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DISTRIBUTION OF RADIOSTRONTIUM IN DEVELOPING BONES AND TEETH

Microautoradiographic study with ^{85}Sr

by

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Exact knowledge of the distribution pattern of radiostrontium and its alteration with time is essential for the understanding of the complex changes which gradually become manifest in the skeleton as a result of continuous irradiation. Available distribution studies have been based upon impulse counting techniques or autoradiography (PECHER 1941, 1942, COMAR et coll. 1952, KIDMAN et coll. 1952, JOWSEY et coll. 1953, ENGSTRÖM et coll. 1957, OWEN et coll. 1957, DOWNIE et coll. 1959, JEE & ARNOLD 1960, NILSSON & ULLBERG 1962). However, the high-energy radiation of ^{90}Sr (0.54 MeV for ^{90}Sr and 2.24 MeV for the disintegration product ^{90}Y) is not suitable for microautoradiographic investigations. APPELGREN et coll. (1963) in their studies of radiostrontium uptake in osteosarcomas, found that the extranuclear β radiation (11.5 keV) of ^{85}Sr could be utilized for autoradiography. This quality has been taken advantage of in the present study of ^{85}Sr distribution in young growing rats during the immediate post-injection period.

Submitted for publication 29 April 1964.

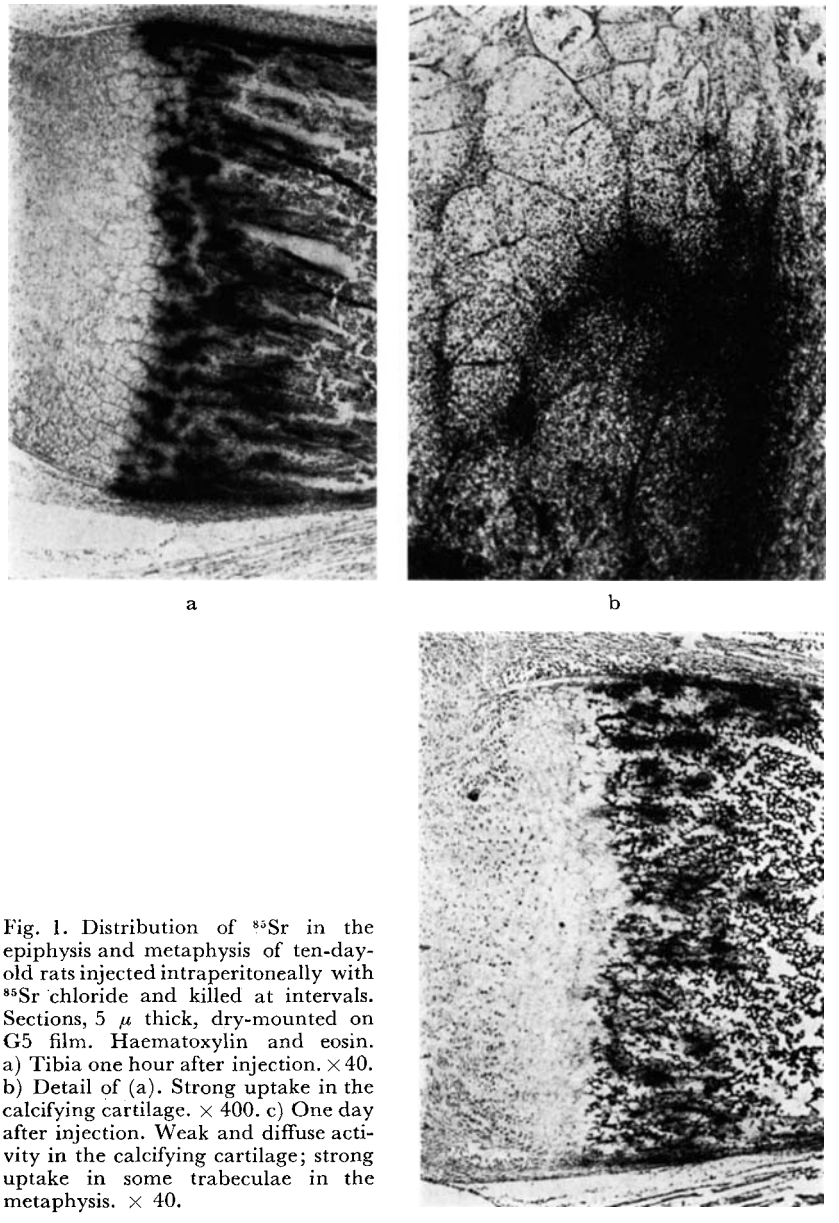

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Fig. 1. Distribution of ^{85}Sr in the epiphysis and metaphysis of ten-day-old rats injected intraperitoneally with ^{85}Sr chloride and killed at intervals. Sections, 5 μ thick, dry-mounted on G5 film. Haematoxylin and eosin. a) Tibia one hour after injection. $\times 40$. b) Detail of (a). Strong uptake in the calcifying cartilage. $\times$ 400. c) One day after injection. Weak and diffuse activity in the calcifying cartilage; strong uptake in some trabeculae in the metaphysis. $\times$ 40.

Material and Methods. Ten-day-old albino rats were injected intraperitoneally with 0.25 ml carrier-free ^{85}Sr chloride (Amersham) corresponding to an approximate total dose of 50 μC ^{85}Sr per animal. The animals were killed one

hour, and one, two, four and sixteen days after the injection of ^{85}Sr under ether anaesthesia by immersion in a mixture of carbon dioxide and hexane (— 75° C) and the head and a hind leg cut off and embedded in carboxymethyl cellulose. Sagittal sections, 5 μ thick, were taken through these structures using ULLBERG's (1954, 1958) technique. The sections were taken on Scotch tape No. 688, dried overnight in a cold room (— 10° C), and mounted on Ilford G5 nuclear emulsion plates with an emulsion thickness of 5 μ. The surface of the emulsion was coated with a thin layer (0.6 μ) of urea alkyd by immersion in a solution of 8 parts acetone, 1 part urea alkyd, and 0.3 parts hardener. The hardener consisted of equal parts of ethyl glycol and absolute alcohol to which 1 % HCl was added. (After the addition of the hardener, the urea alkyd solution tolerates storage for 24 hours at room temperature before becoming opaque from excessive polymerization.)

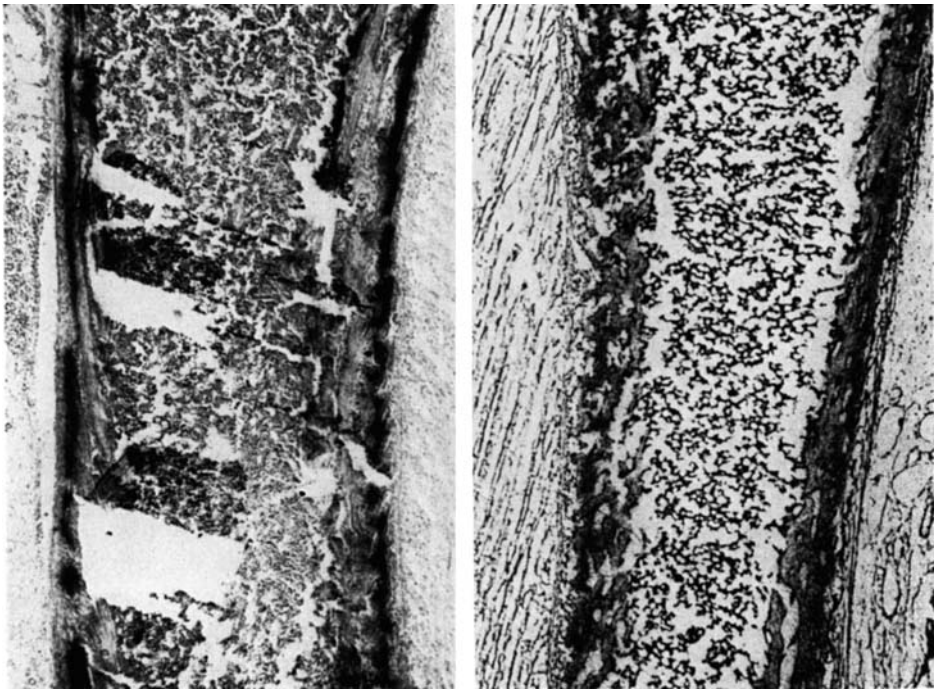
The plates after immersion were placed vertically to allow excess fluid to drain off and the solvents to evaporate. The emulsion surface by this stage was 'dry' and adhesive, and the tape with the sections could be mounted.

The films were exposed under slight pressure for 4 to 7 days, the tape backing then being removed with acetone. A sponge drenched with acetone was pressed against the back of the tape for about 5 min. The tape backing, consisting of polyvinyl chloride, then separated from the adhesive and was removed with a pair of forceps. (If the tape borders were not evenly cut before the application of the tape, irregularities caused adhesion between the adhesive and the backing of the tape, resulting in the concomitant removal of the sections.) The tape adhesive was then dissolved in xylene within two hours.

After removal of the tape the photographic plates with adhering sections were passed down through an ethyl alcohol series (two minutes in each), developed, fixed and rinsed. The sections adhering to the G5 plates were then stained with haematoxylin and eosin or according to van Gieson's method and mounted under a cover slip with Canada balsam.

Results

Bone. The uptake of ^{85}Sr was greatest in the epiphysis and metaphysis of the long bones one hour after intraperitoneal injection (Fig. 1a). The activity was weak and diffuse throughout the epiphyseal cartilage but there was a very strong accumulation of ^{85}Sr in the zone of calcifying cartilage. Activity was also very strong in the primary trabeculae of the metaphysis but declined towards the diaphysis. Not only was the activity marked and diffuse in these regions but there were also a striking number of 'hot spots', near which lines and streaks with high activity between cartilage cells were also evident (Fig. 1b).



a b

Fig. 2. Distribution of ^{85}Sr in the tibia of ten-day-old rats injected intraperitoneally with ^{85}Sr chloride and killed at intervals. Sections, 5 μ thick, dry-mounted on G5 film. a) One hour after injection. Strong uptake in the periosteum, somewhat less in the endosteum. Van Gieson. × 40. b) One day after injection. Diffuse activity throughout the compact bone with strongest activity near the periosteum. Haematoxylin and eosin. × 40. c) Sixteen days after injection. Zone of strong activity near the endosteum; weak and diffuse activity in the compact bone. Haematoxylin and eosin. × 80.

A strong uptake was present in the ossification centres of the epiphysis. In the diaphysis, most of the activity was limited to the periosteum (Fig. 2a).

One to two days after injection, the activity in the calcifying cartilage was weak and diffuse and the activity had also decreased in the primary trabeculae adjacent to this zone. The activity was still high in the other parts of the primary trabeculae and in the secondary trabeculae but was now more uniformly distributed with fewer hot spots (Fig. 1c). Accumulation was evident both on the surface and within the bone tissue of the trabeculae. The activity in the periosteum and endosteum had declined but in the cortex it was both strong and diffuse (Fig. 2b).

Four to sixteen days after injection the activity in the epiphysis and metaphysis was weak and diffuse. Although there were some differences in the degree of activity in different trabeculae there were no hot spots. The uptake of ^{85}Sr was much greater in the cortex of the diaphysis than in the epiphysis and metaphysis. The diffuse activity declined slowly while the apposition lines with their greater activity became more evident at ever increasing distances from the periosteum (Fig. 2c).

Teeth. One hour after injection a narrow zone of strong activity appeared in the dentine immediately adjacent to the predentine. There was slight uptake in deeper parts of the dentine (Fig. 3). At longer post-injection intervals the new dentine formed adjacent to the pulp contained diffusely distributed activity and the strongly active zone was displaced farther and farther from the pulp (Fig. 4). The dentine formed prior to injection also had increased diffuse activity (Fig. 4b).

In the enamel the distribution pattern one hour after injection differed to a great extent from tooth to tooth and even from cusp to cusp of a particular tooth. Uptake in the lower second molar can serve to illustrate the different phases. Strontium uptake immediately under the high ameloblast layer in the cervical region was moderate in a broad and diffusely demarcated zone (Fig. 3c). The height of the ameloblast layer decreases towards the tip of the cusp and in this region there was only slight uptake. A fairly strong uptake of ^{85}Sr throughout the entire enamel was evident at the tip of the cusp under the low ameloblasts; the enamel at this site stained basophilic but was eosinophilic in other regions (Fig. 3).

One day after injection these areas could still be easily distinguished but the distribution in the enamel steadily became more homogeneous and the entire enamel stained basophilic (Fig. 2, a and b).

Sixteen days after injection there was diffuse uptake in the upper third molar, a tooth which at the time of injection had not begun to be mineralized; the uptake was much the same in the enamel and dentine.

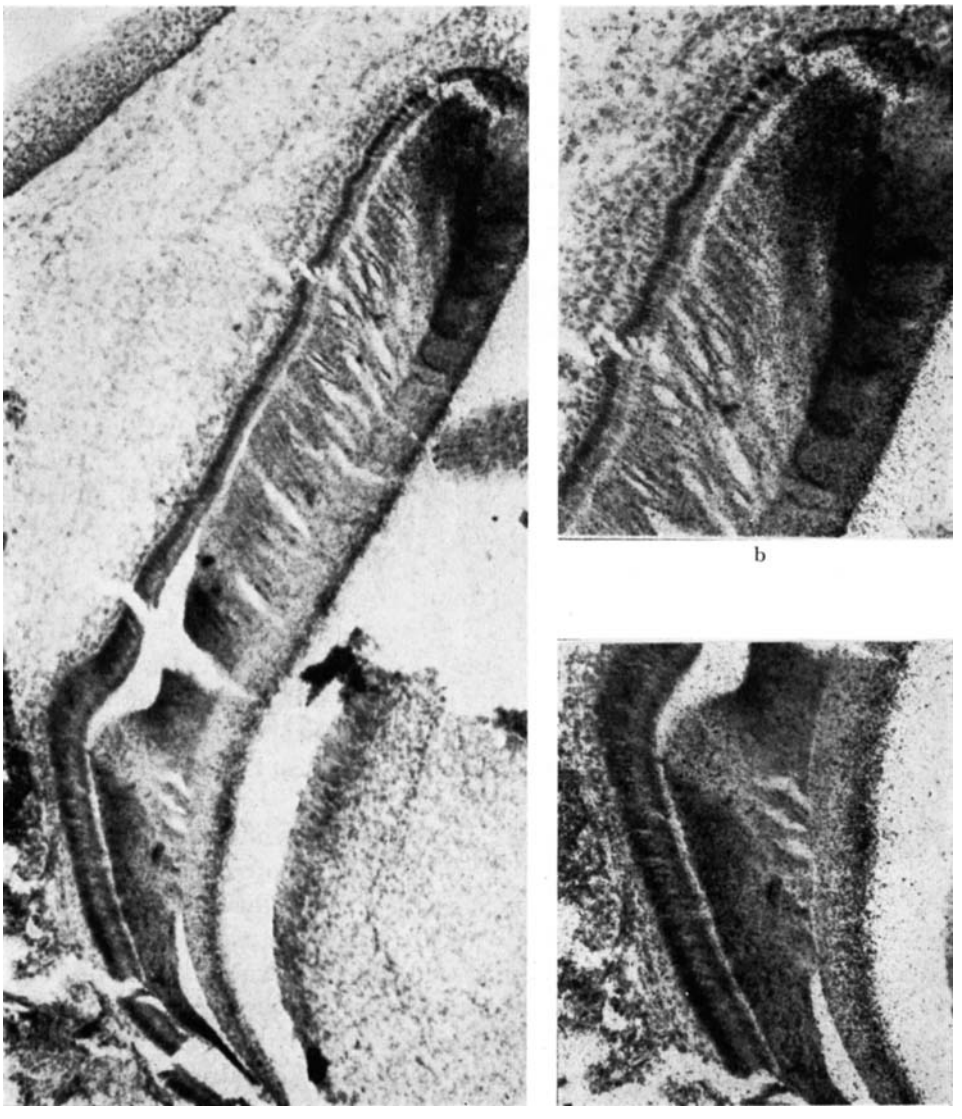
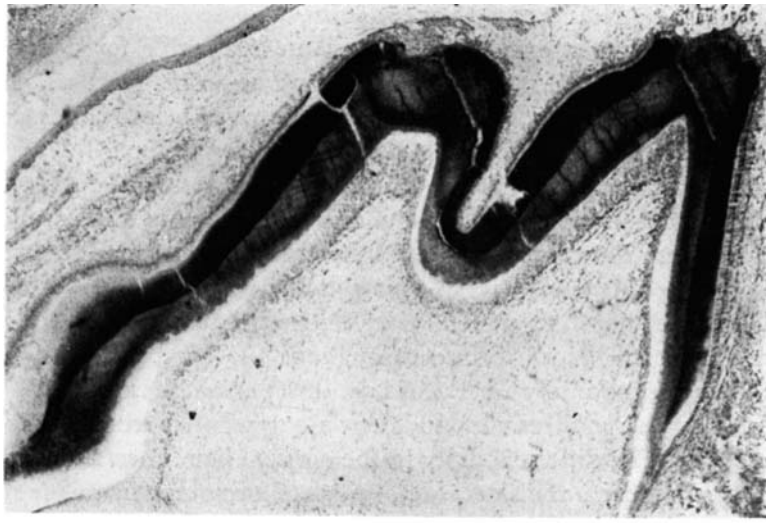


Fig. 3. a) Distribution of ^{85}Sr in the bud of the lower second molar of a ten-day-old rat one hour after intraperitoneal injection of ^{85}Sr chloride. Sections, 5 μ thick, dry-mounted on G5 film. Haematoxylin and eosin. × 110. b) Detail of (a) × 175. c) Detail of (a) × 160. Uptake in the enamel is mainly concentrated to the cervical region and to the tip of the cusp; under the low ameloblast layer in the latter the activity is spread throughout the enamel. The matrix of the enamel stains basophilic. In the cervical region, the activity extends about half-way through the matrix. The high ameloblast layer is a sign that matrix formation is taking place. A zone of strong activity lies in the dentine adjacent to the predentine; dentine formed prior to the injection has a weak and diffuse activity.



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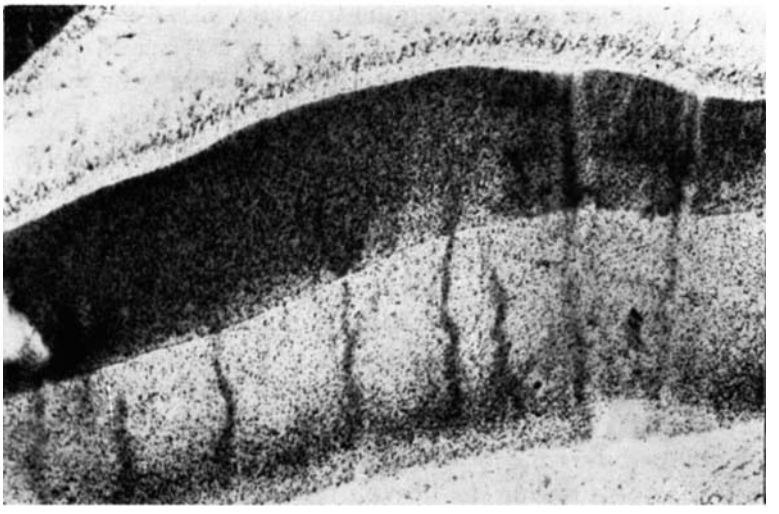

b

Fig. 4. a) Distribution of ^{85}Sr in the first upper molar of a twelve-day-old rat injected intraperitoneally with ^{85}Sr chloride two days previously. Section, 5 μ thick, dry-mounted on G5 film. Haematoxylin and eosin. $\times$ 30. b) Detail of (a) $\times$ 160. Diffuse uptake throughout the enamel; the zone of strong activity in the dentine is now situated at some distance from the pulp; diffuse uptake in the dentine formed before and after the injection.

Discussion

The radiation from strontium 85 proved quite suitable for detailed autoradiographic studies of strontium metabolism. The technique used eliminates contact between the tissues and water, or other fluids, before exposure so that there is no risk of redistribution or loss of the injected isotope.

The strong initial uptake of strontium in the epiphysis and metaphysis was greatest in the calcifying cartilage. The marked reduction in this uptake one day after injection illustrates the rapid breakdown of this cartilage in growing bone.

Uptake in the diaphysis was initially limited mainly to certain sites but subsequently became more diffuse throughout the cortex. This observation supports the assumption of mineral uptake in separate phases (JONES & COPP 1951). However, activity remained highest along what represented the earlier apposition lines. The diffuse activity in the cortical bone then slowly declined, which probably reflects the slow rate of mineral exchange in this region. The initial strong activity in the periosteum sank deeper into the cortex as the time after injection increased and by sixteen days had partially disappeared at the endosteum. This illustrates the classical concept of growth of a long bone, periosteal deposition and endosteal resorption.

The weak and diffuse uptake of radiostrontium in the dentine laid down before injection and the gradual increase of activity correspond with what has been observed after the injection of ^{45}Ca and can probably be associated with the increase in the hardness of dentine in young animals (KUMAMOTO & LEBLOND 1956). Strontium circulating in the blood gave a degree of activity to the dentine formed after injection.

The pattern of strontium uptake in the enamel would seem to support DIAMOND & WEINMANN's (1940) theory of phased mineralization. The high ameloblast layer in the cervical region is a sign that matrix formation is taking place (ORBAN 1953); the newly-formed enamel matrix has a moderate uptake of strontium. When the enamel matrix has attained its full thickness the ameloblast layer becomes reduced in height; mineral deposition seems to recommence only after this has taken place. The later uptake is much greater than that which occurs during the formation of the matrix. The change in stainability that was evident during the heavy strontium uptake has previously been studied in decalcified teeth by CHASE (1935), among others. He observed this change during the phase of development preceding the appearance of the final, fully mineralized enamel. His observations seem to fit in with what was seen during the present study. The initial distribution pattern persists for a long time after injection but a subsequent uptake of strontium from the circulating blood produces diffuse activity throughout the enamel.

SUMMARY

A method for dry-mounting tissue sections in microautoradiography is described. The distribution of strontium 85 in developing bones and teeth has been studied up to sixteen days after a single intraperitoneal injection in albino rats.

ZUSAMMENFASSUNG

Eine Methode für die Trockenmontierung von Gewebsschnitten für die Mikro-Autographie wird beschrieben. Die Verteilung von Strontium 85 in wachsenden Knochen und Zähnen wurde an Albinoratten nach einmaliger intraperitonealer Injektion bis zu 16 Tagen verfolgt.

RÉSUMÉ

Les auteurs décrivent une méthode de montage à sec de coupes de tissues en microautoradiographie. Ils ont étudié la distribution de strontium 85 dans les os en développement et les dents jusqu'à seize jours après une injection intrapéritonéale unique chez des rats albinos.

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