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HISTOGENESIS OF Sr^{90}-INDUCED OSTEOSARCOMAS

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The steadily increasing use of nuclear energy has been followed by, and necessitated study of the biological effects of radioisotopes and especially their carcinogenic properties. Much work has been done on the carcinogenic effects of radiostrontium administered by various routes to different species (see 25). Interest has centred around the relationship between radioactive dose, latent period, tumour incidence, and site of tumour growth (2, 4, 5, 31, 38 and others). What cannot be found are detailed reports dealing with the histogenesis of the tumours. To help fill this gap, serial observations have been made on mice to follow the morphological pattern from the earliest discernible changes after the injection of Sr^{90} to the development of overt tumours and from this to ascertain the tissue in which the tumours originate. These serial observations also cover the period during which proliferation becomes autonomous tumour growth and is able to sustain this autonomy in an environment free from Sr^{90}. These observations also permitted study of the correlation between site of tumour formation and the distribution and relative intensity of radiation.

MATERIAL AND METHODS

Three hundred and fifty male CBA mice, 75 to 85 days old and weighing 21 to 22 g., were injected intraperitoneally with 0.67 μC Sr^{90} $(NO_3)_2$ per gram body weight. Comparable male CBA mice were kept as controls. All mice were fed and kept under uniform conditions (9) and measures were taken to prevent coprophagy.

For study of the changes occurring shortly after the injection of Sr^{90}, mice were killed in groups of 10 after 6, 12 and 24 hours, and after 2, 4, 8, and 16 days. Groups of 10 control mice were killed at comparable periods. Only the femurs were studied in these series.

For study of changes occurring later, mice were killed in groups of 10 at monthly intervals from one to 10 months after the injection of Sr^{90}. Groups of 10 control mice were killed after the corresponding intervals. Before being killed, the mice were weighed, anaesthetised with mebumal®, and examined roentgenologically. All animals were autopsied. Femur, tibia, humerus, the thoracic and lumbar vertebrae, the ribs, and the calotte from each mouse were examined histologically.

As these groups were killed and examined, it became apparent that changes of particular interest for the study of tumour histogenesis appeared 3 to 4 months after the injection of Sr^{90}. For this reason, another 180 mice were injected with Sr^{90} and then killed in groups of 10 at weekly intervals beginning at 105 days after injection and continuing until 224 days. Two control mice were killed at each interval. A femur and the lumbar vertebrae from each animal were examined histologically.

For histological study tissues were fixed in Stieve's fluid (34), decalcified under vacuum in 20 per cent formic acid, dehydrated, embedded in paraffin, and sectioned at 5 μ. All sections were stained with Ehrlich's haematoxylin and eosin (34) and with van Gieson's stain. Particular sections were stained with Lillie's azure-eosinate (15), PAS (32), Foot and Foot's silver (34), and with Ladewig's modification of Mallory's connective tissue stain (34). Alkaline phosphatase activity was demonstrated histochemically by applying Fredricsson's cobalt method (32) to sections decalcified by Greep's method (32) in buffered formic acid at pH 4.9. Control sections were placed in boiling water for 10 minutes to abolish enzyme activity.

RESULTS

Early changes were evident 4 and 8 days after the injection of Sr^{90} as an increase in the number of osteoblasts. At 4 days, this increase was mainly limited to the trabeculae immediately adjacent to the calcification zone of the epiphyseal cartilage. By 8 days the osteoblasts lining the cortical endosteum of the distal femur were enlarged. In both the 4-day and the 8-day specimens

numerous osteoblasts contained cytoplasmic vacuoles (Fig. 13). The osteocytes were intact; no empty lacunae were observed. By 8 days there was an increase in the number of osteoclasts and the formation of Howship's lacunae was especially evident in the periosteum of the distal femur. The blood vessels of the marrow close to the epiphyseal plate were greatly distended and sometimes surrounded by small extravasations, by 4 days. This appearance was even more evident after 8 days and the endothelial lining of many capillaries was obviously thickened. The formation of argyrophilic fibres, at first extremely fine and only visible under phase-contrast, was apparent after 8 days between the trabeculae in the metaphysis (Fig. 14). Formation of these fibres was also apparent adjacent to the endosteum distally in the femur. During this initial period there were no changes in the cells of the epiphyseal plate, but by 4 days there was a distinct decrease in the number of argyrophilic fibres between the columns of cartilage cells and between individual cells (Fig. 9). After 8 days there were some vacuoles in the intercellular substance of the cartilage and even some micronecroses. The mineralised zone of cartilage was now narrow and formation of osteoid tissue was less evident towards the metaphysis.

During the period from 16 days to 2 months osteoblast depletion occurred and became more accentuated with time (Fig. 15) even although small groups of enlarged osteoblasts persisted throughout the period. The cells between and along the metaphyseal trabeculae became fusiform. The numbers of osteoclasts varied somewhat during the period but there was a general tendency towards increase in this region. Many of the osteoclasts lying close to the epiphyseal cartilage had an acidophilic and heavily vacuolated cytoplasm. Perivascular oedema was present throughout the bone marrow. The number of argyrophilic fibres had increased and by 2 months fuchsinophilic collagen fibres became evident. The fibres were clearly visible under phase-contrast.

By one month after injection, the chondrocyte columns in the epiphyseal plate were disorganised and the cells were greatly hypertrophic. Mineralisation had practically ceased within degenerated areas of the mineralisation zone and the cartilage matrix appeared oedematous. These changes became more extensive with time to result in a very irregular epiphyseal plate (cf. Fig. 6). In sections stained with azure-eosinate, the normal-

ly blue nuclei of the chondrocytes stained a deep reddish purple. The number of argyrophilic fibres was still diminished.

From 2 to 4 months after the injection of Sr^{90} the normal trabecular pattern was largely obliterated and partially replaced by coarse-fibred bone, a tight network of strongly basophilic fibres. Few osteocytes were present in the newly-formed bone. The interstices between the islands of newly-formed, coarse-fibred bone and pre-existing trabeculae were largely filled by collagen and argyrophilic fibres (Fig. 16). Large numbers of osteoclasts and widespread breakdown of pre-existing bone were especially apparent at 3 months. At places within the areas of active breakdown of cortical bone there was apposition of newly-formed bone (Fig. 17).

Destruction and disorganisation in the epiphyseal plate were now still more severe than previously (Fig. 6). The width of the plate varied greatly; in places it was only 2 or 3 cells wide.

All these changes seem to be manifestations of radiation injury in the bones. In the femur the changes were most severe in the distal metaphysis but did occur to some extent proximally as well. Changes of the same nature were observed in the tibia, humerus, vertebrae and to a much milder degree, in the bones of the skull.

Phase of tumour formation. The first intramedullary tumour (an osteosarcoma "bud") was observed in the proximal tibia 3 months after the injection of Sr^{90}. Most of the tumours appeared after 4 to 6 months. During the period 7 to 9 months after the injection of Sr^{90} tumour growth continued and resulted in a breaking through of the cortex between 8 and 10 months after injection (Fig. 1 A, B, C). The tumours generally originated at some point along the endosteum. A few osteosarcoma buds were observed in the medullary cavity without any demonstrable connection with the endosteum.

Tumours seldom originated in the metaphyseal region but usually somewhat towards the diaphysis (Fig. 31 A). The pattern was similar for the femur, tibia, and humerus.

Developmental patterns for the main two types of tumours will be described separately. The tumours have been classified as fibroblastic or osteoblastic osteosarcoma as described in a previous publication (25).

Predominantly osteoblastic osteosarcomas. Their development could be followed more readily in the endosteum of the diaphysis

than in the metaphysis where the radiation injury was more severe. Up to 4 months after the injection of Sr^{90} there were no obvious changes in the diaphyseal region but by 4 to 6 months there was an increase in the number of osteoclasts along the endosteum (Figs. 20, 21) and a degree of fibrosis which was succeeded by apposition of coarse-fibred bone. The abundant collagen fibres along the endosteum gave it a ragged appearance (Fig. 22). In places along the endosteum these fibres were gathered into denser bud-like formations (Figs. 23—25). Towards the periphery of these buds the cells were quite pleomorphic and several were in mitosis. The bony tissue in these buds was arranged as trabeculae or fine spicules lined by osteoblasts (Fig. 28). The newly-formed bone was more basophilic than the pre-existing bone. There were few collagen or argyrophilic fibres in the interstices within the buds (Fig. 29) but these were abundant towards the periphery (Fig. 30). The bony tissue of the buds was distinguishable by its stainability and morphology from the islands of completely unorganised coarse-fibred bone which had earlier developed in the metaphysis (Fig. 18). Small foci of osteoblast proliferation were still evident during this period (Figs. 19, 27).

The over-all cellularity of the metaphysis was still depressed, the irregular masses of coarse-fibred bone in this region still persisted (cf. Figs. 16, 17) and the number of osteoblasts varied widely. Bone formation, distinguishable from the previously formed coarse-fibred tissue by stainability and the abundance of osteocytes, now became evident (Fig. 18). At first this bone appeared as a tight network of narrow spicules enclosing osteoblasts. Fine and, later, coarse fibres were abundant at the periphery. The cells at the periphery were pleomorphic (Fig. 26); the number of mitoses varied. In the femur, as was mentioned previously, these osteosarcoma buds seldom developed in the metaphysis but usually a millimeter or so towards the diaphysis.

Predominantly fibroblastic osteosarcoma. Osteoblasts were sparse along great stretches of the endosteum but osteoclasts were abundant. At the same time the bone marrow was aplastic and oedematous and consisted largely of fatty tissue. The reticular cells were enlarged and their nuclei hyperchromatic. A degree of pleomorphism was evident but most of the reticular cells were fusiform with elongated nuclei. The great distension of the sinusoids compressed the reticular tissue (Fig. 40). Endothelial

cells lining the capillaries were also swollen (Fig. 38). Collagen fibres gradually appeared in the thickened sinusoid walls (Fig. 39). Formation of collagen and argyrophilic fibres, arranged in parallel bundles and as irregular strands, increased with time (Figs. 42, 44). Islands of fatty tissue and groups of haemosiderin-containing macrophages were often enclosed in this tissue. The tissue became denser and cell pleomorphism and mitotic activity increased (Figs. 41, 43). Bone was sparse in this tissue, little more than a few narrow strands. From the serial observations it appeared that these tumours developed from what was originally a bone-forming bud. The growing tumour tissue at the periphery gradually lost the property of forming bone. By the time the tumours broke through the cortex they usually consisted of a small intramedullary bone centre surrounded by a broad zone of predominantly fibroblastic tissue.

Intramedullary growth of the osteosarcoma buds. This phase of tumour development, preceding break through of the cortex, generally covered the period 7 to 9 months after the injection of Sr^{90}. Tumour tissue gradually filled the medullary cavity (Figs. 31 B, 47). A tissue made up of strongly pleomorphic cells and abundant collagen fibres and which was gradually ossified was usually situated proximally and distally to the osteosarcoma buds. The width of this tissue collar varied widely and sometimes it was very narrow (Fig. 31 A). The cortex often remained surprisingly intact in spite of the medullary cavity being largely filled out by tumour tissue, particularly in the case of bones containing predominantly osteoblastic tumours with a relatively compact trabecular structure (Figs. 31 B, 32). Some tumours, on the other hand, vigorously infiltrated and broke through the cortex at an early stage while the intramedullary tumour was still relatively small. Tumour cells were observed in greatly dilatated Haversian canals. The predominantly fibroblastic tumours displayed a much greater osteolytic tendency. The cortex could be rapidly broken through and most of the cortical bone in the area destroyed (Fig. 46). Periosteal appositional ossification and small subperiosteal islands of cartilage were sometimes present close to the point where the cortex was broken through (Fig. 46). In some bones multiple osteosarcoma buds developed along the endosteum and gradually formed a confluent tumour mass. If the microtumours happened to be widely separated, these could independently break through the cortex close to their particular

point of origin. The incidence of demonstrable multiplicity for the osteosarcoma buds was greater among the groups of mice killed at 7-day intervals than among those killed at 30-day intervals, an indication that the intramedullary buds rapidly fused (Fig. 2). As for the other bones examined, osteosarcoma buds were demonstrated in the thoracic vertebrae of 4 mice and in the skull bones of one mouse, but never in the ribs.

Extramedullary tumour growth.

The majority of these tumours were observed after 10 months. The first tumour, a fibroblastic osteosarcoma proximally in a tibia, was detected 202 days after the injection of Sr^{90}. Altogether

Table I.

Site, number and type of tumours with an extramedullary extent in 100 mice killed in groups of ten at monthly intervals from one to 10 months after Sr^{90}-injection.

Tumour site	Number of tumours	Tumour type	
		Osteoblastic	Fibroblastic
Femur	11	10	1
Tibia	4	2	2
Humerus	2	2	—
Lumbar vertebrae	6	6	—
Saccral vertebrae	6	6	—
Coccygeal vertebrae	5	5	—
Thoracic vertebrae	2	2	—
Ileum	2	2	—
Skull	3	2	1
Total	41	37	4

Table II.

Number of mice with tumours with an extramedullary extent and total number of tumours in relation to time after injection of Sr^{90}.

Days	Number of mice	Number of tumour-bearing mice	Total number of tumours	Mean number of tumours, tumour-bearing mice
181—210	10	1	1	1.0
211—240	10	2	2	1.0
241—270	10	6	12	2.0
271—300	10	8	26	3.3
	40	17	41	2.4

41 tumours were present in 17 mice. The site, type, and number of tumours are shown in Table I, the time required for tumour induction in Table II, and tumour multiplicity in Fig. 3.

DISCUSSION

The observations made on the present series of mice injected with Sr^{90} accord with known details (13, 14, 16, 17, 18, 21, 24). Little mention, however, has been made of the early changes in the skeleton after the injection of Sr^{90} and the early phases in the histogenesis of tumours. The skeletal changes can suitably be discussed under the following headings.

A. The immediate manifestations of radiation injury.

The first changes observed after the injection of Sr^{90} are in the bone marrow of the metaphysis, the site of great initial accumulation of Sr^{90} (27). Calculations of the radiation dose also give an indication of the intense radiation within this region (2, 4, 20, 30, 38). The early morphological changes in the metaphyseal bone tissue would then seem to be an immediate consequence of radiation. Early radiation injury in bone tissue after external irradiation has been described in detail (6, 22, 23) but relatively little is known of the corresponding changes after internal irradiation (8, 33).

In the present series changes in the metaphyseal bony tissue appeared by 4 days as an increase in the number of osteoblasts followed somewhat later by an increase in the number of osteoclasts, increased formation of argyrophilic fibres, and swelling of vascular endothelium with escape of a fibrin-containing transudate. The increase in the number of osteoblasts between 4 and 8 days was only temporary; by 16 days to one month the number of osteoblasts had declined. The decline in the number of osteoblasts can probably be interpreted as a manifestation of radiation injury since it was accompanied by such morphological changes as the appearance of cytoplasmic vacuoles. According to *Gates* (6), osteoblasts are more radio-sensitive than osteoclasts, a conclusion which is compatible with the observed pattern of changes here. By 2 months after the injection of Sr^{90}, degenerative changes — vacuolisation and an intensely acidophilic cytoplasm

— appeared in the osteoclasts. Concomitantly with the decline in the number of osteoblasts in the metaphyseal region, there was an increase in fusiform cells which formed abundant argyrophilic fibres and even some fuchsinophilic collagen fibres. Fibre formation became more evident between 2 and 3 months at the same time as osteoclast activity and lysis of pre-existing bone were pronounced in the regions exposed to intense radiation (cf. *Jee,* 11). Several reports (1, 20, 29, 33) have mentioned that radiation injury in bone tissue becomes manifest as necrosis of osteocytes and matrix. Changes of this type were rarely seen in the present series. The vigorous breakdown of bone, then, probably reflected the relative radio-resistance of the osteoclasts. As trabeculae and cortical bone were broken down they were replaced by a tissue with relatively few cells but abundant fibres. The extracellular substance with its fibres gradually condensed to form large islands of a strongly basophilic, coarse-fibred bone enclosing only a few osteocyte lacunae. In all likelihood this represents a reparative process. The cells in the areas where radiation was intense (i.e. in the metaphysis) probably had osteoblastic potentialities but under the existing irradiation were incapable of forming normal bone.

The changes in the epiphyseal plate resulting from external irradiation have been described by *Melanotte* (22). In this region changes seemed to develop more slowly after the administration of Sr^{90}, at least in the dose administered here. The cartilage cells were affected so that maturation and arrangement in orderly columns were gradually interfered with to result in suppression of enchondral ossification (Fig. 7) as has been pointed out by *Litvinov* (17) and *Nikitin* (24). There is no ready explanation for the great reduction in the number of argyrophilic fibres between the cell columns which appeared by 4 days after injection. This, like the decrease in alkaline phosphatase activity at 4, 16, and 30 days after the injection of Sr^{90} (Figs. 10, 11), may have reflected abnormalities in the metabolism of the cartilage cells.

B. Development of tumours.

1. *Intramedullary osteosarcoma buds.* The tumours could arise at practically any point along the endosteum of the long bones. But the most common site in the femur, humerus, and tibia was situated a few mm. from the portion of the metaphysis

where radiation was initially greatest and where radiation injury was most severe. Radiation injury was manifest as a morphological disorganization of the epiphyseal plate, depressed enchondral ossification, and increased break down of bone and apposition of a strongly PAS-positive, coarse-fibred bone. The regions in which the majority of the tumours arose corresponded to the regions with severe depletion of bone marrow throughout long periods after the administration of Sr^{90} (26). One possible explanation for the high tumour incidence in this region is that radiation injury was not sufficiently intense to suppress the proliferative capacity of the tissue, to the same degree as in the metaphyseal region. The presence of multiple, spatially independent osteosarcoma buds within a single bone demonstrates the multicentric genesis of the tumours (cf. ref. 27).

2. *Development of the intramedullary buds and the attainment of autonomy.*

From 4 to 6 months after the injection of Sr^{90} there was extensive intramedullary proliferation. The newly-formed tissue became more cellular and pleomorphic cells more abundant. Cellular proliferation, the formation of argyrophilic and collagen fibres, and increased osteoclast activity were now evident in the diaphyseal region where these changes had previously been of minor degree. The level of radioactivity was much lower here than in the metaphysis. This and the fact that the tissues of the diaphyseal region were probably less responsive than these of the growing zone may explain why the changes in the diaphysis did not appear earlier. The successive changes leading to the formation of fibroblastic and osteoblastic tumours have been described above. It is of interest to know just when the osteosarcoma buds become capable of autonomous growth. Changes of the type referred to here as osteosarcoma buds have often been observed (3, 13, 16, 17, 18, 29, 30, 38) but the properties of these buds have not been established. Through transplantation experiments it could be demonstrated that both fibroblastic and osteoblastic buds are capable of autonomous growth. Tissue from the femoral medullary cavity was removed from 15 mice 5 months after the injection of Sr^{90}. Part of the tissue was retained for histological study and part was transplanted either subcutaneously or intraperitoneally into mice of the same CBA strain. Five tissue samples did not happen to contain tumour buds and one

recipient mouse died shortly after injection. The mice which had received transplants of fibroblastic or osteoblastic tumour buds developed osteosarcomas which histologically resembled the parent tissues (Figs. 33—36) by 3 to 4 months after transplantation. The osteoblastic osteosarcoma transplants metastasised to the same degree as did transplants obtained from fully developed extramedullary osteoblastic osteosarcomas. Once formed, then, the osteosarcoma buds can follow their course of development in an environment free of Sr^{90}.

C. Histogenesis.

1. *Osteoblastic osteosarcoma.*

The majority of the predominantly osteoblastic osteosarcomas arise in the endosteum as has been reported previously (3, 10, 13, 16, 28, 38). Only a few tumours have developed in the medullary cavity as islands without any demonstrable contact with the endosteum. Neither in this series nor in previous series (3, 25, 30, 36) did tumours arise in the periosteum. Sr^{90} accumulation is much greater in the endosteum than in the periosteum (27) and, as *Owen* (30) has pointed out, the periosteum is exposed to relatively less radiation than the endosteum because of the cylindrical shape of the bones. It is impossible to assess the importance of other factors such as circulatory arrangements and oxygen supply.

Within the endosteum it is the fusiform cells in the osteogenic connective tissue which probably undergo neoplasia. Within the areas damaged by radiation the recognisable osteoblasts decrease in number and are partly or entirely replaced by fusiform cells which have the property of forming abundant argyrophilic and collagen fibres. These cells may be poorly differentiated osteoblasts or they may be relatively radio-resistant elements. After serial transplantation of originally strongly bone-forming tumours, the osteoblasts lose their distinguishing morphological characteristics and become transformed to fusiform cells which form abundant extracellular fibres (25). The propensity of osteoblasts in an unnatural environment to transform into cells resembling fibroblasts has been observed by *Lombard* (19) in tissue cultures.

2. *Fibroblastic osteosarcoma.*

These tumours can seemingly arise from the same tissue as the osteoblastic tumours and from the reticular cells of the medullary cavity. Tumours which develop from an osteosarcoma bud of osteoblastic type can, as they grow, gradually form tissue of predominantly fibroblastic type. The original osteosarcoma bud is often the sole osteoblastic tissue component by the time the tumour reaches its full extramedullary extent. Tumours which arise from the reticular cells contain little or no bony tissue in their intramedullary portion from the beginning.

D. The mechanism of tumour induction.

It should be pointed out that the skeletal changes are only one facet of the reaction of the body to internal radiation from Sr^{90}. The changes in the bone marrow and blood as well as the complex changes during the initial exposure and even possible effects on the endocrine glands or upon the ageing process can conceivably have certain consequences. The loss of weight which occurred 7 months after the injection of Sr^{90} (Fig. 4) did not depend solely upon tumour growth since comparable loss of weight did not occur in mice which survived long periods with transplanted tumours.

Kaplan's (12) observations indicate that a virus-like agent may have a place in the genesis of leukaemia in mice exposed to ionizing radiation. Radiation-induced leukaemia could be transmitted by means of cell-free extracts. *Gross* (7) has also demonstrated that a virus like agent is associated with naturally-occurring leukaemia in mice and that it can induce leukaemia when injected into newborn mice of an otherwise resistant strain. According to *Sjögren* (35) polyoma virus can also induce osteosarcoma in mice which have not been exposed to radiation.

It is unlikely that the tumours in these series which occurred after the injection of Sr^{90} were indirectly virus induced. Blood serum samples from both tumour-bearing and control mice in these series were examined for the presence of antibodies against polyoma virus. No signs of a serological response to this agent could be detected. Attempts were also made to transmit the osteosarcomas by injecting 250 12-hour-old CBA mice subcutaneously with cell-free extracts prepared in various ways (37) of extramedullary osteosarcomas. No tumours developed during an observation period of one year.

REFERENCES

1. *Andersson, A. C. and Goldman, M.:* Pathologic sequelae in beagles following continuous feeding of Sr^{90} at a toxic level. Univ. Calif., Davis, Fourth Annual Progress Report UCD-104, 1961, 92.
2. *Bensted, J. P., Blackett, N. M., and Lamerton, L. F.:* Studies on the development of radiation-induced bone tumours. Acta Un. Int. Cancer, 1959, *15*, 559.
3. *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.
4. *Finkel, M. P., Bergstrand, P. J., and Biskis, B. O.:* The latent period, incidence and growth of Sr^{90}-induced osteosarcomas in CF1 and CBA mice. Radiology, 1961, *77*, 269.
5. — *and Biskis, B. O.:* Radiocarcinogenesis. Argonne Nat. Lab., Summaries of current work, 1960, Oct. 15, 22.
6. *Gates, O.:* Effects of radiation on normal tissues. XII. Effects on bone, cartilage and teeth. Arch. Path. 1943, *35*, 323.
7. *Gross, L.:* Mouse leukemia: An egg borne virus disease. Acta Haematol. 1955, *13*, 13.
8. *Heller, M.:* „Bone", in Histopathology of Irradiation from External and Internal Sources, edited by W. Bloom, New York, McGraw-Hill, 1948, 70.
9. *Holmberg, B. and Nelson, A.:* Synpunkter på uppfödning av laboratoriedjur. Svensk Läkartidn., 1957, *54*, 3261.
10. *Jee, W. S. S. and Arnold, J. S.:* Effect of internally deposited radioisotopes upon blood vessels of cortical bones. Proc. Soc. exp. Biol. Med., 1960, *105*, 351.
11. — , — : The toxicity of plutonium deposited in skeletal tissues of beagles. Lab. Invest. 1961, *10*, 797.
12. *Kaplan, H. S.:* Radiation-induced leukemia in mice: A progress report in Radiation Biology and Cancer, Univ., Texas Press, Austin, 1958, 289.
13. *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. Book 2, 367.
14. *Kuzma, J. F. and Zander, G. E.:* The histogenesis of osteogenic sarcoma as induced by radioactive calcium and strontium. Amer. J. Path. 1957, *33*, 607.
15. *Lillie, R. D.:* Histopathologic technic and practical histochemistry. The Blakiston Company, New York, Toronto, 1953.
16. *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, 1956, 285.
17. — : Morphological changes in bone tissue of rats during chronic intoxication by radioactive strontium. Arkhiv Patologii 1957, *19*, 26.

18. *Litvinov, N. N.:* A further study of the appearance and development of bone sarcoma in cases of injury by radioactive strontium (Sr^{90}) and by yttrium (Y^{91}). U.S. Atomic Energy Commission, Translation Series AEC-tr-4473, 1959, 157.
19. *Lombard, L. S.:* Cytologic changes in irradiated tissue cultures of osteogenic cells. Argonne Nat. Lab., Summaries of current work, 1960, Oct. 15, 28.
20. *MacPherson, S.:* Radiation damage in bone in relation to radiation dose from Sr^{90}. Radiation Damage in Bone. Internat. Atomic Energy Agency, Vienna 1960, 35.
21. *Makarycheva, R. L.:* Dynamics of the development of osteogenic sarcomas in experiments. U.S. Atomic Energy Commission, Translation Series AEC-tr-4473, 1959, 147.
22. *Melanotte, P. L. and Follis, R. H.:* Histochemical studies on x-irradiated cartilage and bone. (Abstract) Amer. J. Path., 1957, *33*, 607.
23. — , — : Early effects of x-irradiation on cartilage and bone. Amer. J. Path., 1961, *39*, 1.
24. *Nikitin, B. M.:* Morphogenesis of bone tumours occurring under the influence of penetrating radiation. Med. Radiol. 1960, *5*, 13.
25. *Nilsson, A.:* Sr^{90}-induced osteosarcomas. Acta vet. scand. (In press).
26. — : Effects of radiostrontium on blood and bloodforming tissues in mice. Acta vet. scand. (In press).
27. — *and Ullberg, S.:* Uptake and retention of Sr^{90} in mouse tissues studied by whole animal autoradiography and impulse counting. Acta Radiol. (In press).
28. — , — : Uptake and retention of Sr^{90} in Sr^{90}-induced osteosarcomas. Acta Radiol. (In press).
29. *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.
30. — , *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.
31. *Parmley, W. W., Jensen, J. B., and Mays, C. W.:* Skeletal self-absorbtion of beta-particle energy. Some aspects of internal irradiation. Pergamon Press, Oxford 1962.
32. *Pearse, A. G. E.:* Histochemistry, Theoretical and Applied. J. & A. Churchill, London 1960.
33. *Ray, R. D., Thomson, D. M., Wolff, N. K., and Laviolette, D.:* Bone Metabolism. II. Toxicity and metabolism of radioactive strontium (Sr^{90}) in rats. J. Bone Surg., 1956, *38 A*, 160.
34. *Romeis, B.:* Mikroskopische Tecknik. Leibniz Verlag, München 1948.
35. *Sjögren, H. O. and Ringertz, N.:* Histopathology and transplantability of polyoma induced tumours in strain A/Sr and free coisogenic resistant (I.R.) substrains. J. Nat. Cancer Inst. 1962. Personal communication. (In press).

36. *Strel'tsova, V. N.:* Principles governing the development of osteogenic sarcoma induced by radioactive isotopes. Problems Oncol. (U.S.S.R.), 1959, *5*, 1.
37. *Thorell, B. and Yamada, E.:* The distribution of adenosintriphosphatase activity in virus-induced leukemia cells. Biochim. Biophys. Acta 1959, *31*, 104.
38. *Vaughan, J.:* The relation of radiation damage to radiation dose in bone. Radiation Damage in Bone, Internat. Atomic Energy Agency, Vienna 1960, 7.

SUMMARY

Five hundred and thirty mice were injected intraperitoneally with Sr^{90} and then killed in groups at intervals from 6 hours to 10 months later. The mice were examined roentgenologically before being autopsied and histological specimens were prepared from the femur, tibia, humerus, thoracic and lumbar vertebrae, ribs, and calotte.

The first change — an increase in the number of osteoblasts in the metaphysis — was seen at 4 and 8 days after the injection of Sr^{90}. After 8 days there was an increase in the number of osteoclasts. At the same time fibre formation commenced in the marrow adjacent to the endosteum.

From 2 to 4 months after injection abundant argyrophilic and collagen fibres as well as a coarse-fibred, basophilic, acellular bone were formed. By 4 to 6 months, intramedullary tumours, osteosarcoma buds, appeared near the distal epiphyseal plate in the femur and the proximal plates in the tibia and humerus, i. e. adjacent to the regions where the radiation reaction was most severe. The majority of the osteoblastic tumours originated in the endosteum, probably from cells in the osteogenic connective tissue. Fibroblastic osteosarcomas arise from the endosteum and also from reticular cells in the medullary cavity.

The osteosarcoma buds are often multiple along the endosteum of a bone. The intramedullary tumours are autonomous and transplantable from an early stage in their development.

ZUSAMMENFASSUNG

Die Histogenese der Sr^{90}-induzierten Osteosarkome.

530 Mäuse wurden in Gruppen von je 10 Tieren nach 6, 12 und 24 Stunden, 2, 4, 8 und 16 Tagen und monatsweise nach 1 bis 10 Monaten sowie ausserdem jeden siebenten Tag vom 105. bis zum 224. Tage nach der Sr^{90}-Behandlung getötet. Die Mäuse wurden vor der Tötung mittels Röntgen durchleuchtet und danach obduziert. Die histologische Untersuchung umfasste Femur, Tibia, Humerus, Brust, Lendenwirbel, Rippen und Schädeldach.

Die frühesten Veränderungen kennzeichneten sich deutlich durch Osteoblastvermehrung in der Metaphysenregion nach 4 bis 8 Tagen nach der Sr^{90}-Injektion. Nach 8 Tagen ist auch Zunahme der Osteoklasten und beginnende Bildung von phasenkontrastreichen Fädchen sichtbar.

Während der Zeitperiode von 16 Tagen bis zu 2 Monaten nach der Sr^{90}-Injektion beobachtet man eine Abnahme der Osteoblastenanzahl,

und die Zellen in den Hohlräumen zwischen den Trabekeln werden fusiform und fibroblastenähnlich.

2 bis 4 Monate nach der Sr^{90}-Injektion tritt starke Neubildung der argyrophilen und kollagenen Fädchen sowie eine Apposition eines „coarse fibered" stark basophilen acellularen Knochens ein. Nach 4—6 Monaten geschieht eine successive Vermehrung intramedullarer Tumoren, von „Osteosarkomknöpfchen", welche gewöhnlich in einem gewissen Abstand von den Gebieten erscheinen, wo die Strahlreaktion am stärksten ist, d. h. im Anschluss an die Epiphysenplatte distal im Femur bzw. proximal in der Tibia und im Humerus. Der Hauptteil der osteoblastischen Tumoren entwickelt sich vom Endost, wahrscheinlich aus Osteoblastvorstadien im osteogenen Bindegewebe. Fibroblastische Osteosarkome werden in engem Kontakt mit Endosten oder mit Retikularzellen in der Markhöhle gebildet.

Die „Osteosarkomknöpfchen" haben eine ausgeprägte Tendenz, sich multipel am Endost entlang sogar in ein und demselben Bein zu bilden, und es hat sich auch gezeigt, dass diese intramedullaren Tumoren in einem frühen Entwicklungsstadium autonom und transplantabel sind.

SAMMANFATTNING

De Sr^{90}-inducerade osteosarkomens histogenes.

530 möss ha avlivats i grupper om 10 djur i vardera efter 6, 12 och 24 timmar, 2, 4, 8 och 16 dygn samt månadsvis efter 1 t.o.m. 10 månader och dessutom med 7 dagars intervall från 105 t.o.m. 224 dagar efter Sr^{90}-behandlingen. Mössen ha röntgats före avlivningen samt därefter obducerats. För histologisk undersökning har rutinmässigt uttagits femur, tibia, humerus, bröst och ländkotor samt revben och skalltak.

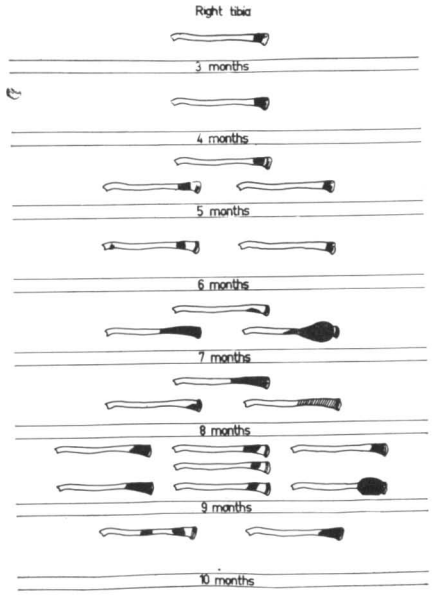
De tidiga förändringarna karakteriseras av en osteoblastökning i metafysregionen iakttagbar 4 och 8 dygn efter Sr^{90}-injektionen. Efter 8 dygn kan även en ökning av osteoklaster samt en begynnande bildning av faskontrastrika trådar iakttagas.

Under tidsperioden 16 dygn till 2 månader efter Sr^{90}-injektionen ses en minskning av antalet osteoblaster och cellerna i hålrummen mellan trabeklerna bli fusiforma och fibroblastlika.

2 till 4 månader efter Sr^{90}-injektionen ses en starkt ökad bildning av argyrofila och kollagena trådar samt apposition av ett „coarse fibered" starkt basofilt, acellulärt ben. Efter 4—6 månader sker en successivt ökad bildning av intramedullära tumörer, „osteosarkomknoppar", vilka vanligen bildas på något avstånd från de områden där strålreaktionen är starkast, d v s i anslutning till epifysplattan i distala femur resp. proximala tibia och humerus. Huvuddelen av de osteoblastiska tumörerna utgå från endostet sannolikt från osteoblastförstadier i den osteogena bindväven. De fibroblastiska osteosarkomen bildas i nära kontakt med endostet eller från retikularceller i märghålan.

„Osteosarkomknopparna" ha en utpräglad tendens att bildas multipelt längs endostet inom ett och samma ben, och det har även visats att dessa intramedullära tumörer äro autonoma och transplantabla i ett tidigt utvecklingsstadium.

(Received April 4. 1962).



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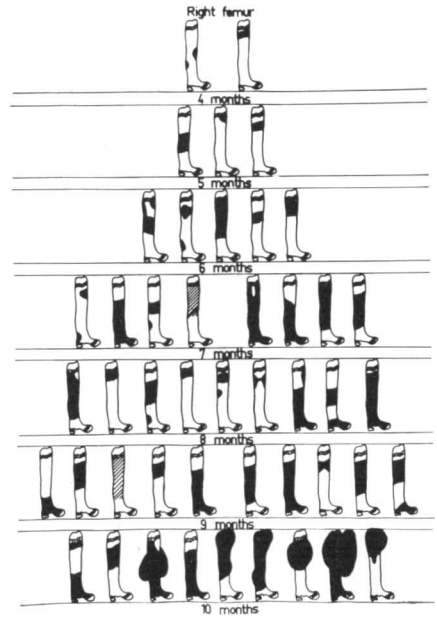

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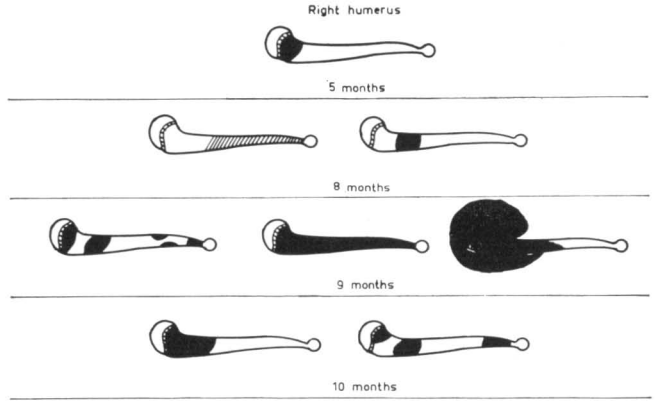


C

Fig. 1. A. B. C. Localisation and number of intramedullary osteosarcoma buds and osteosarcomas with an extramedullary extent different times after injection of Sr^{90}. Ten mice were examined on each occasion.

Predominantly osteoblastic osteosarcoma. ▬

Predominantly fibroblastic osteosarcoma. ▥

A. Right tibia. — B. Right femur. — C. Right humerus.

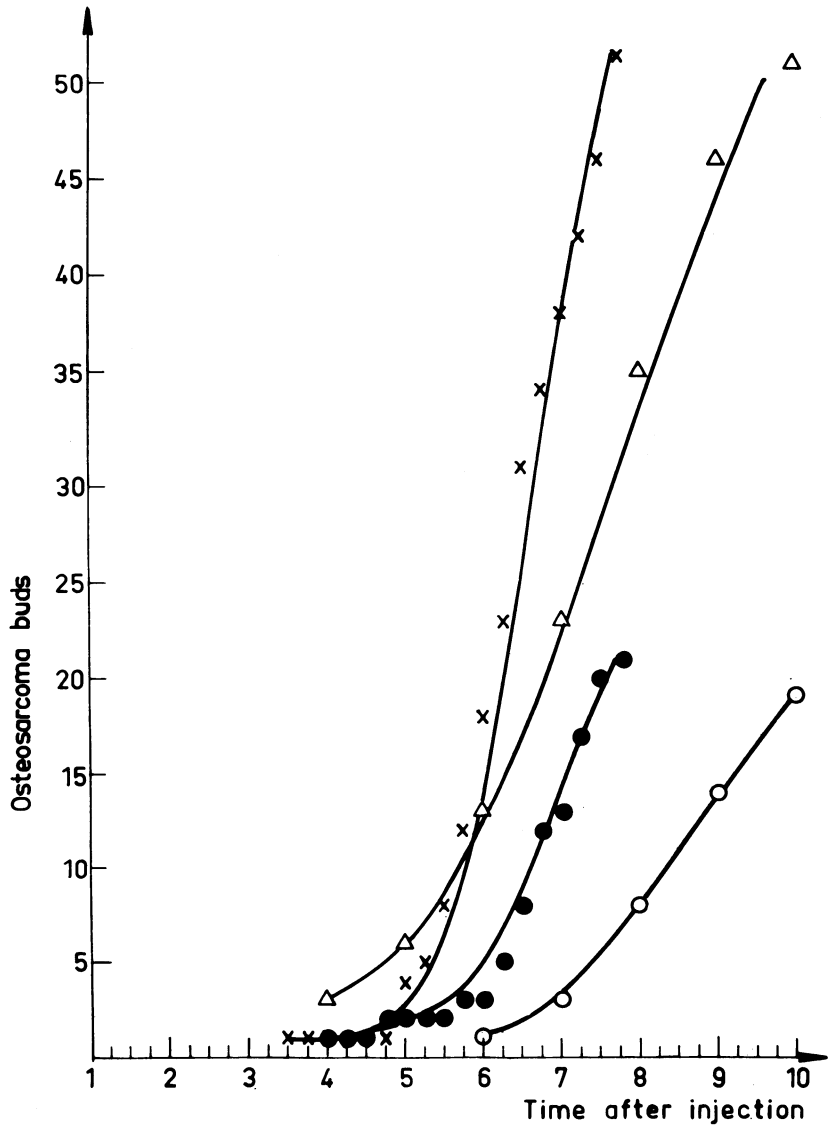


Fig. 2. Occurrence of osteosarcoma buds after injection of Sr^{90}. Ten mice were examined on each occasion. Osteosarcoma buds in lumbar vertebrae — mice killed at weekly (•——•) and at monthly (o——o) intervals. Osteosarcoma buds in right femur — mice killed at weekly (x——x) and at monthly (△——△) intervals.

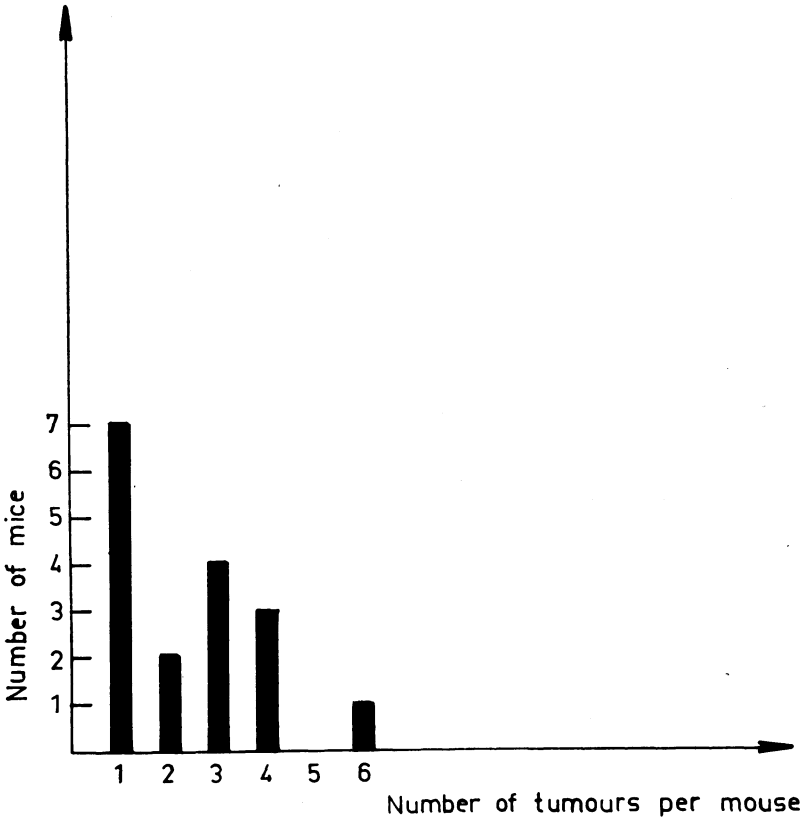


Fig. 3. The incidence of multiple tumours with an extramedullary extent.

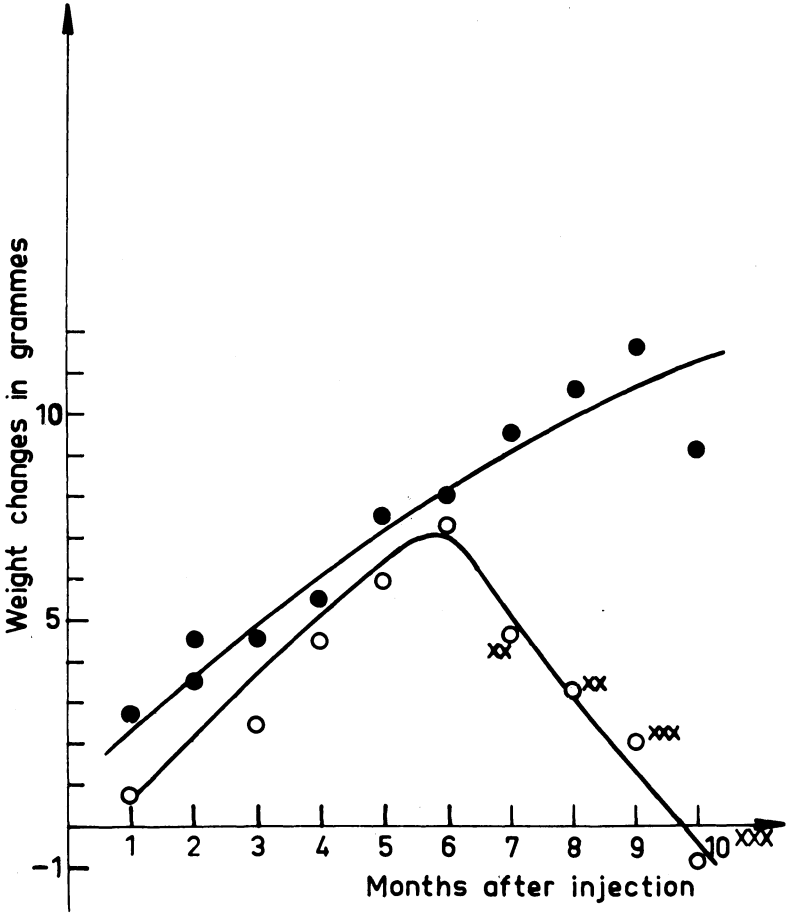


Fig. 4. Monthly weight changes in control groups (•——•) and in Sr^{90}-injected groups (o——o). Each value represents the mean weight change of 10 animals.

** Significant ($0.01 > p > 0.001$) decrease in weight.
*** Highly significant ($p < 0.001$) decrease in weight.

Fig. 5. Normal epiphyseal plate from an 80-day-old mouse. The cartilage cells are arranged regularly in parallel columns; abundance of cartilage remnants in the primary trabeculae. van Gieson, × 100.

Fig. 6. Epiphyseal plate of the femur, 3 months after the injection of Sr^{90}. The plate is very irregular and the cartilage cells are no longer arranged in columns. The cartilage cells vary in size; many of them are greatly hypertrophied. Few osteoblasts along the primary trabeculae but formation of abundant collagen. van Gieson, × 100.

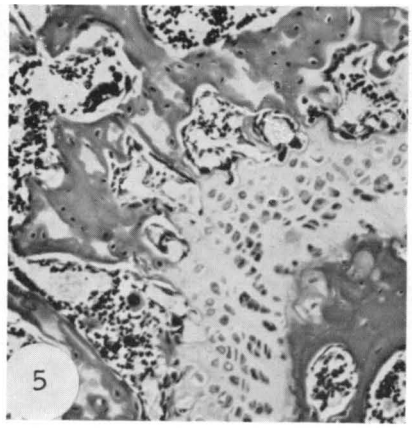
Fig. 7. Epiphyseal plate, femur, 8 months after the injection of Sr^{90}. Enchondral ossification has ceased. The majority of the cells in the epiphyseal plate are dead. van Gieson, × 100.

Fig. 8. Normal epiphyseal plate, femur, 80-day-old mouse. Abundant argyrophilic fibres between the cartilage cells. Foot and Foot, × 250.

Fig. 9. Epiphyseal plate, femur, 4 days after the injection of Sr^{90}. Argyrophilic fibres now much less evident. Foot and Foot, × 250.

Fig. 10. Normal epiphyseal plate, 80-day-old mouse. High alkaline phosphatase activity in the zones of maturation and calcification. Fredricsson's cobalt method, × 100.

Fig. 11. Epiphyseal plate, femur, 16 days after the injection of Sr^{90}. Enzyme activity has decreased in comparison with that in Fig. 10. Fredricsson's cobalt method, × 100.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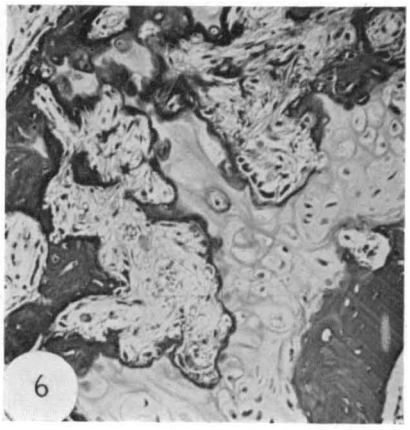
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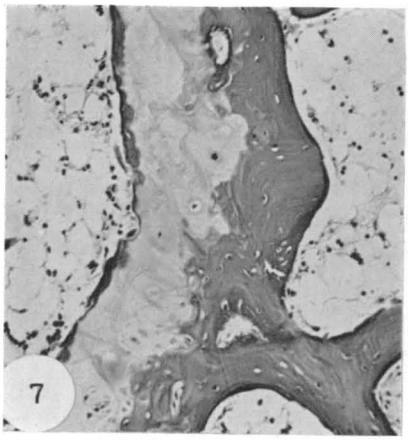
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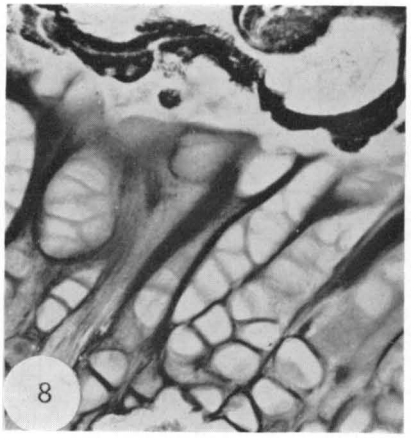
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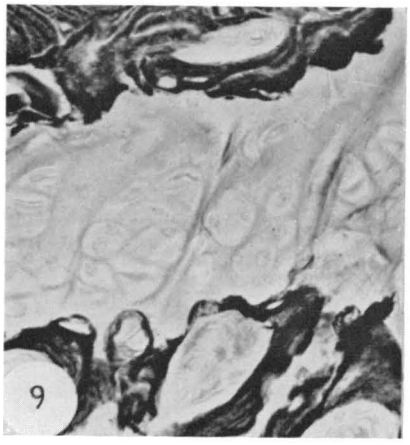
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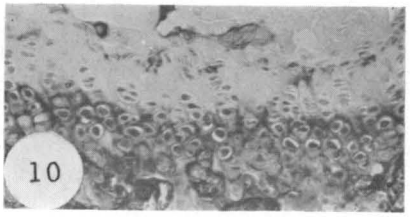
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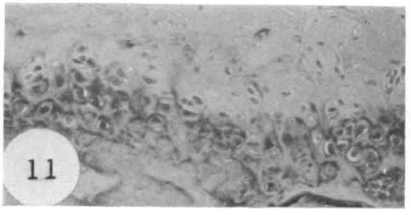
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Fig. 12. Normal metaphysis, femur, 85-day-old mouse, with osteoblasts lining the trabeculae. van Gieson, × 400.

Fig. 13. Metaphysis, femur, 4 days after the injection of Sr^{90}. Increased number of osteoblasts, several of which display cytoplasmic vacuoles. van Gieson, × 400.

Fig. 14. Metaphysis, femur, 8 days after the injection of Sr^{90}. Note the formation of fine fibres in the interstices between the trabeculae. Phase-contrast, × 400.

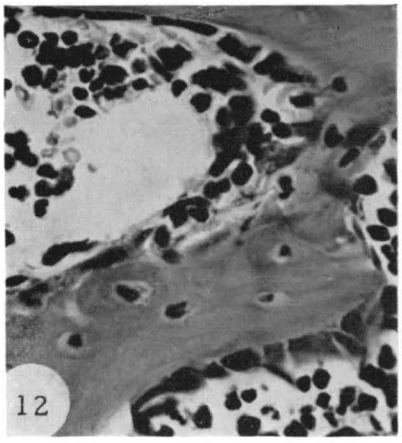
Fig. 15. Metaphysis, femur, 16 days after the injection of Sr^{90}. Reduction in number of osteoblasts and in marrow cellularity. Collagen fibres scarcely evident. van Gieson, × 400.

Fig. 16. Metaphysis, femur, 3 months after the injection of Sr^{90}. Abundant collagen fibres, few cells. van Gieson, × 400.

Fig. 17. Distal femur, 4 months after the injection of Sr^{90}. Compact bone and PAS-positive, acellular, coarse-fibred bone. PAS, × 250.

Fig. 18. Metaphysis, femur, 6½ months after the injection of Sr^{90}. Upper right, normal trabeculae; upper left, newly-formed coarse-fibred basophilic bone. Centre, formation of a small osteosarcoma bud. Note the abundant cellularity. van Gieson, × 100.

Fig. 19. Enlargement of portion of Fig. 18 showing a group of proliferating osteoblasts. van Gieson, × 400.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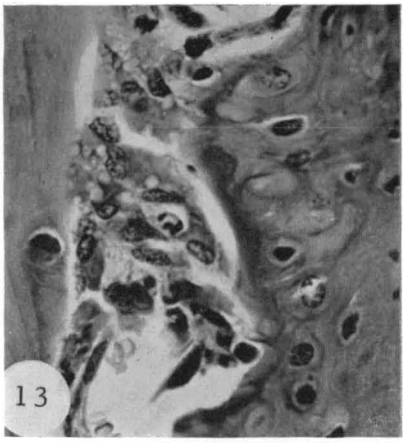
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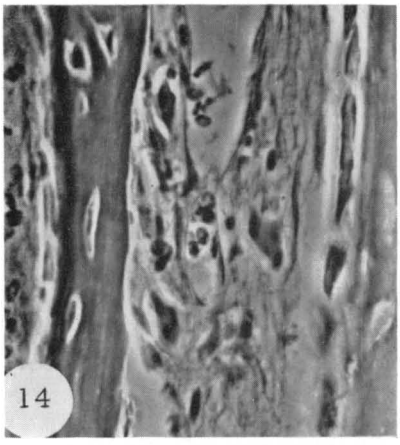
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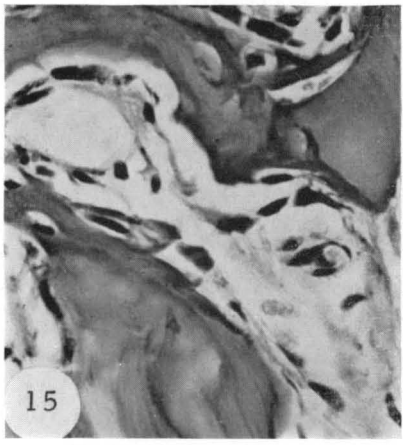
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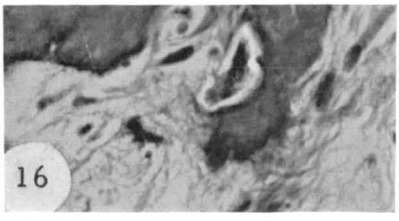
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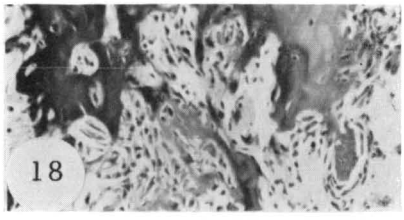
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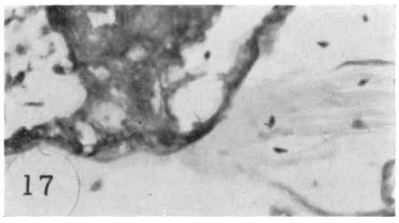
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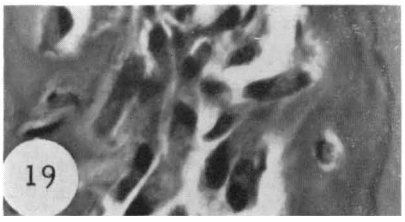
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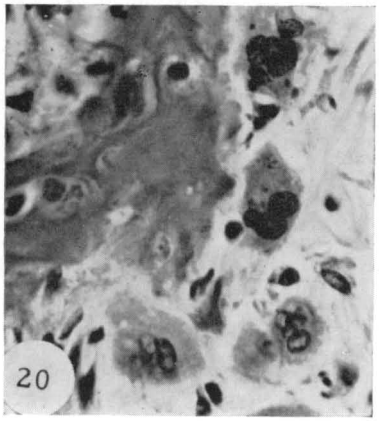
Fig. 20. Vertebra, diaphysis, 7 months after the injection of Sr^{90}. Increased number of osteoclasts and the breaking down of newly-formed bone. van Gieson, × 400.

Fig. 21. Femur, diaphysis, 5 months after the injection of Sr^{90}. Note the osteoclasts along the endosteum. H & E, × 400.

Fig. 22. Femur, diaphysis, 5 months after the injection of Sr^{90}. Formation of bone and collagen fibres along the endosteum. van Gieson, × 100.

Fig. 23. Osteosarcoma bud, 6 months after the injection of Sr^{90}. Femur diaphysis. Note the large osteocyte lacunae in the newly-formed bone and the proliferation of osteoblasts. van Gieson, × 100.

Fig. 24. Tibia, diaphysis, 5 months after the injection of Sr^{90}. Apposition of bone with a spur-like projection at the apex of the newly-formed bone. van Gieson, × 250.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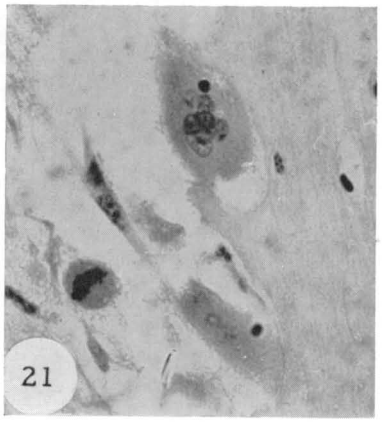
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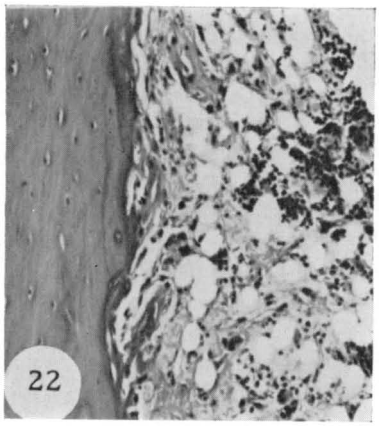
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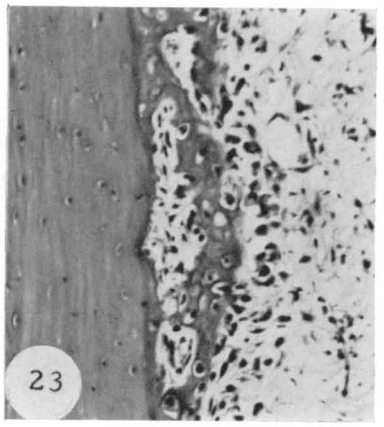
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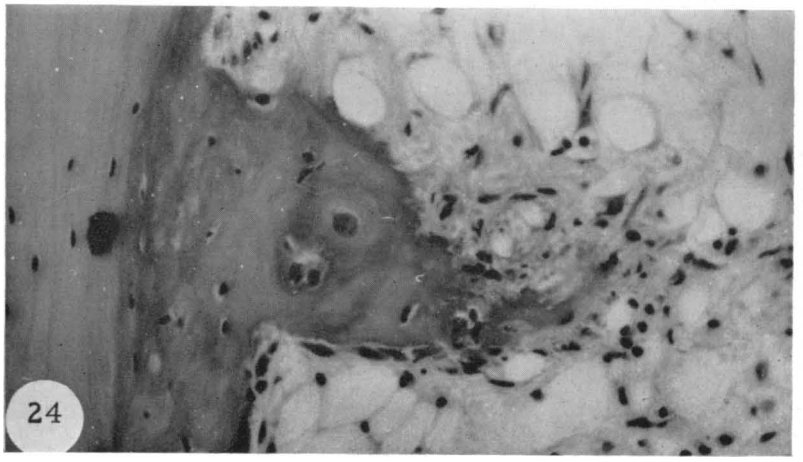
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Fig. 25. Femur, diaphysis, 4 months after the injection of Sr^{90}. An osteosarcoma bud. van Gieson, × 100.

Fig. 26. Enlargement of a portion of Fig. 26. Proliferation of pleomorphic cells at the periphery of the bud. Azure-eosinate, × 400.

Fig. 27. Proximal humerus, 5 months after the injection of Sr^{90}. Proliferation of osteoblast-like cells in a bud. van Gieson, × 400.

Fig. 28. Enlargement of portion of Fig. 23. Lacunae containing large, hyperchromatic osteocytes in newly-formed bone. Proliferation of osteoblasts. van Gieson, × 400.

Fig. 29. Femur diaphysis, 7½ months after the injection of Sr^{90}. Few argyrophilic fibres between the bony trabeculae in central part of an osteosarcoma bud. Foot & Foot, × 250.

Fig. 30. Same bone as in Fig. 29 showing more abundant argyrophilic fibres at the edge of the osteosarcoma bud. Foot & Foot, × 250.

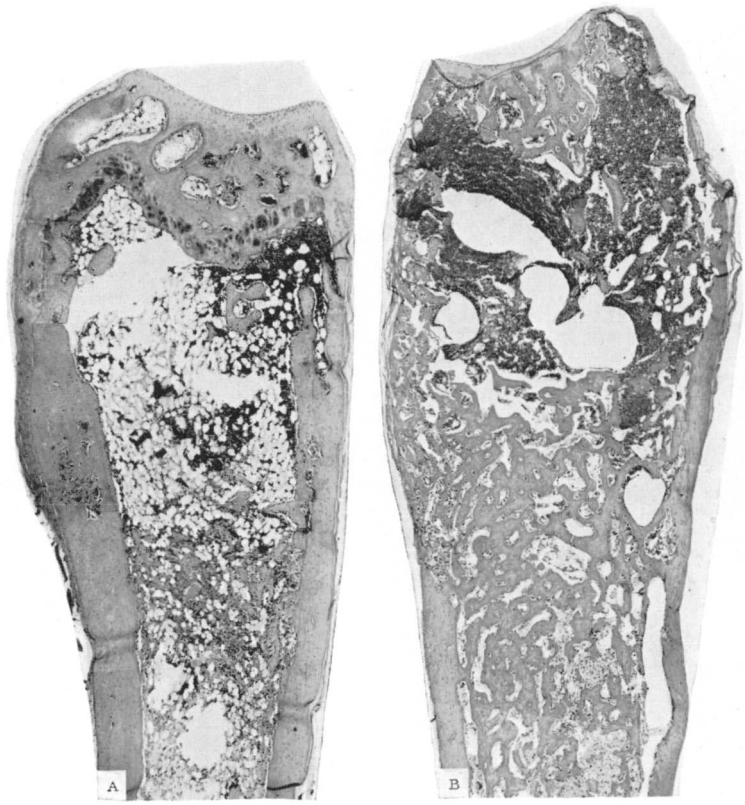
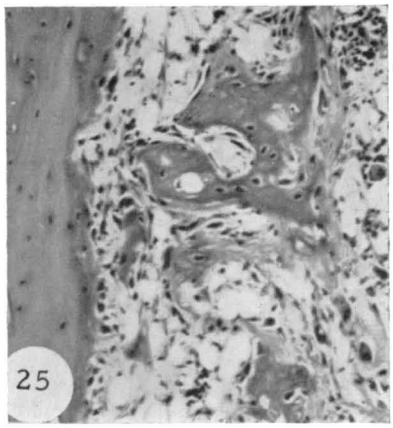


Fig. 31. Femur, (A) 6 months and (B) 8 months after the injection of Sr^{90}, showing different phases in the development of tumours. The osteosarcoma bud in A occupies the usual site for these buds in the femur and in the humerus and tibia. Regenerating bone marrow distally in both A and B. Very little destruction of cortical bone in B in spite of the medullary cavity being practically filled with tumour tissue. van Gieson, × 20.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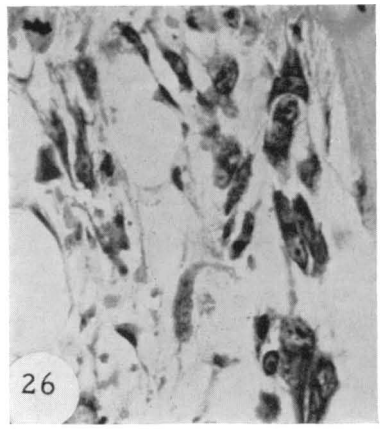
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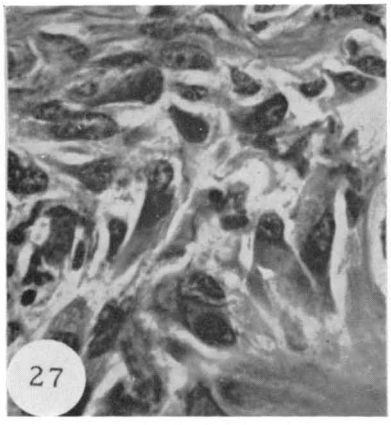
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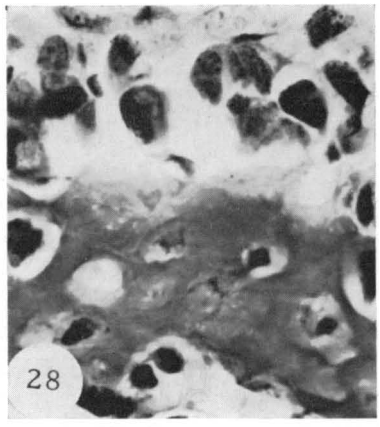
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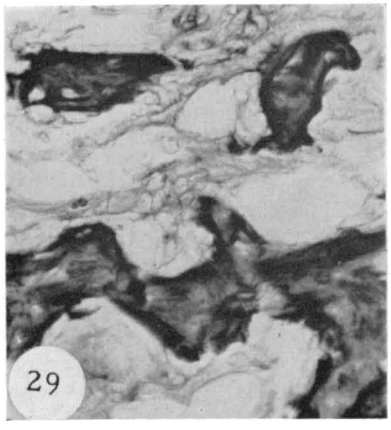
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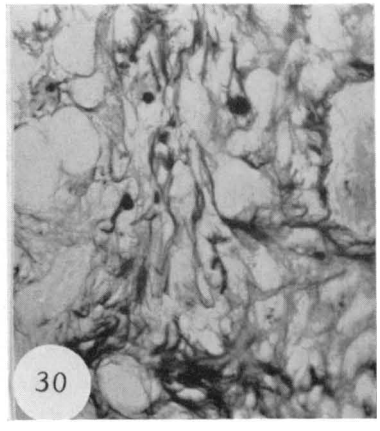
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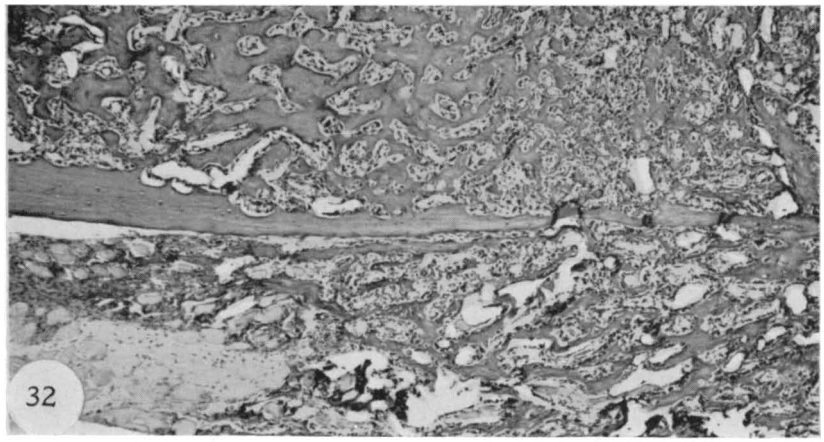
Fig. 32. Osteoblastic osteosarcoma 10 months after the injection of Sr^{90}. Extension through the cortical bone. van Gieson, × 40.

Fig. 33. Osteosarcoma bud in the diaphysis of the femur, 5 months after the injection of Sr^{90}. This bud was excised and transplanted subcutaneously in another mouse. van Gieson, × 100.

Fig. 34. Transplant of tumour shown in Fig. 33. In 5 months the transplant had attained a diameter of 2 cm. Compare the histological appearances of the parent tumour and transplant. van Gieson, × 100.

Fig. 35. Bud (1×1 mm.) of fibroblastic osteosarcoma in the distal femur, 6 months after the injection of Sr^{90}. Bud transplanted intraperitoneally into another mouse. van Gieson, × 400.

Fig. 36. By 5½ months after transplantation of the bud shown in Fig. 35 there were numerous tumours in the abdominal cavity. Compare the histological appearance of the parent tumour and the transplants. van Gieson, × 400.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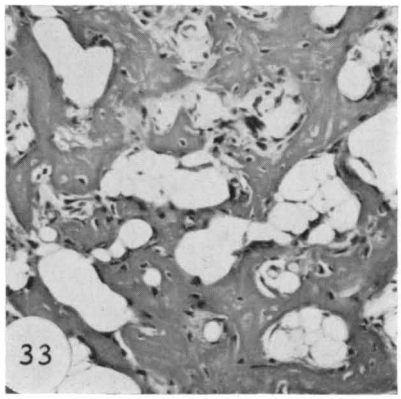
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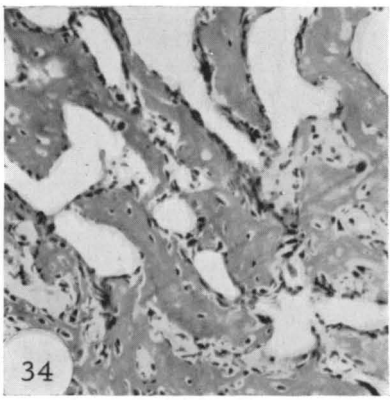
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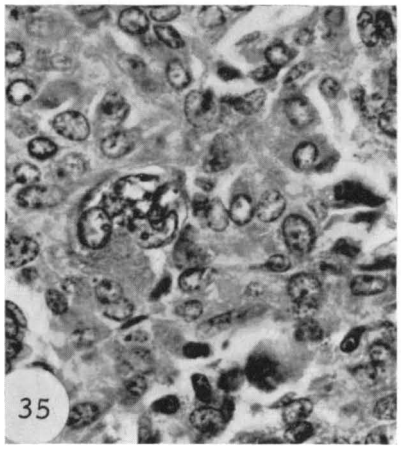
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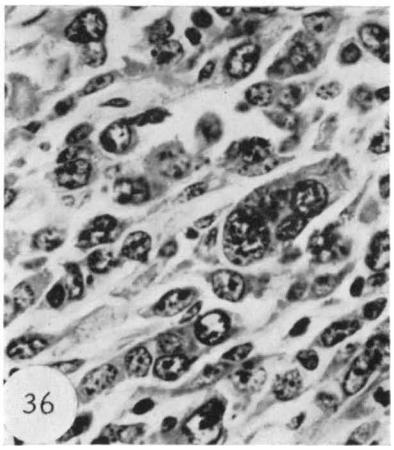
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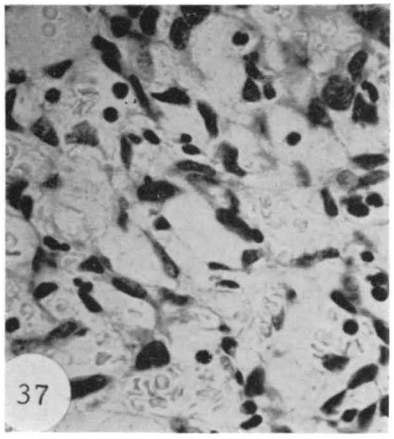
Fig. 37. Vertebra, 5½ months after the injection of Sr^{90}. Aplastic bone marrow, proliferation of reticular cells. van Gieson, × 400.

Fig. 38. Femur, 6½ months after the injection of Sr^{90}. Aplastic bone marrow, dilatated capillaries, swelling of the endothelium. van Gieson, × 1000.

Fig. 39. Femur, 5 months after the injection of Sr^{90}. Aplastic marrow with dilatated sinusoids. Note the thickened sinusoidal walls and the variations in the morphology of the littoral cells. van Gieson, × 400.

Fig. 40. Femur, 5½ months after the injection of Sr^{90}. Dilatated sinusoid, proliferation of reticular cells. van Gieson, × 400.

Fig. 41. Vertebra, 7 months after the injection of Sr^{90}. Proliferation of pleomorphic cells in the medullary cavity. van Gieson, × 400.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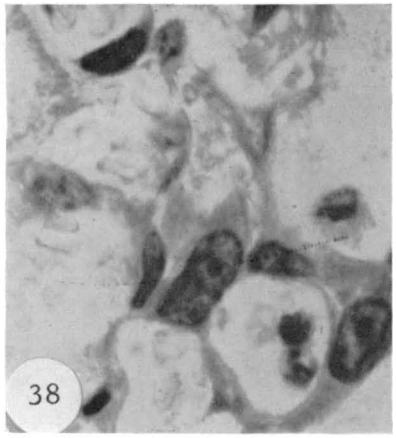
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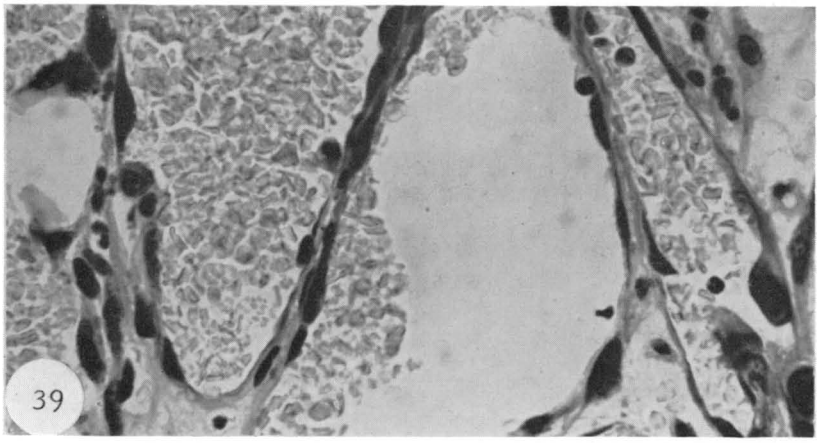
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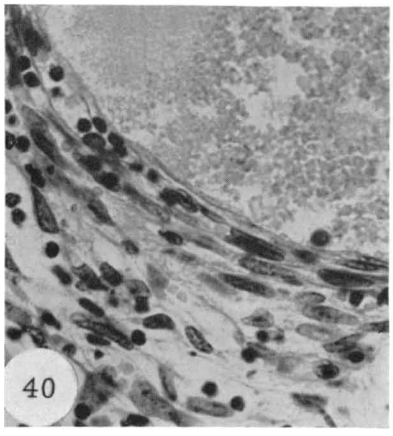
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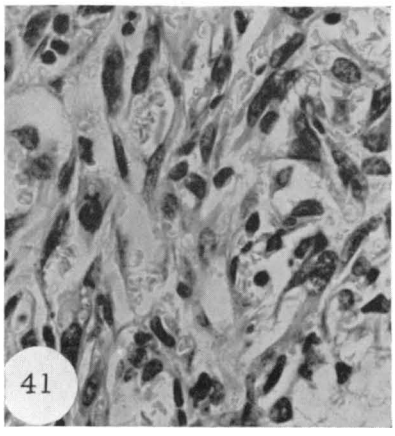
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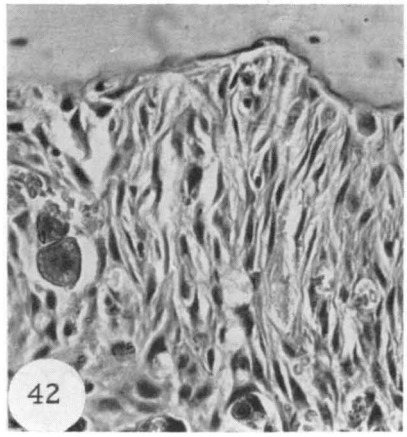
Fig. 42. Humerus, 8 months after the injection of Sr^{90}. Fibroblastic osteosarcoma bud. H & E, × 250.

Fig. 43. Femur, 7 months after the injection of Sr^{90}. Fibroblastic osteosarcoma bud. Little formation of extracellular substances and pronounced pleomorphism. Azure-eosinate, × 400.

Fig. 44. Same bone as in Fig. 43. Abundant argyrophilic fibres. Foot & Foot, × 400.

Fig. 45. Fibroblastic osteosarcoma, tibia, 7 months after the injection of Sr^{90}. Three mitotic figures. H & E, × 400.

Fig. 46. Fibroblastic osteosarcoma, extension through the cortical bone. Island of cartilage on the periosteal side. van Gieson, × 40.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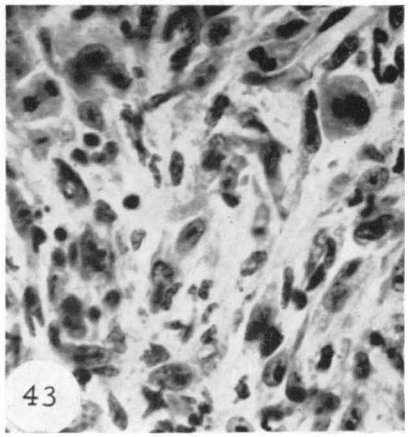
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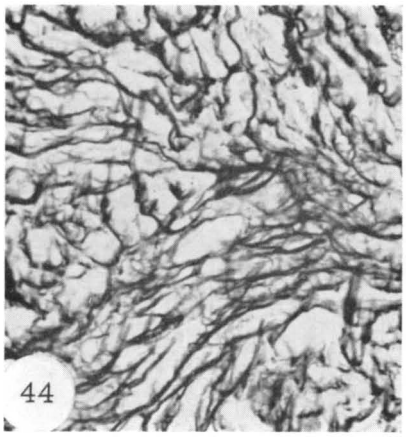
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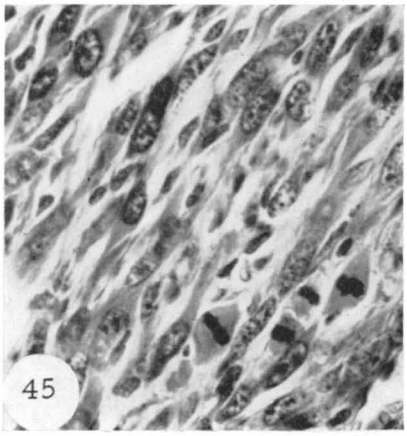
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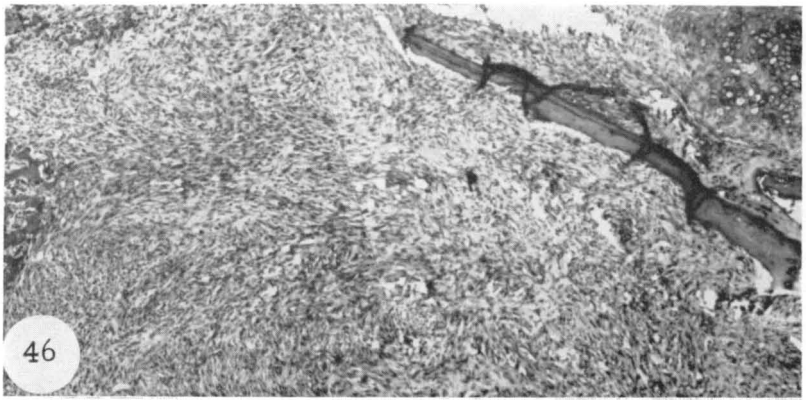
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Fig. 47. Fibroblastic osteosarcoma bud, 7 months after the injection of Sr^{90}. General view of the bone shown in Fig. 43. van Gieson, × 20.