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AUTORADIOGRAPHIC LOCALIZATION OF STRONTIUM 85 IN OSTEOSARCOMAS

by

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The only isotopes that have been used up to now in autoradiographic distribution studies with radiostrontium seem to be ^{90}Sr and ^{89}Sr. These are both exclusively beta emitters with the relatively high maximum energies of 0.55 and 1.5 MeV respectively.

Strontium 85 has been used mainly in experiments connected with external measurements. Its 0.513 MeV gamma radiation has then been registered by scintillation detectors; ^{85}Sr also emits short-range extranuclear electrons (main energy about 11.5 keV) which theoretically are very suitable for high resolution autoradiography.

The present authors, in earlier investigations with transplanted bone-forming osteosarcomas (3, 4), found a more rapid strontium turnover in tumours than in normal bone. The autoradiograms revealed a very rapid uptake of ^{90}Sr in the actively growing tumour tissue soon after injection, followed later by a rapid decrease in concentration.

In the present investigation, the early uptake of radiostrontium in transplanted osteosarcomas has been studied in more detail by means of autoradiography. An improved autoradiographic resolution was obtained, mainly

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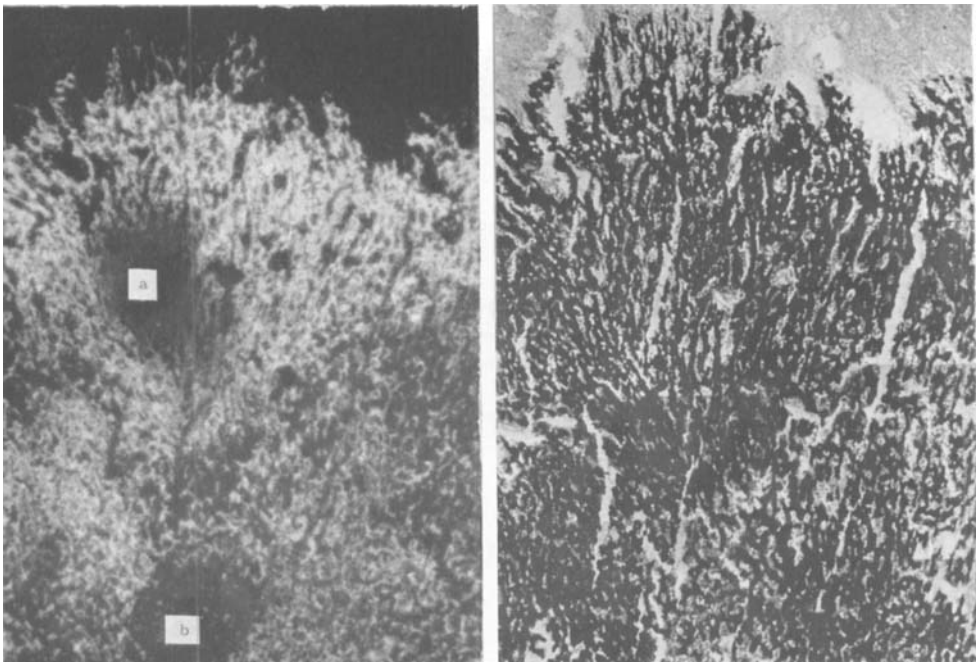

Fig. 1. *Left:* Autoradiogram of part of a transplanted osteosarcoma, 4 hours after injection of ^{85}Sr; white areas correspond to high radiostrontium uptake; high uptake is evident in bone trabeculae; two areas (a) and (b) show very low ^{85}Sr uptake. *Right:* Section corresponding to the autoradiogram, stained with von Kossa's silver; heavy mineralization is generally related to ^{85}Sr uptake; the trabeculae in areas corresponding to the ^{85}Sr-free parts, (a) and (b) in the autoradiogram, are slightly more slender in neighbouring parts of the tumour. × 40.

because of the more favourable radiation properties of ^{85}Sr as compared with ^{90}Sr.

Methods. ^{85}Sr nitrate, in HNO_3 solution, was purchased from Oak Ridge, Tennessee, USA. The specific activity was 8.718 mC/g.

Inbred CBA mice were used as experimental animals. Small pieces (about 2 × 2 × 2 mm) of ^{90}Sr-induced osteosarcomas were transplanted to the subcutaneous dorsal region of the neck of other CBA mice. The tumours had been used for transplantation in several generations of animals and had lost their original content of ^{90}Sr; in two months they had reached a diameter of 2.5 to 3 cm.

Strontium 85 (80 μC) was injected intraperitoneally after development of the transplanted tumour, and autoradiograms were obtained of sagittal whole-body sections through the mice which were killed four hours later by immersion in a dry ice-and-acetone-mixture.

Section thicknesses varied from 5 to 20 μ. The autoradiographic exposure

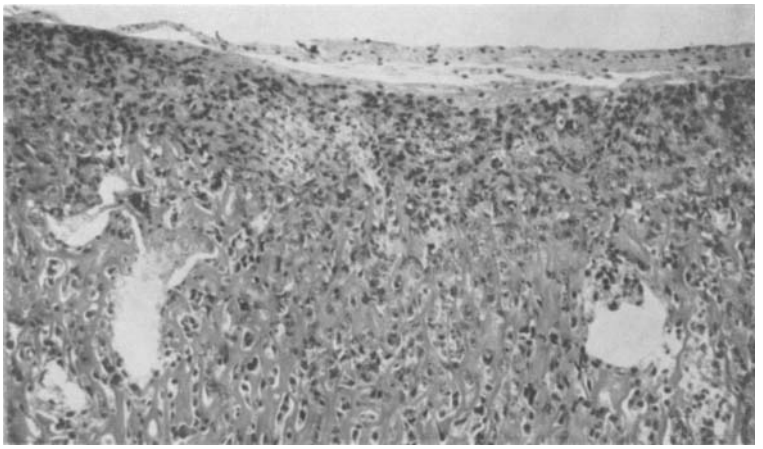

Fig. 2. Transplanted osteosarcoma with highly mineralized tumour bone (lower part of view) and a peripheral narrow zone of soft tumour tissue (upper part of view) where trabecular bone has not yet been formed. van Gieson. × 100.

was made by apposition against Ilford G 5 nuclear plates (emulsion thickness 4 μ), or films of the Schumann type (Kodak SWR) with the silver bromide layer confined to the surface of the emulsion. The exposure time was 16 days. The autoradiographic method has been described earlier (5, 6). The sections were stained by the von Kossa method after the autoradiographic exposure. Sections for histologic examination, 5 μ in thickness, were also stained with haematoxylin-eosin, azure-eosinate (Lillie), or according to van Gieson.

Results

The most peripheral part of the tumour consists of a narrow zone of a cellular soft tissue without detectable mineralization (Figs 1 and 2); there is no noticeable ^{85}Sr uptake in this area (Fig. 1). The next zone contains well-developed mineralized trabeculae (Fig. 3) with highly active osteoblasts (Fig. 4) and mostly intact blood vessels. This area shows the most active uptake of ^{85}Sr in the autoradiograms.

The lines of high uptake of ^{85}Sr in the autoradiograms correspond well with the bone trabeculae in the von Kossa-stained sections (Fig. 1). A number of small islands (10 μ to 1 mm in diameter) are seen in the zone of generally intact, obviously fast-growing tumour tissue, and these in the autoradiograms present evidence of little or no uptake of ^{85}Sr. The uptake of ^{85}Sr gradually rises to match that of the surroundings at the periphery of these 'dead' islands (Fig. 1). The degree of mineralization occasionally appears in the corresponding areas of the von Kossa-stained sections. Similar islands, with varying degrees of cellular degeneration, from a hardly noticeable granulation of the osteoblasts to complete necrosis of the tumour bone, may be found in the histologic sections (Fig. 5).

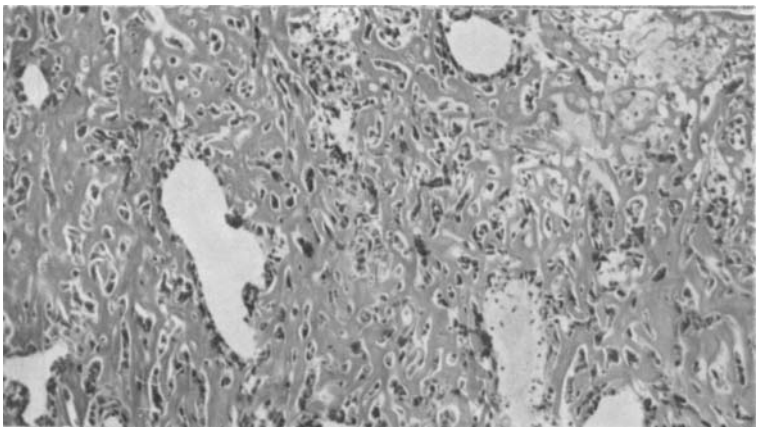

Fig. 3. Transplanted tumour. Highly mineralized; necrotic area at upper right corner. van Gieson. × 100.

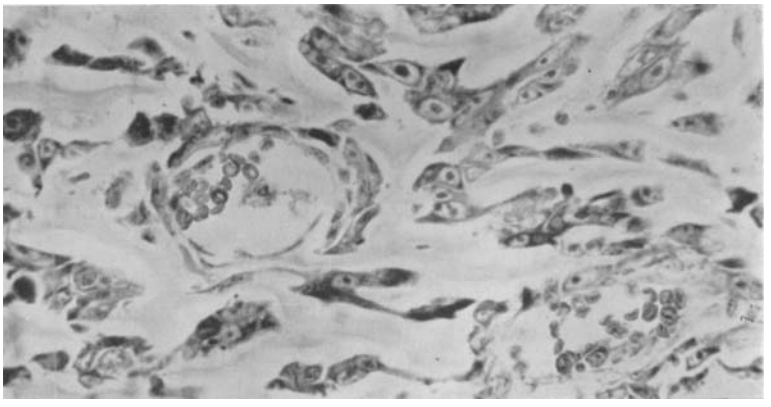

Fig. 4. Area from a transplanted tumour with blood vessels, well developed tumour bone, and highly active osteoblasts. Azure-eosinate. × 400.

Discussion

The use of ^{85}Sr in autoradiography has led to a striking improvement in resolution compared with earlier experiences with ^{90}Sr. This is probably mainly due to the contribution of the short-range extranuclear electrons emitted by ^{85}Sr (1, 2).

The monoenergetic electrons of ^{85}Sr have predominant lines at about 11.5 keV, in the neighbourhood of the energy of tritium (max. energy 18 keV). The range for such electrons is 2.2 μ in water. The electron radiation may be expected to dominate in causing blackening of the photographic emulsion in autoradiography with thin sections and emulsions; in work with sections

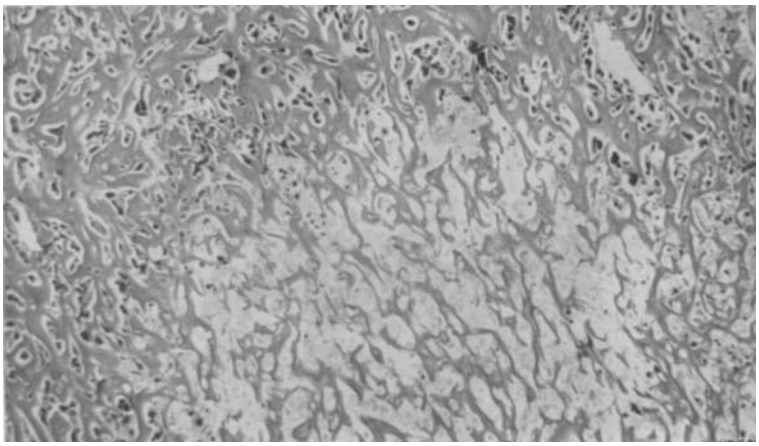

Fig. 5. Transplanted osteosarcoma with necrosis of the tumour bone. Empty lacunae and debris between the narrow trabeculae. van Gieson. × 100.

considerably thicker than the range of the electrons, however, it can be expected that the electromagnetic (roentgen) radiation of ^{85}Sr (energy 13.3 keV) significantly contributes to the blackening of the emulsion. If the absorption in water is considered, the roentgen radiation of ^{85}Sr (half thickness about 3.3 mm) is less advantageous for autoradiographic resolution than the beta radiation of ^{90}Sr (range about 1.8 mm, or half thickness about 0.25 mm); the situation will be the converse, however, if the absorption in the photographic emulsion is considered. With an emulsion consisting of 50 per cent silver bromide and 50 per cent gelatine (v/v) the half thickness of the ^{90}Sr betas is about 70 μ while that of the 13.3 keV roentgen radiation of ^{85}Sr is only about 50 μ.

Attention has not been paid in the above calculation to the fact that the autoradiographic resolution with ^{90}Sr is impaired by the influence from the yttrium 90 daughter.

Concerning the uptake of radiostrontium in the tissues, the results agree with the previous observation of a very high early uptake of radiostrontium in tumour as compared with normal bone. The distribution within the tumour tissue, however, was highly irregular, with marked differences in uptake, both between the various zones from the periphery and inwards, and within the zones.

The appearance of strontium 85-free areas in zones of rapid tumour growth may probably be related to blood vessel damage. Autoradiography appears to be a sensitive method for discovering metabolic changes in such areas, before any histologic signs can be detected.

Acknowledgement

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SUMMARY

Improved resolution has been obtained with strontium 85 as compared with strontium 90, in autoradiographic investigations of the distribution of radiostrontium in bone-forming osteosarcomas. A much higher uptake of radiostrontium was found in tumour than in normal bone, the distribution being highly irregular and related to the varying degrees of mineralization and degenerative changes.

ZUSAMMENFASSUNG

Strontium 85 zeigte ein besseres Auflösungsvermögen als Strontium 90 in autoradiographischen Untersuchungen über die Verteilung von radio-aktiven Strontium in knochenbildenden Osteosarkomen. Es ergab sich, dass beträchtlich mehr radio-aktives Strontium im Tumor als im normalen Knochengewebe aufgespeichert wurde; die Verteilung des Strontium erfolgte recht unregelmässig, je nach dem Mass der Knochenbildung oder der Entartungsprozesse.

RÉSUMÉ

Dans l'étude autoradiographique de la distribution du radiostrontium dans les ostéosarcomes ostéogéniques, le strontium 85 donne une meilleure résolution que le strontium 90. On trouve une fixation de radiostrontium beaucoup plus élevée dans la tumeur que dans l'os normal, sa distribution étant très irrégulière et liée aux variations de la minéralisation et aux modifications dégénératives.

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