PRELIMINARY NOTES

Bone Kinetics of Calcium-45 and Pyrophosphate Labeled with Technetium-96: An Autoradiographic Evaluation

A. Guillemart, A. Le Pape, G. Galy, and J-C Besnard

Faculté de Médecine, Tours, ERA—C.N.R.S. 697, Tours, and Hôpital Neuro-Cardiologique, Lyon, France

The uptake of calcium-45 and of pyrophosphate labeled with the long-lived technetium-96 isotope were compared by means of liquid-emulsion microautoradiograms of the epiphyseal plates of 10-week-old rabbits, at 30 min, and 3 and 48 hr after i.v administration. For both tracers, thin sections confirm the significant role of the blood supply, especially shortly after injection. However, other more specific mechanisms lead to a mixing of the calcium in the mineral mass and to a linear deposition of technetium facing the osteoid surfaces. These findings suggest that the tropism of tin-reduced technetium pyrophosphate is not governed by the mineral pool but rather by exchanges inside a still poorly calcified organic matrix.

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It is widely accepted that the pyrophosphate ion adsorbs strongly on hydroxyapatite crystals and could therefore play an important role in calcium homeostasis (*1–2*). A simplistic explanation for the bone tropism of technetium-stannous-pyrophosphate (*3*) would be that technetium binds to bone because the pyrophosphate moiety binds on crystals. However, this hypothesis is highly controversial and other factors, such as local blood flow (*4*) and organic components of bone tissue (*5–6*), have been proposed. In order to obtain basic information required for a better understanding of the mechanism involved in skeletal scintigraphy, we compared the movements of calcium from blood to the mineral components of bone with the accumulation of the technetium-stannous-pyrophosphate complexes.

In a previous study (*7*), the relatively long-lived technetium isotopes Tc-95m and Tc-96 were shown to be suitable for the autoradiographic investigation of growing long bones. In the present work, using the same experimental model, we compared the distributions of calcium and of Tc-96-labeled pyrophosphate at 30 min, and 3 and 48 hr after an i.v. dose. The histologic technique has been improved to achieve thinner sections and higher resolution.

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For reprints contact: Alain Guillemart, Laboratoire de Biophysique Médicale, U.E.R. de Médecine, 37000-Tours, France.

MATERIALS AND METHODS

Labeled compounds. *Pyrophosphate.* The lyophilized form of stannous pyrophosphate was freshly reconstituted with an isotonic saline solution of pertechnetate (*8*). The technetium was extracted from a target of natural molybdenum bombarded with cyclotron-accelerated deuterons (*7*).

During the irradiation, several technetium isotopes were obtained. It has been observed that technetium-95m, which is present in a concentration of less than 20 μCi per ml, cannot be detected autoradiographically unless the exposure time exceeds 2 mo. In fact, only technetium-96 has the half-life (4.3 days) and decay characteristics suitable for autoradiography. In the present study, the injected solution contains 750 μCi per ml of technetium-96 for 0.65 mg of stannous chloride and 30 mg of tetrasodium pyrophosphate. A chromatogram on Whatman No. 1 paper with methanol/water (85/15 v:v) established the quality of the labeling. Animals were injected through an ear vein with 1.5 ml of the solution within 30 min after its preparation.

Calcium. In a separate study, animals were similarly injected with a 0.9% NaCl solution containing 500 μCi of carrier-free calcium-45 as chloride.

Animal experiment. Due to the small amount of technetium-96 available, six 10-week-old rabbits from the same litter were injected with either labeled pyrophosphate or calcium. For each tracer one animal was

killed after 30 min, and 3 and 48 hr, respectively. The distal femur and proximal tibia were immediately excised and refrigerated to prevent cytolysis. Small specimens were cut from longitudinal sections of the growing zones and fixed in chilled 0.1 *M* cacodylate-buffered (pH 7.5), 10% formaldehyde solution.

Autoradiography. Macroautoradiograms were obtained from tissue blocks embedded in paraffin and placed in direct contact with x-ray film.* For the microautoradiograms, the fixed specimens were dehydrated in propylene oxide and embedded in araldit M resin.† Sections 2-μm thick were cut with an ultramicrotome equipped with a glass knife, fixed on glass slides with a thin layer of gelatin subbing solution, then dipped in nuclear tracking emulsion‡ diluted to 33% with water. After 3 wk of exposure at 4°C, the microautoradiograms were developed with D19b,‖ fixed with Hypam,§ then stained through the photographic emulsion using Mallory's phosphotungstic hematoxylin.

Controls. To achieve technical reproducibility, the complete processing from bone excision to slide staining was achieved on at least 15 fragments per animal. During the fixation step, the bath solutions never contained more than 1% of the osseous radioactivity.

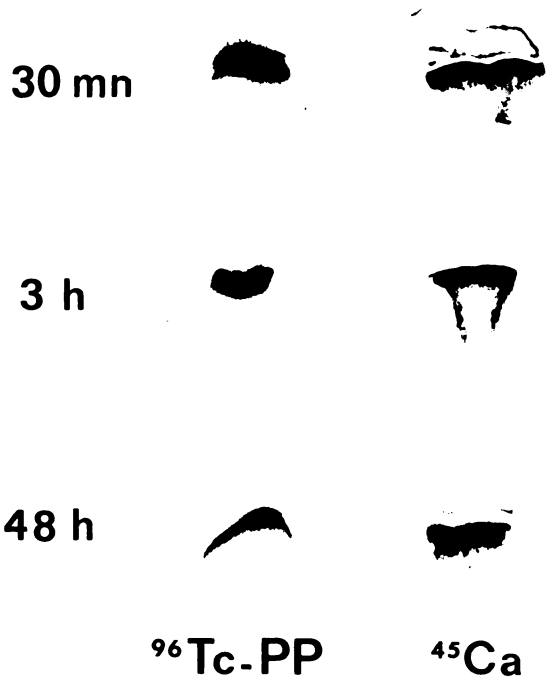

FIG. 1. Crude distribution of calcium-45 (right side) and technetium-96 stannous pyrophosphate (left side) obtained from longitudinal sections of femur epiphyseal plate, respectively, 30 min and 3 and 48 hr after i.v. administration of tracer. Note on 48 hr autoradiograms displacement of line of highest density.

RESULTS

On macroautoradiograms (Fig. 1), no striking difference could be observed between the distributions of calcium and technetium. The early figures (30 min and 3 hr) revealed a maximum of labeling on the diaphyseal side of the epiphyseal plate. At 48 hr, this maximum had migrated toward the medullary cavity, with movement corresponding to the bone growth. There was also a feeble and diffuse radioactivity detectable in the newly formed area, indicating a continuous deposition of the tracer from the circulation.

In the microautoradiograms obtained 30 min after injection of calcium (Fig. 2), the osseous trabeculae and the calcified and hypertrophic cartilage were labeled. At

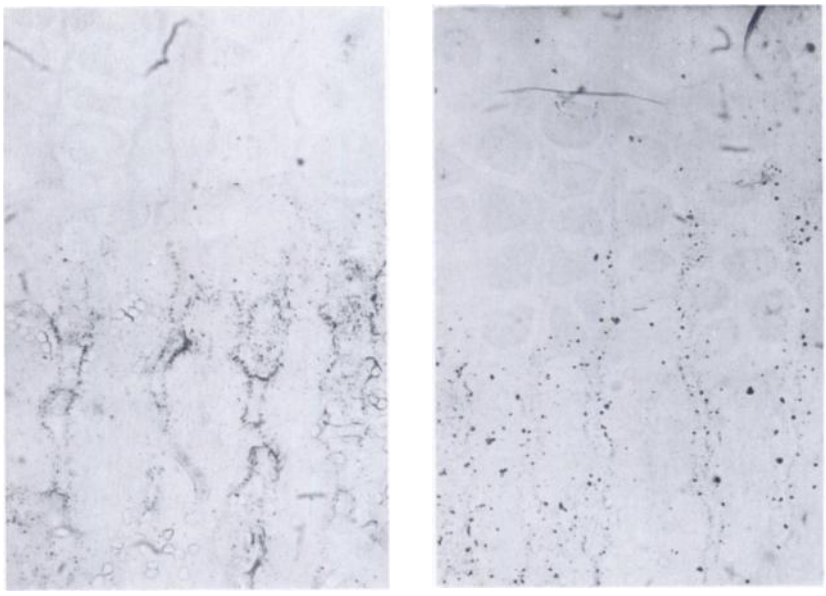

FIG. 2. Microautoradiograms obtained 30 min after injection of tracer. For calcium-45, silver grains are overlying newly created osseous trabeculae and longitudinal septa in calcified cartilage and lower part of hypertrophic cartilage. In contrast, technetium-96 deposits in linear patterns on both sides of osseous trabeculae.

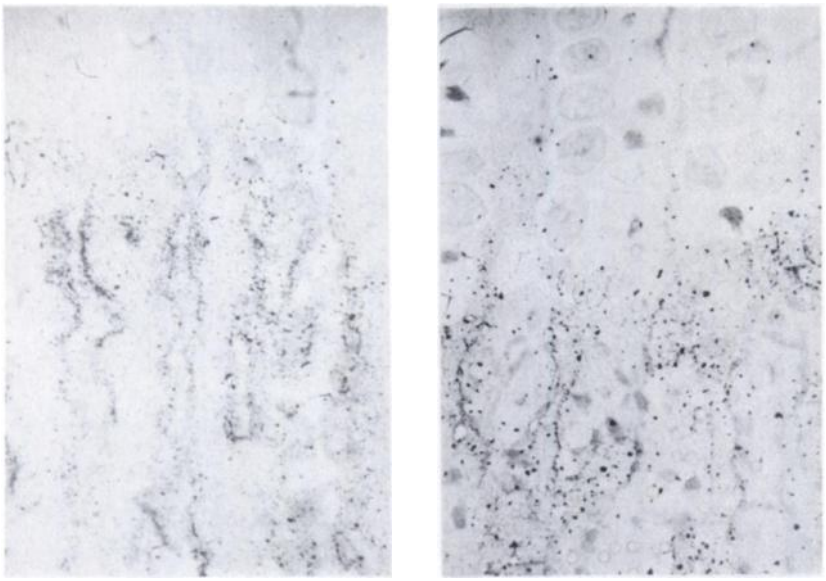

FIG. 3. Three hours after injection, the distribution of calcium-45 is very similar to 30 min one. At this stage, technetium-96 has almost entirely disappeared from cartilaginous structures.

this early stage, the technetium was found in linear patterns overlying the osteoid surfaces (Fig. 2). Immediately beneath the calcified cartilage, a high density of silver grains could be seen, abutted on the longitudinal septa rather than facing the vascular buds. Moreover, a moderate amount of tracer was present in the calcified cartilage.

On the 3-hr slides (Fig. 3), the distribution of the calcium was very similar to that seen at 39 min, although incorporation inside the osseous trabeculae was more obvious. In contrast, very little technetium was visible in the calcified cartilage, but the linear patterns in the diaphysis remained unchanged.

In the 48-hr preparations, both tracers were found essentially in the osseous trabeculae that were forming and calcifying at the time of injection (Fig. 4). The radioactive calcium was also incorporated in the calcium pool, whereas the technetium remained in linear deposits overlying the osteoid layer. Even at this stage, the diffusion of technetium inside the osseous trabeculae was very limited.

In the present study, attention was focused essentially on extracellular bone structures. However, as it has been previously reported (*7*), a 3-hr incorporation of technetium was found on multinucleated cells considered as osteoclasts abutting on heavily labeled osseous trabeculae. A similar pattern was observed at 48 hr but not in 30-min plates.

DISCUSSION

The 3-hr distribution of Tc-96 pyrophosphate has been discussed previously (*7*). Thanks to thinner sections, the distribution can be more precisely studied, especially for the intense labeling observed in close contact with the cartilage. In this area, it appears that silver grains are

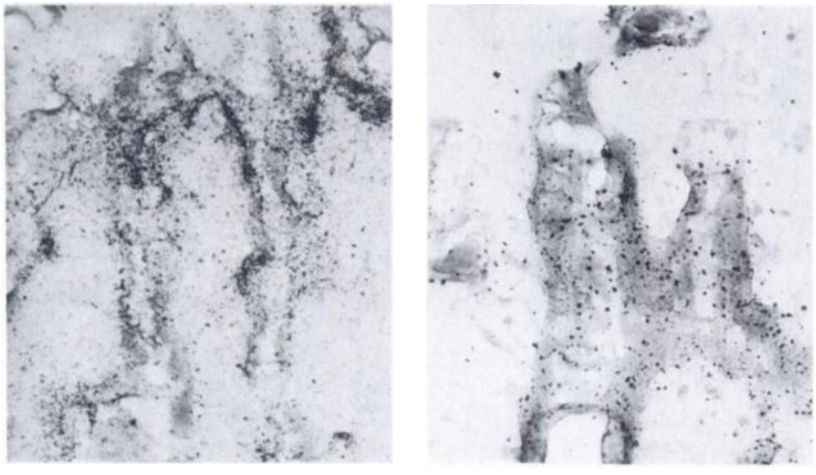

FIG. 4. Forty-eight hour microautoradiograms obtained in diaphysis, 500 μm beneath calcified cartilage. Calcium-45 is well mixed in osseous trabeculae. Very different is repartition of technetium-96, which remains in linear deposits overlying osteoid structures.

facing the longitudinal septa rather than the vascular buds (Fig. 3). Thus a linear pattern is formed all along the osseous trabeculae, with densities corresponding to the abundance of the local vascular supply.

The 30-min findings do emphasize the importance of the blood flow for both tracers. In fact, the general arrangement of the labeled deposits parallels the bone's vascular lattice. However, this does not exclude the possibility of other mechanisms. With radiocalcium, incorporation into the bone mineral is observed at every stage, which agrees with the well-established concept of "volume-seeker" (*9*). In contrast, the movements of technetium seem to follow two directions: at the level of the calcified cartilage, the labeling is essentially established at 30 min, which favors a rapidly reversible exchange; here the tracer does not interact durably with tissue structures. In the diaphysis, however, the linear patterns persist throughout the experiment, which could be interpreted as a local retention of technetium. If so, technetium bound to pyrophosphate should preferably be considered as a "surface-seeker" rather than a "volume-seeker." A possible diffusion of the tagged material during processing has been considered. Movements of radioactivity observed in the growth cartilage 30 min after injection are far more pronounced than on 3-hr slides, despite a very similar pattern on the diaphysial side. This together with the very weak amount of radioactivity released during fixation, is interpreted as an in vivo diffusion of radioactivity before the processing of the specimens.

Within the osteoid tissue, the radioactive deposits are arranged in bands expanding with time (Figs. 4 and 5) but without specific uptake by some substructure. Thus the linear deposits observed at 48 hr (Fig. 4) are far more diffuse than those previously described by Jones et al. (*10*) at the calcified front of trabeculae, 24 hr after administration of tritiated diphosphonate. Such differences in findings necessitate a cautious interpretation. We have observed previously (*7*) that the resolution obtainable with Tc-96-labeled macroaggregates is undoubtedly coarser than that with tritium or Ca-45, and the apparent spreading of silver grains that results from these physical limitations could be emphasized by biological processes.

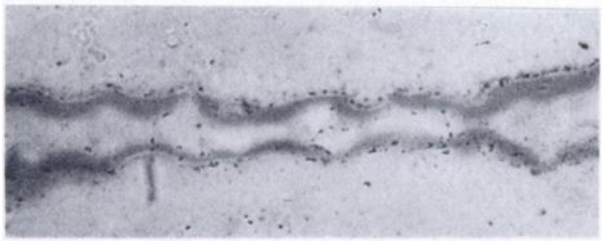

FIG. 5. Microautoradiogram of newly created osseous trabecula 3 hr after injection of Tc-99m pyrophosphate. Silver grains are abutted on each side of bone spicule, an area rich in poorly calcified and immature organic matrix.

The phosphorus compounds act as carriers for reduced technetium; the latter could be released, permitting separate, local metabolism for phosphorus and technetium, as suggested by Van Langevelde et al. (*11*). In an alternative hypothesis, the differences in tissue distribution could reflect the chemical differences between polyphosphates and diphosphonates (*2*).

In another connection, the tagged material appears within an extracellular matrix rich in immature and still poorly cross-linked collagen (*12*). This finding emphasizes the importance of organic components for the retention of technetium, as already demonstrated by Rosenthall and Kaye (*13*).

In conclusion, the data presented suggest the existence of differences in the histologic partition of calcium and pyrophosphate and give some credence to possible dissimilarities between the technetium and phosphate moities in their binding by bone.

FOOTNOTES

* PE-4006, Kodak.
† Merck-Darmstadt.
‡ K5, Ilford.
‖ Kodak.
§ Ilford.

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