CBA mice. Radiology 1961, *77*, 269.
15. *Finkel, M. P. and Biskis, B. O.:* The induction of malignant bone tumours in mice by radioisotopes. Acta Un. Int. Cancer. 1959, *15*, 99.
16. *Finkel, M. P., Biskis, B. O. and Bergstrand, P. J.:* Radioisotope toxicity: Significance of chronic administration. Symposium on "Radioisotopes in the Biosphere" at the Univ. Minn., Minneapolis 1959.
17. *Finkel, M. P., Biskis, B. O. and Scribner, G. M.:* Toxicity of Sr^{90} and of Ca^{45} in mice. III. Effect of Sr^{90} upon life span and neoplasms of bone and the blood-forming tissues. Argonne Nat. Lab. Report ANL-5841, 1957, 51.

18. *Finkel, M. P., Flynn, R. J., Lestina, J. and Czajka, D. M.:* Radiostrontium at "Optimum Carcinogenic Level" in the dog: Effect upon morbidity of total blood exchange shortly after injection. Argonne Nat. Lab. Report ANL-5732, 1957, 15.
19. *Finkel, M. P., Lisco, H. and Brues, A. M.:* Toxicity of Sr^{89} in mice malignant bone tumours. Argonne Nat. Lab. Report ANL-5378, 1955, 106.
20. *Finkel, M. P. and Scribner, G. M.:* Toxicity of Strontium^{90} and Calcium^{45} in mice. II. Status of experiments 625 days after injection. Argonne Nat. Lab. Report ANL-5597, 1956, 16.
21. *Finkel, M. P., Telleksen, B. J., Lestina, J. and Biskis, B.:* The influence of dosage pattern upon the toxicity of Sr^{90} in mice. Argonne Nat. Lab. Report ANL-5732, 1957, 21.
22. *Gomori, G.:* Microscopic histochemistry. Principles and practise. Chicago Univ. Press 1952.
23. *Gomori, G.,* cited from *Bourne, G. H.:* The biochemistry and physiology of bone. Acad. Press, New York 1956.
24. *Gutman, A. B.:* Tumours of the skeletal system: Medical aspects. Bull. N. Y. Acad. Med. 1947, *23,* 512.
25. *Holmberg, B. och Nelson, A.:* Synpunkter på uppfödning av labolatoriedjur. (Swedish). Svenska Läk. Tidn. 1957, *54,* 3261.
26. *Jaffe, H. L.:* Tumours and tumerous conditions of the bones and joints. Lea and Febiger, Philadelphia 1958.
27. *Jaffe, H. L. and Bodansky, A.:* Diagnostic significance of serum alkaline and acid phosphatase values in relation to bone disease. Bull. N. Y. Acad. Med. 1943, 19, ser. 2, 831.
28. *Jowsey, J., Rayner, B., Tutt, M. and Vaughan, J.:* The deposition of Sr^{90} in rabbit bones following intravenous injection. Brit. J. exp. Pathol. 1953, *34,* 384.
29. *Krayevskiy, N. A. and Litvinov, N. N.:* Study of the development in animals of bone tumours arising under the influence of radioactive substances. U. S. Atomic Energy Commission, Translation Series AEC-tr-3661, 1959, *2,* 367.
30. *Kuzma, J. F. and Zander, G.:* Cancerogenic effects of Ca^{45} and Sr^{89} in Sprague-Dawley rats. Arch. Path. 1957, *63,* 198.
31. *Lagerstedt, S.:* Cytological studies on the protein metabolism of the liver in rat. Acta anatom. suppl. IX, 1949.
32. *Lillie, R. D.:* Histopathologic technic and practical histochemistry. The Blakiston Company, New York, Toronto 1953.
33. *Lindbom, Å., Söderberg, G. and Spjut, H. J.:* Osteosarcoma. Acta radiol. 1961, *56,* 1.
34. *Lisco, H., Finkel, M. P. and Brues, A. M.:* Carcinogenic properties of radioactive fission products and of plutonium. Radiology 1947, *49,* 361.
35. *Litvinov, N. N.:* Dynamics of the formation and development of bone sarcomas after damage by radioactive strontium and yttrium. U. S. Atomic Energy Commission, Translation Series AEC-tr-3077, Vopr. Onkol. 1956, *3,* 285.

36. *Litvinov, N. N.:* Morphological changes in bone tissue of rats during chronic intoxication by radioactive strontium. Arkkhv. Pathologii 1957, *19,* 26.
37. *Litvinov, N. N.:* Osteogenic sarcomata in dogs affected by Sr^{90}. Vopr. Onkol. 1959, *5,* 675.
38. *MacDonald, I. and Budd, J. W.:* Osteogenic Sarcoma. II. Roentgenographic interpretation of growth pattern in bone sarcoma. Surg. Gyn. Obst. 1946, *82,* 81.
39. *Martland, H. S. and Humphries, R. E.:* Osteogenic sarcoma in dial painters using luminous paint. Arch. Path. 1929, *7,* 406.
40. *Newman, S. B., Borysko, E. and Swerdlow, M.:* New sectioning techniques for light and electron microscopy. Science 1949, *110,* 66.
41. *Owen, M., Sissons, H. A. and Vaughan, J.:* The effect of a single injection of high dose of Sr^{90} (500—1000 μc/kg) in rabbits. Brit. J. Cancer 1957, *11,* 229.
42. *Owen, M. and Vaughan, J.:* Radiation dose and its relation to damage in the rabbit tibia following a single injection and daily feeding of Sr^{90}. Brit. J. Cancer 1959, *13,* 424.
43. *Palade, G. E.:* A study of fixation for electron microscopy. J. exp. Med. 1952, *95,* 285.
44. *Pearse, A. G. E.:* Histochemistry. Theoretical and applied. J. and A. Churchill, Second edition, 1960.
45. *Pritchard, J. J.:* "The osteoblasts" cited from Bourne, G. H. In the biochemistry and physiology of bone. Acad. Press, New York 1956, 179.
46. *Romeis, B.:* Mikroskopische Technik. Leibniz Verlag, München 1948.
47. *Sissons, H. A. and Vaughan, J.:* Ear tumours in rabbits receiving strontium-90. Nature 1960, *185,* 399.
48. *Strel'tsova, V. N.:* Principles governing the development of osteogenic sarcoma induced by radioactive isotopes. Problems Oncol. 1959, *5,* 1.
49. *Weinmann, J. P. and Sicher, H.:* Bone and Bones. Mosby, St. Louis 1947, 386.
50. *Yokoro, K.:* Morbid anatomical and haematological studies on the effects of internal irradiation with Sr^{89} in mice. Acta haemat. Jap. 1958, *21,* 817.

SUMMARY

Sr^{90} $(NO_3)_2$ was given as a single intraperitoneal dose to 200 male CBA mice. The dose corresponded to 0.67 μC/g. body weight. Osteosarcomas developed in 90 per cent of the mice and lymphatic leukaemia in about 5 per cent. The first and the last of the osteosarcomas were detected 181 and 462 days respectively after the injection of Sr^{90}. The bone tumours were generally multiple with a mean of 2.2 tumours per mouse and in 10 per cent of the animals 4 or more tumours developed. About 90 per cent of the tumours originated in the parts of the skeleton posterior to the 13th thoracic vertebra. Most of the

tumours originated in the long bones, particularly the femur and the tibia, followed by the vertebrae, especially the 6th lumbar and first sacral vertebrae. Metastases, particularly in the lungs and liver, were observed in a few mice; all these originated from boneforming tumours and were themselves boneforming.

The skeletal tumours can be divided into 2 histological types.

I. Predominantly non-boneforming, fibroblastic osteosarcoma.

II. Predominantly boneforming, osteoblastic osteosarcoma.

The majority (83 per cent) were of the osteoblastic type. Both types of tumour could occur in one animal and even within one bone. An argyrophilic stroma was formed in inverse ratio to the amount of bone formed.

The number of mitoses was significantly higher in the fibroblastic than in the osteoblastic tumours; the fibroblastic tumours grew much more rapidly.

Serum alkaline phosphatase activity was highly significantly increased for mice with osteoblastic tumours and highly significantly decreased for mice bearing fibroblastic tumours.

Both types of tumour could be transplanted isologously.

ZUSAMMENFASSUNG

Sr^{90}-induzierte Osteosarkome.

200 männlichen CBA-Mäusen wurde $Sr^{90}(NO_3)_2$ intraperitoneal in Einzeldosen verabreicht. Die Dosierung war 0,67 μC per g. Körpergewicht. 90 % der Mäuse bekamen Osteosarkome und ca. 5 % lymphatische Leukämie. Betreffend der Knochentumoren erhielt man den ersten und letzten Fall 181 respektive 462 Tage nach der Sr^{90}-Injektion. Die Knochentumoren haben eine ausgeprägte Neigung multipel aufzutreten. Die Anzahl der Tumoren im gesamten Material der Maus ist 2,2, in aber nicht weniger als 10 % aller Fälle sind 4 oder mehr Geschwülste beim gleichen Tier vorgekommen. Ca. 90 % der Tumoren sind vom hinteren Teil des Skelettes kaudal des 13. Brustwirbels ausgegangen. Die Mehrzahl der Geschwülste stammt von den Röhrenknochen, vor allem vom Femur und von der Tibia. Danach kommen die Wirbel, wobei besonders der 6. Lenden- und 1. Kreuzwirbel Prädilektionsplätze zu sein scheinen. Metastasen wurden bei einer minderen Anzahl Mäuse beobachtet. Sämtliche waren knochenbildende und gingen von knochenbildenden Muttertumoren aus. Prädilektionsstellen sind Leber und Lunge.

Den histologischen Untersuchungen gemäss kann das Tumormaterial folgendermassen eingeteilt werden:

I. Überwiegend schwach knochenbildende fibroblastische Osteosarkome.

II. Überwiegend stark knochenbildende osteoblastische Osteosarkome.

Die Mehrzahl der Geschwülste (83 %) ist vom osteoblastischen Typ. Tumoren beider Arten kommen gleichzeitig bei ein und demselben Tier vor, sogar an ein und demselben Knochen. Die histo-

logische Untersuchung hat auch das Vorkommen eines silberpositiven Stromas erwiesen, das im umgekehrten Verhältnis zur Knochenbildung steht.

Eine Untersuchung von Mitosfrequenzen zeigt, dass diese signifikant höher in den fibroblastischen als in den osteoblastischen Tumoren ist. Dieses stimmt gut mit der schnellen Zuwachsgeschwindigkeit der fibroblastischen Tumoren überein.

In den osteoblastischen Geschwülsten ist das Vorkommen von alkalischen Serumphosphatasen im Vergleich zum Normalserum stark signifikant erhöht, wogegen eine starke signifikante Senkung beim Vorkommen von fibroblastischen Tumoren vorliegt.

Beide Tumortypen sind isolog transplantabel.

SAMMANFATTNING

Sr^{90}-inducerade osteosarkom.

Till 200 CBA hanmöss har $Sr^{90}(NO_3)_2$ administrerats i intraperitoneala engångsdoser. Doseringen har varit 0,68 μc/g kroppsvikt. 90 % av mössen ha erhållit osteosarkom och ca 5 % lymfatisk leukemi. Vad bentumörerna beträffar erhölls första respektive sista fallet 181 respektive 462 dagar efter Sr^{90}-injektionen. Bentumörerna ha en utpräglad benägenhet att uppträda multipelt. Antalet tumörer per mus i hela materialet är 2,2 men i ej mindre än 10 % av fallen har 4 eller flera tumörer förekommit hos samma djur. Ca 90 % av tumörerna ha utgått från bakkroppsskelettet kaudalt om 13:e bröstkotan. Flertalet svulster emanera från rörbenen, framför allt femur och tibia. Därnäst kommer kotorna, där speciellt 6:e länd- och 1:a korskotorna synes vara predilektionsställen. Hos ett litet antal möss ha metastaser iakttagits. Samtliga ha varit benbildande och emanerat från benbildande modertumörer. Predilektionsställen är lever och lungor.

Enligt den histologiska undersökningen kan tumörmaterialet indelas i:

I. Övervägande svagt benbildande, fibroblastiska osteosarkom.
II. Övervägande starkt benbildande, osteoblastiska osteosarkom.

Flertalet tumörer (83 %) är av osteoblastisk typ. Tumörer av båda typerna förekomma samtidigt hos ett och samma djur t. o. m. inom ett och samma ben. Den histologiska undersökningen har även visat att förekomsten av ett silverpositivt stroma står i omvänd proportion till benbildningen.

En undersökning av mitosfrekvensen visar, att denna är signifikant högre i de fibroblastiska än i de osteoblastiska tumörerna. Detta överensstämmer väl med de fibroblastiska tumörernas snabba tillväxthastighet.

I de osteoblastiska tumörerna är förekomsten av alkalisk serumfosfatas starkt signifikant förhöjt i jämförelse med normalserum, under det att en starkt signifikant sänkning föreligger vid förekomst av fibroblastiska tumörer.

Båda tumörtyperna äro transplantabla isologt.

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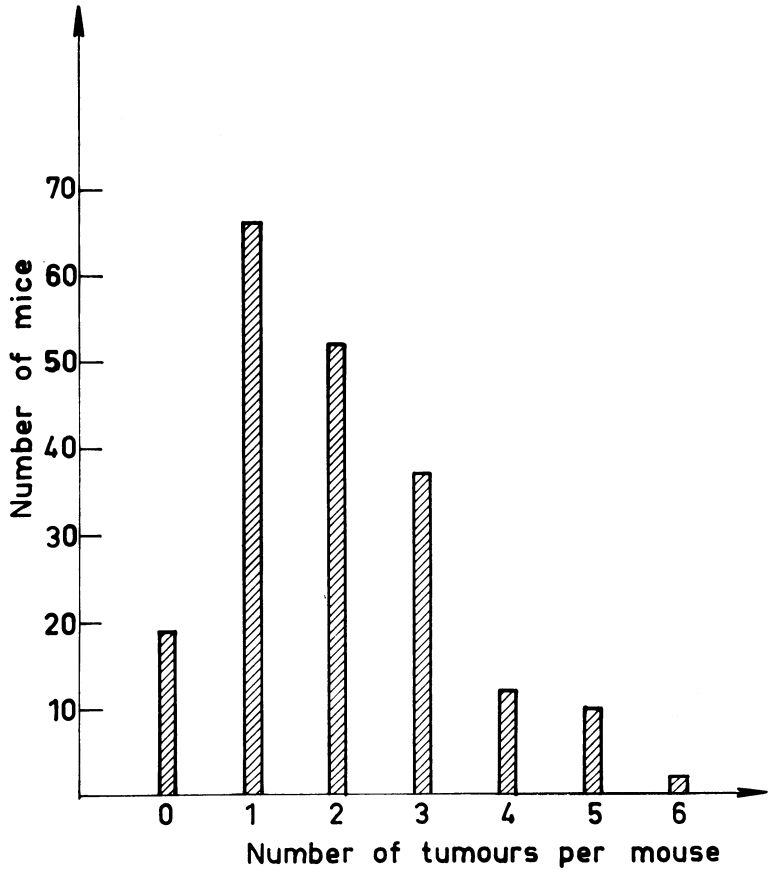

Fig. 1. Histogram showing number of tumours per mouse.

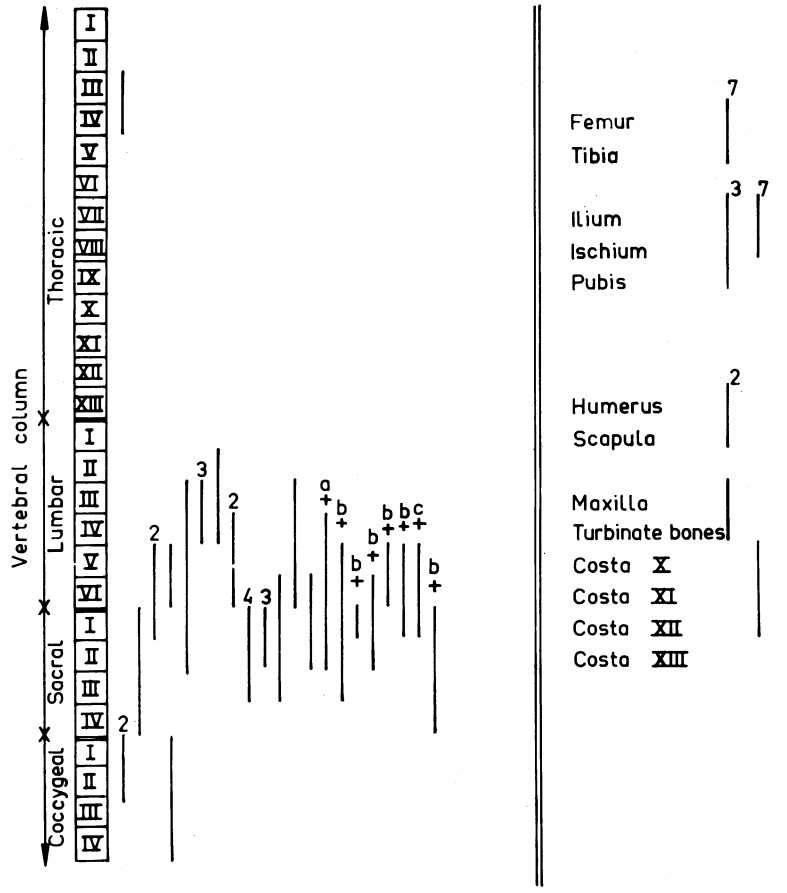

Fig. 2. Site of tumours involving multiple bones. Each line represents one tumour. Figures indicate number of tumours.

a = tumour involving both os ilei
b = „ „ ilium on one side
c = „ „ ilium, femur and tibia on one side.

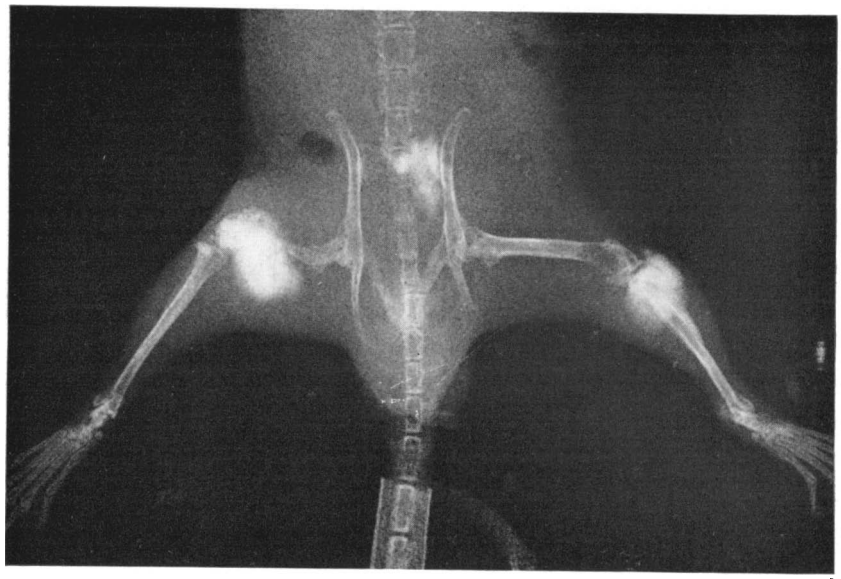

Fig. 3. Roentgenogram of osteoblastic osteosarcomas 274 days after injection of Sr^{90}. Note also the lytic process in distal part of left femur.

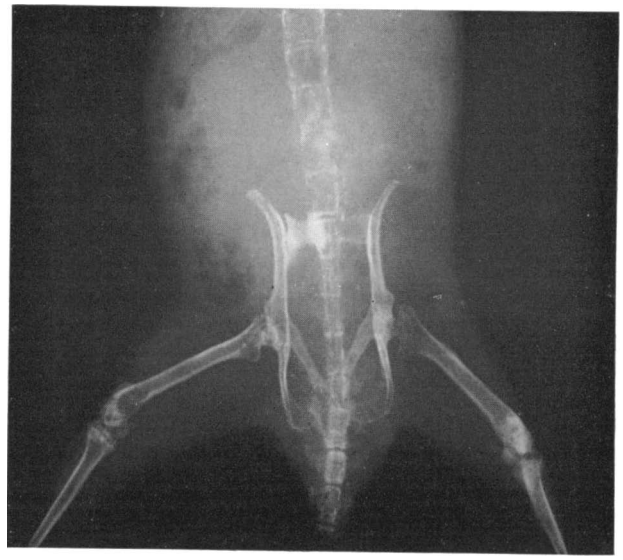

Fig. 4. Roentgenogram of a fibroblastic osteosarcoma in the lumbar vertebrae. Note opacity in transverse process of first sacral vertebra.

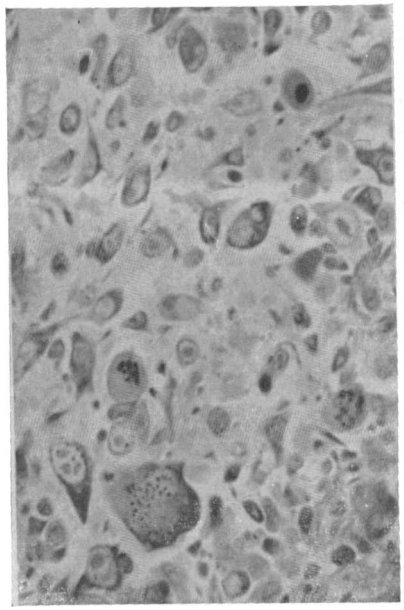

Fig. 5. Strongly pleomorphic fibroblastic tumour. Azure eosinate 400 ×.

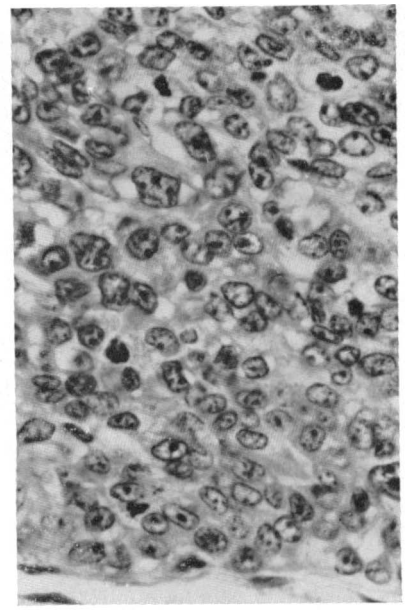

Fig. 6. Fibroblastic tumour showing pleomorphic cells with distinct nucleoli. van Gieson 400 ×.

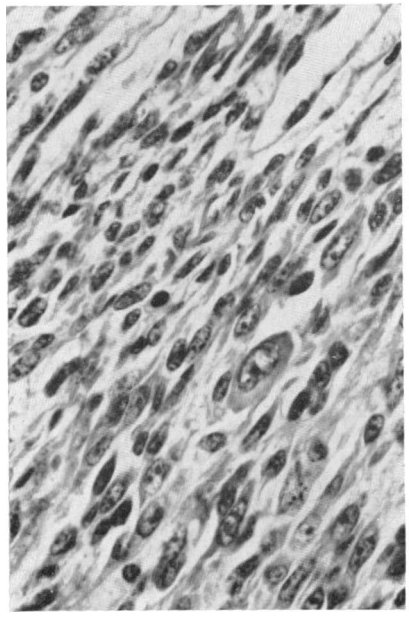

Fig. 7. Fibroblastic tumour with parallel bundles of fusiform cells. van Gieson 400 ×.

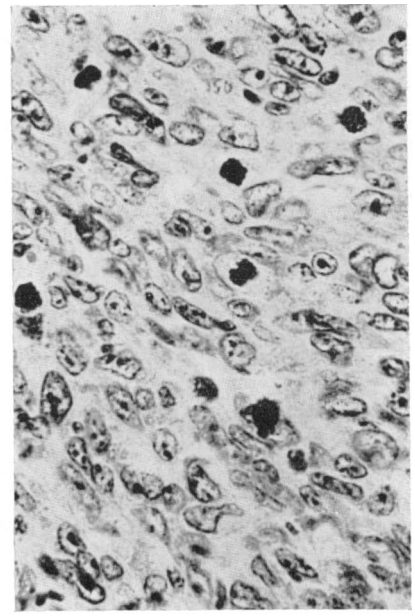

Fig. 8. Fibroblastic tumour with eight mitotic figures in the field. Haematoxylin-eosin 400 ×.

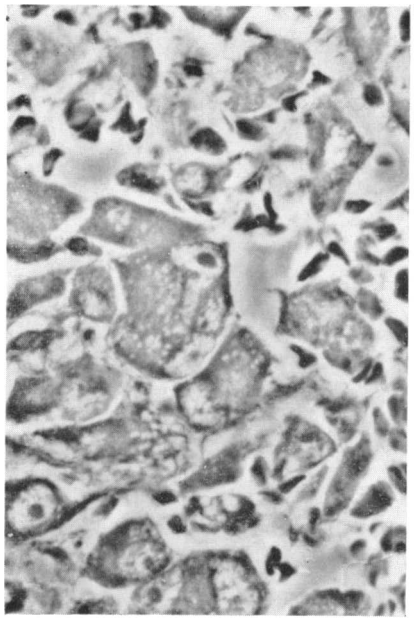

Fig. 9. Fibroblastic tumour showing cytoplasmic vacuolisation. Phase contrast, unstained 700 ×.

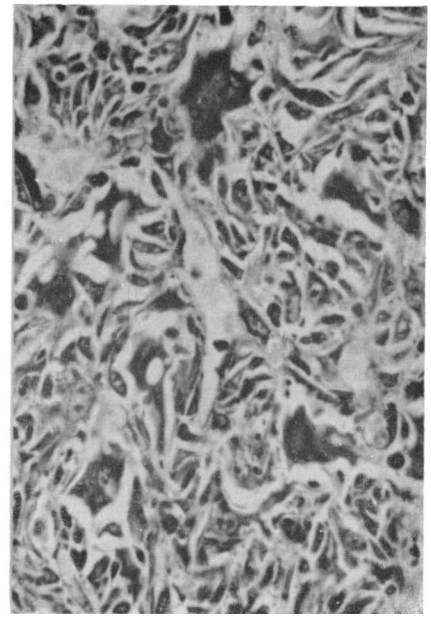

Fig. 10. Osteoblastic tumour with group of osteoclast-like giant cells. Azure-eosinate 100 ×.

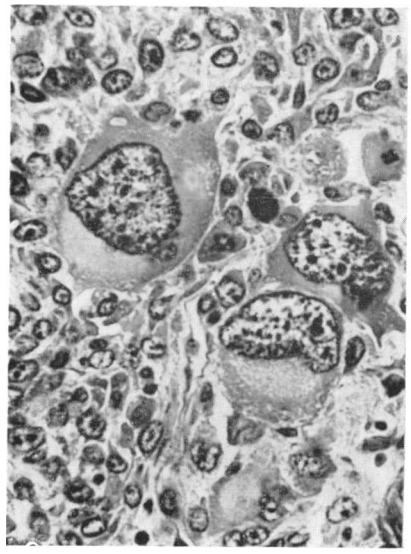

Fig. 11. Group of uninuclear giant cells in a fibroblastic tumour. PAS 400 ×.

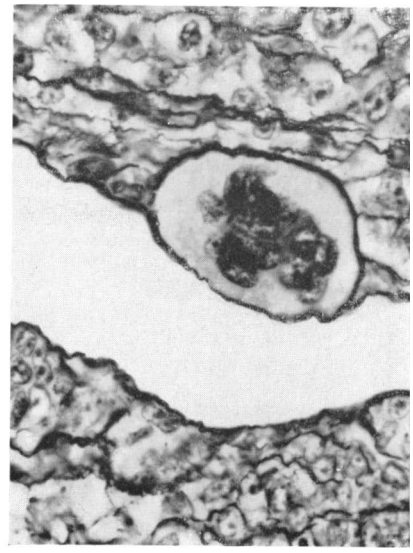

Fig. 12. Multinucleated giant cell surrounded by an argyrophilic sheath in a fibroblastic tumour. Foot and Foot 400 ×.

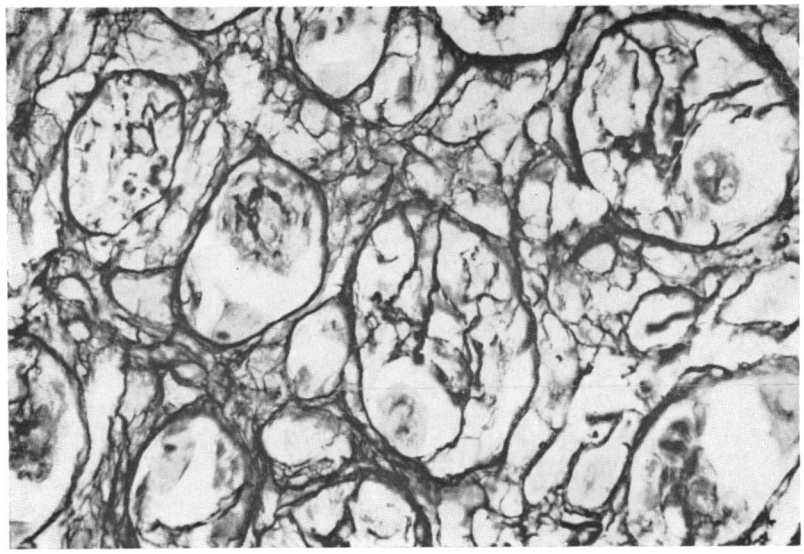

Fig. 13. Fibroblastic tumour demonstrating network of argyrophilic threads surrounding tumour cells. Foot and Foot 400 ×.

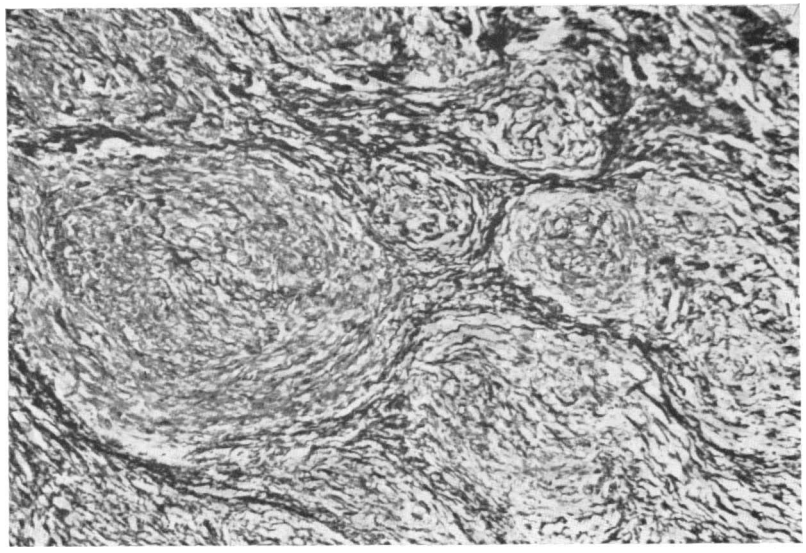

Fig. 14. Fibroblastic tumour with argyrophilic threads arranged circularly and eliptically. Foot and Foot 100 ×.

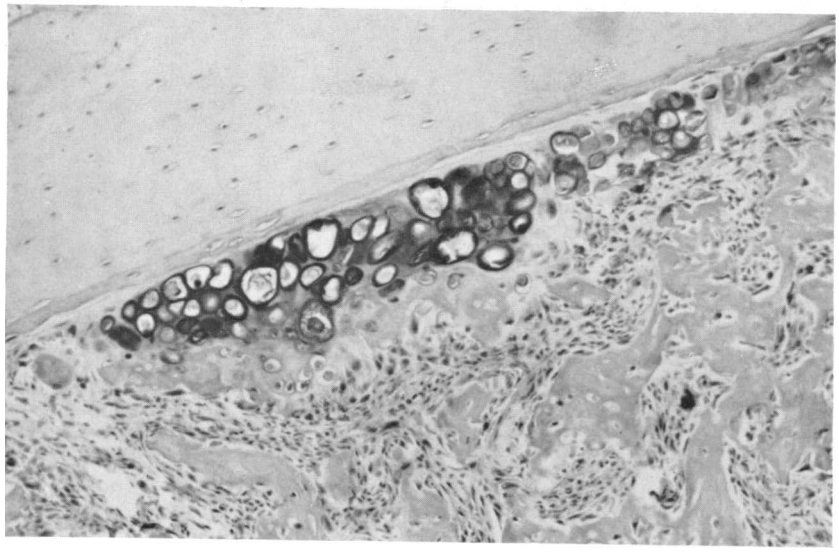

Fig. 15. Osteoblastic osteosarcoma with production of a small island of cartilage adjacent to pre-formed cortical bone. Haematoxylin-eosin 100 ×.

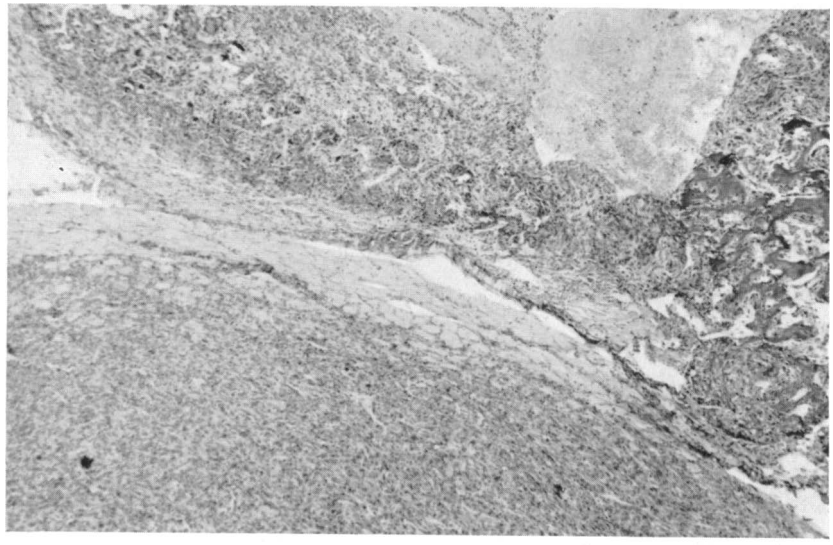

Fig. 16. Section showing an osteoblastic (above) and a fibroblastic osteosarcoma (below) in a femur. Note the strands of muscle tissue separating the two tumours. The general contours of the two tumours can be seen towards the left. van Gieson 40 ×.

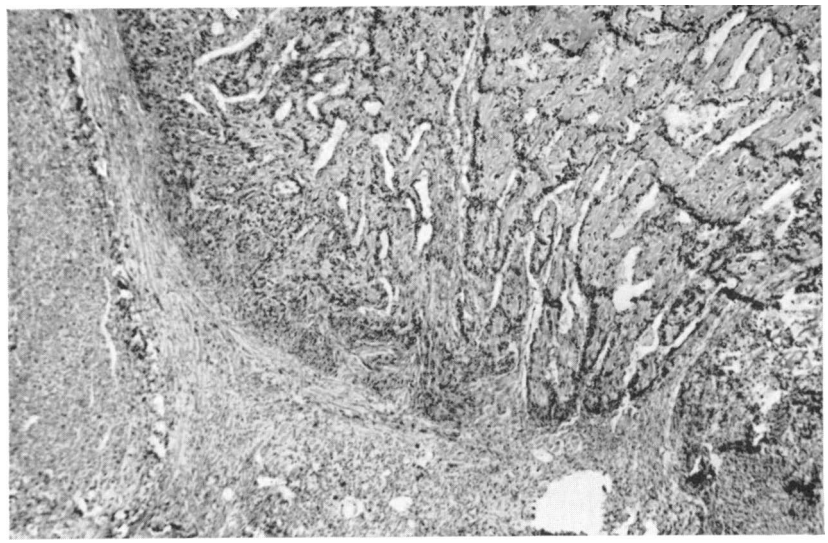

Fig. 17. "Fused" tumour consisting of osteoblastic osteosarcoma from ilium (upper right) and fibroblastic osteosarcoma from femur (left). van Gieson 40 ×.

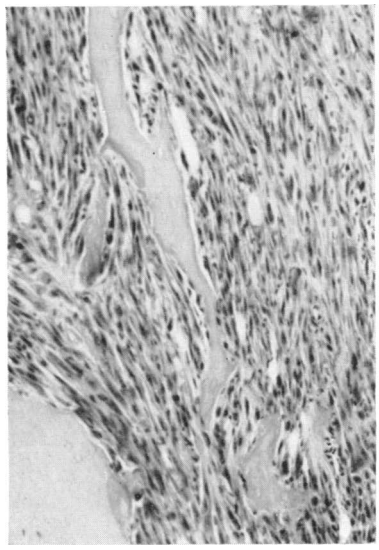

Fig. 18. Fusiform tumour cells with hyperchromatic nuclei from a fibroblastic tumour. Note remnants of pre-formed cortical bone undergoing lysis. Haematoxylin-eosin 100 ×.

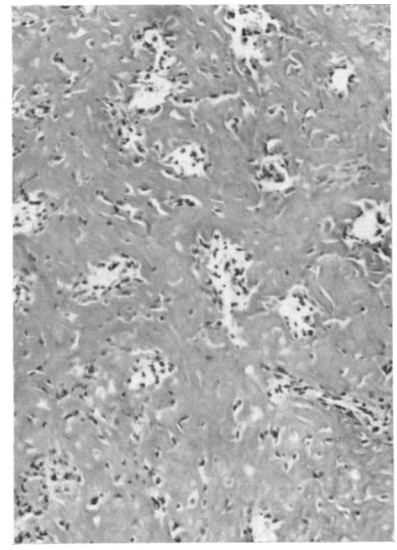

Fig. 19. Osteoblastic tumour with intense formation of tumour bone. van Gieson 100 ×.

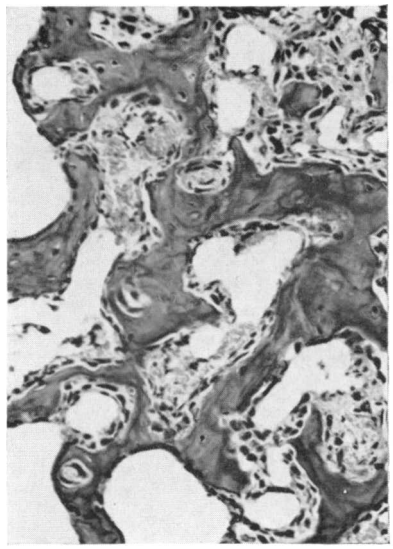

Fig. 20. Osteoblastic tumour. Moderate bone production. Note the layer of osteoblast-like cells with hyperchromatic nuclei outlining the tumour bone. PAS 100 ×.

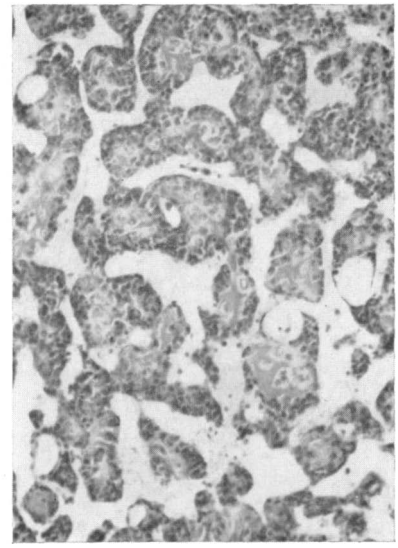

Fig. 21. Osteoblastic tumour. Trabeculae surrounded by osteoblast-like cells and cut in cross-section to give an insular appearance. Haematoxylin-eosin 100 ×.

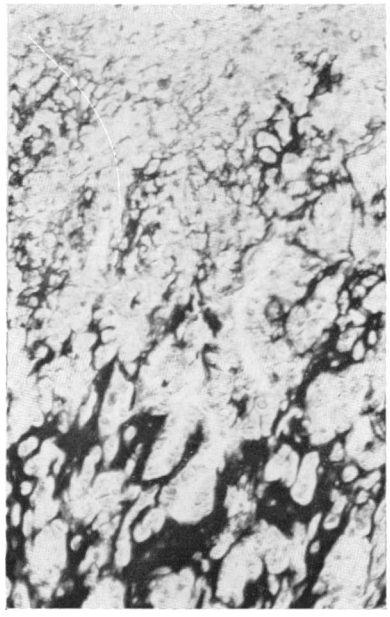

Fig. 22. Osteoblastic tumour showing decreasing bone formation towards the periphery of the tumour. Heidenhain's azan 100 ×.

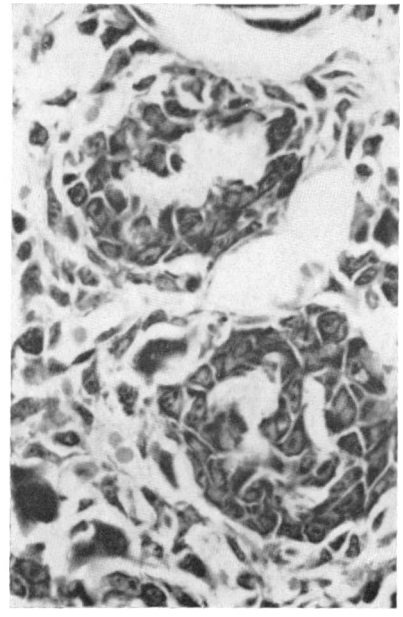

Fig. 23. Osteoblastic tumour. Basophilic osteoblast-like cells surrounding trabeculae cut in cross-section. Azure-eosinate 400 ×.

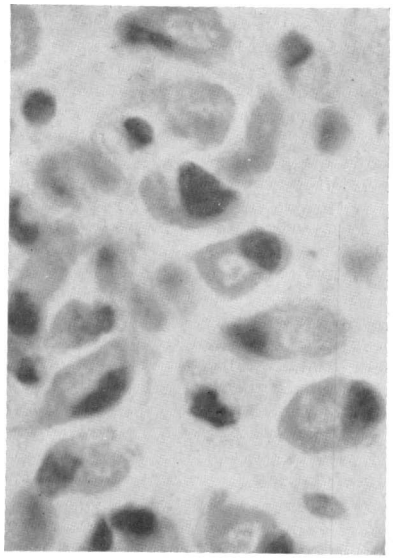

Fig. 24. Osteoblastic tumour showing osteoblast-like basophilic cells with juxtanuclear zones and hyperchromatic, excentric nuclei. Haematoxylin-eosin 100 ×.

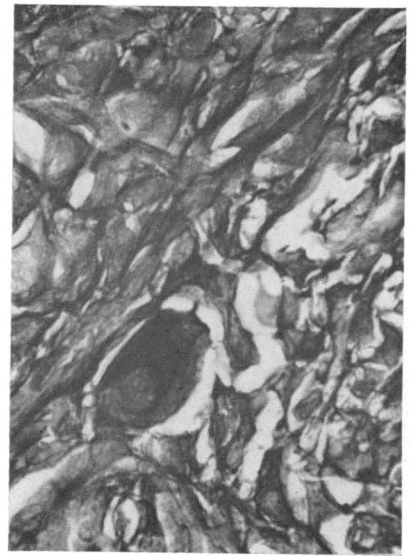

Fig. 25. Osteoblastic tumour. Intense alkaline phosphatase reaction. Fredricsson's Co-method 400 ×.

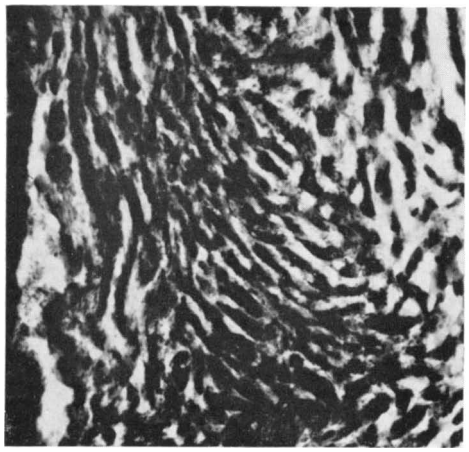

Fig. 26. Osteoblastic tumour. Microradiograph showing strong mineralisation in tumour bone. Ground section 33 ×.

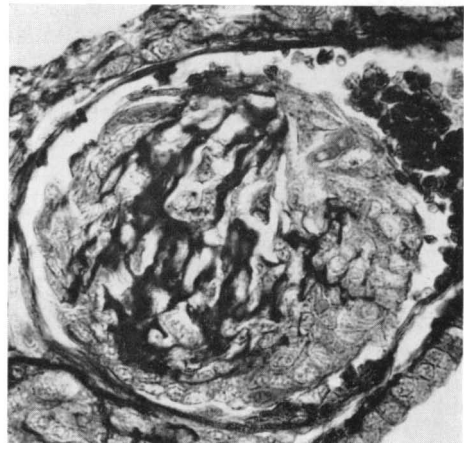

Fig. 27. Lung vessel with tumour embolus from mouse with osteoblastic osteosarcoma. Note production of bone within the vessel. Heidenhain's azan 330 ×.

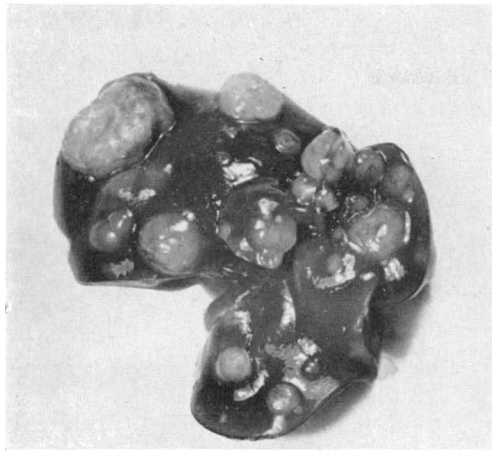

Fig. 28. Liver with multiple metastases from an osteoblastic tumour.

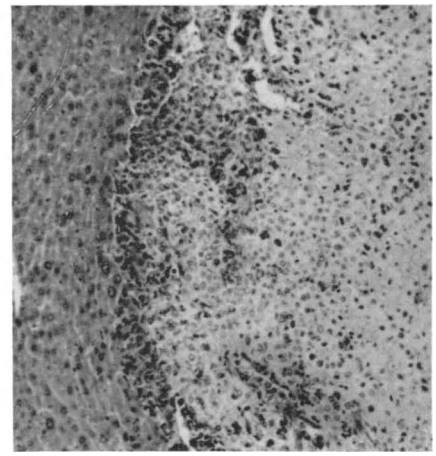

Fig. 29. Metastasis from the same liver. Note strong production of tumour cartilage. Haematoxylin-eosin 83 ×.

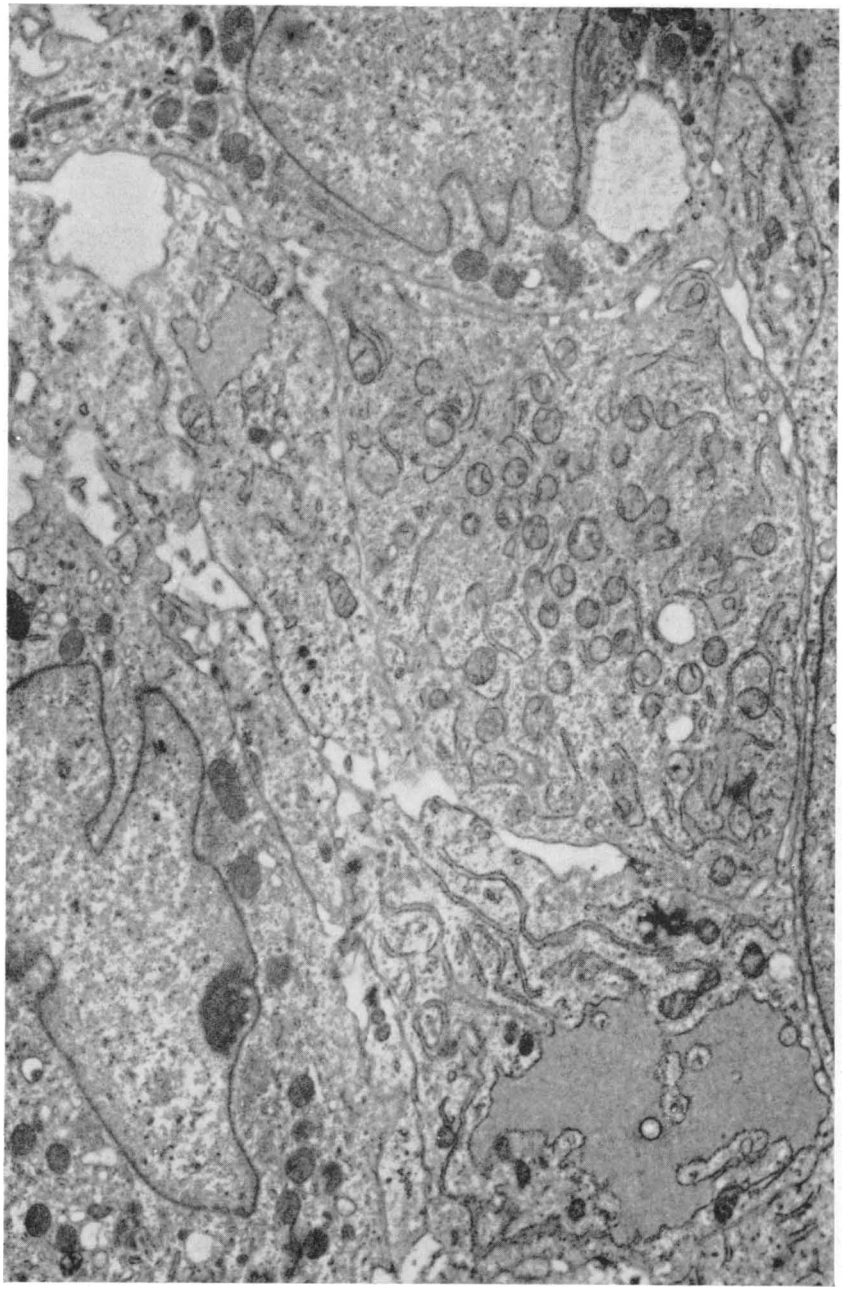

Fig. 30. Fibroblastic tumour showing vesicle formation in endoplasmic reticulum. Many mitochondria and two lobulated nuclei can also be seen. Electron micrograph. 7000 ×.

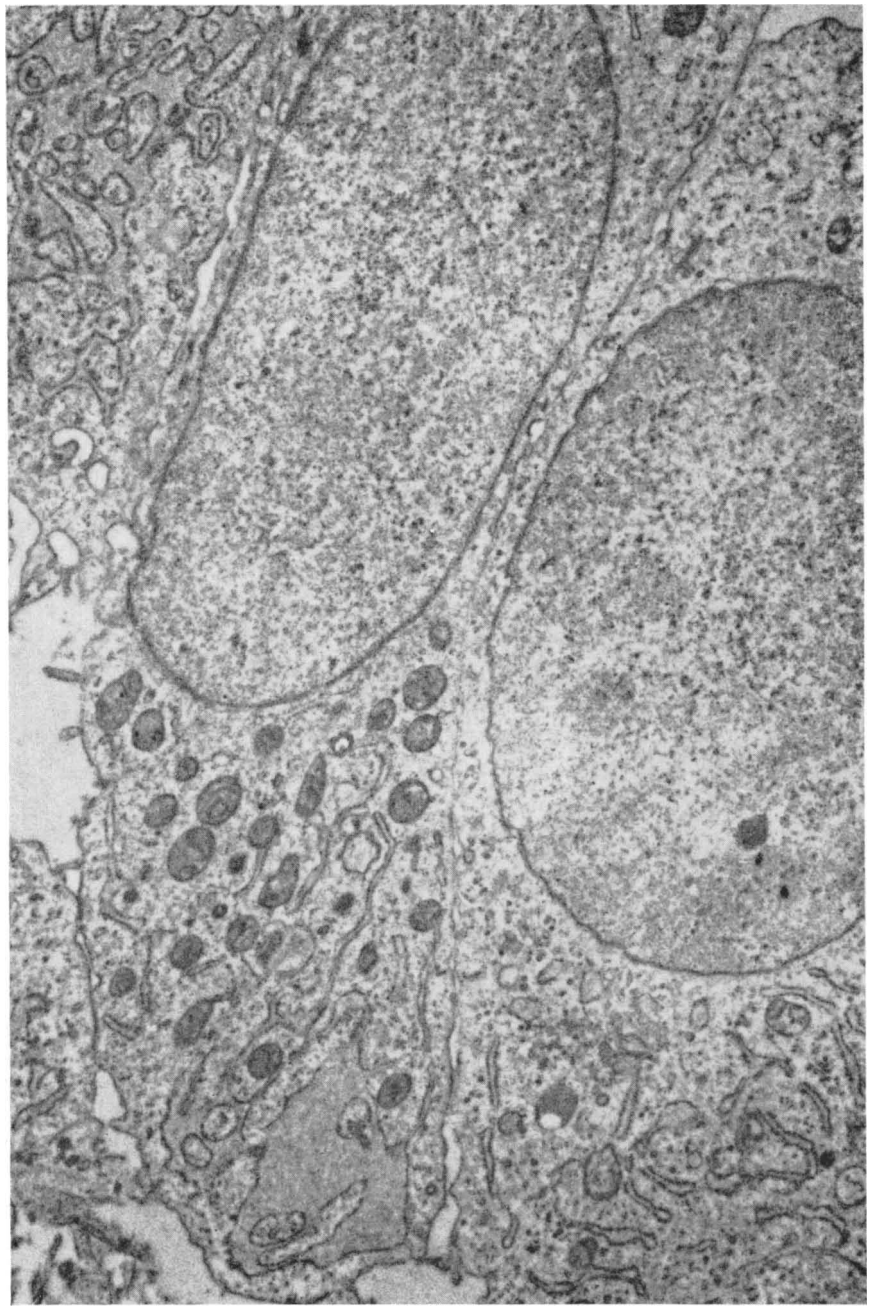

Fig. 31. Fibroblastic tumour. Greatly dilatated canaliculi in endoplasmic reticulum. Double nuclear membrane and several mitochondria. Electron micrograph 12000 ×.

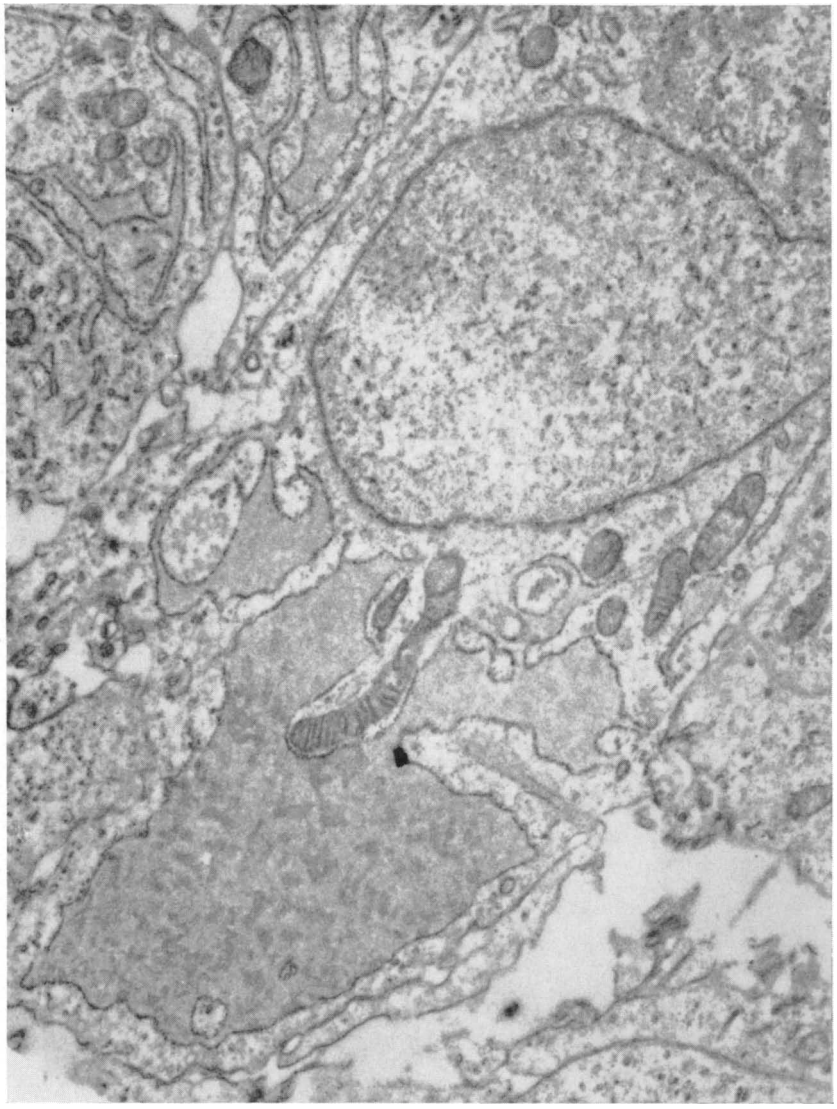

Fig. 32. Fibroblastic tumour. "Lakes" of unidentified substance in endoplasmic reticulum. Note also clearly visible grains of Palade and cristae mitochondriales in longitudinally cut mitochondria. Electron micrograph 15000 ×.