Skeletal Uptake of Pyrophosphate Labeled with Technetium-95m and Technetium-96, as Evaluated by Autoradiography

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For animal experimentation, the 95m and 96 technetium isotopes offer many advantages over technetium-99m. Their long physical half-lives and the emission of extranuclear electrons of low penetrating power make it possible to obtain autoradiograms of a great precision. The uptake of technetium stannous pyrophosphate by the epiphyseal plate was studied using liquid-emulsion microautoradiography, 3 hr after i.v. injection into 10-week-old rabbits.

Microautoradiograms showed a well-defined and rather specific pattern of localization, with intense uptake beneath the epiphyseal disk on the extremities of the vascular buds and a lack of accumulation in the cartilage, whether calcified or uncalcified. In the metaphysis, the label was located where new bone was being laid down and also over the cytoplasm of osteoclasts. We deduce from these results that in normal bone the general distribution of this tracer reflects mainly the arrangement of the blood supply, but the specific sites of accumulation are the bone-forming surfaces and the active resorbing osteoclasts.

J Nucl Med 19: 895–899, 1978

Since 1971, the introduction to nuclear medicine of numerous phosphorus compounds labeled with technetium-99m (*1*)—and more particularly of technetium stannous pyrophosphate (*2*)—has greatly improved the routine clinical practice of bone scanning. The physiopathologic mechanisms contributing to and affecting the tracer deposition, however, are not clearly defined (*3*). Several investigators have emphasized the importance of local vascularity (*4–6*) and of interactions with either mineral surfaces (*7*), or organic components such as enzyme systems (*8*), or collagen (*9*).

The objective of this article is to show the relative contribution of cellular elements and extracellular fluids to the uptake of labeled pyrophosphate in different bone areas of various reactivities. For this purpose, the endochrondral ossification is of great interest because the tracer uptake is important in the extremities of growing long bones (*4*); moreover, many metabolic activities with intense ion movements are at work simultaneously. The combination of the autoradiographic technique with the long-lived 95m and 96 isotopes of technetium makes it possible to point out the substructures of bone tissue and the specific processes responsible for the local accumulation of technetium stannous pyrophosphate.

MATERIALS AND METHODS

Radiopharmaceuticals. These were prepared extemporaneously by reconstituting the lyophilized

Received April 23, 1977; revision accepted Jan. 14, 1978.

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form of stannous pyrophosphate with an isotonic saline solution of pertechnetate. The mixture of Tc-95m and Tc-96 was obtained by irradiation of a target of native molybdenum with cyclotron accelerated deuterons. Most abundant nuclear reactions were of (d,n) and (d,2n) types. After dissolution of the target with nitric acid, the technetium was oxidized into pertechnetate, then extracted with methyl-ethyl-ketone (*10*). The activity of the solution of labeled pyrophosphate (for 0.2 mg stannous chloride and 9 mg sodium pyrophosphate) was 10–12 μCi/ml for Tc-95m and 350–375 μCi/ml for Tc-96.

Animals. The experiments were carried out in 10-week-old male rabbits weighing 1100–1500 g. The animals were injected through the ear vein with about 2–3 ml of the radiopharmaceutical, and killed 3 hr later. The long bones of both lower limbs were immediately excised. Small specimens were cut from longitudinal sections of the growing zones of the long bones (including the epiphyseal disk and the diaphysis adjacent to it), fixed either in Bouin's aqueous solution or in 10% neutral formaldehyde solution, then dehydrated and embedded in paraffin wax.

Microautoradiography. The comparatively long half-lives of Tc-95m (61 days) and Tc-96 (4.3 days) provide a major advantage over Tc-99m in the preparation of histologic material. In this experiment, the elapsed time between the administration of the tracer and the production of the histologic sections was about 1 wk.

Despite the absence of decalcification, it was possible to cut the embedded material with a standard histologic microtome, because the tissue resistance was very much less than that of compact bone. There was no need, therefore, for an ultramicrotome equipped with a glass knife. The section thickness, however, was approximately 7 μm and it seems difficult to obtain thinner sections when carrying out this procedure.

After the sections were fixed on glass slides with a subbing solution, they were dipped in a nuclear tracking emulsion* (*11*). The exposure time was about 3 wk at a temperature of 4°C. The photographic process included development with D19b† and fixation with Hypam‡. After processing was complete, some sections were stained through the photographic emulsion using toluidine blue or Masson's trichrome.

Macroautoradiography. A crude picture of the distribution of the radioactivity within the different structures of the specimens was obtained by direct contact with an x-ray film||. The radioactivity originated from the tissue blocks after the histologic sections were cut.

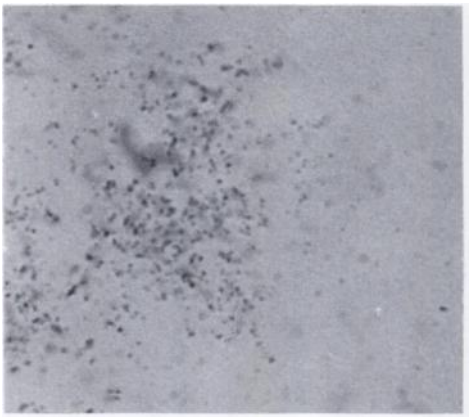

FIG. 1. Microautoradiogram of macroaggregates of human serum albumin labeled with Tc-95m and Tc-96.

RESULTS

Validity of the radionuclide. The decay schemes of Tc-95m and Tc-96 show that there is no emission of beta particles; but there is an abundance of extranuclear electrons of low energy due to internal conversion and Auger electrons. The low penetrating power of these particles contributes to the excellent autoradiographic resolution. Figure 1, obtained with macroagregates of human serum albumin labeled with the described mixture of Tc-95m and Tc-96, shows the close correspondence between the deposits of silver grains and the radioactive sources. In particular, there is a very small response of the photographic emulsion outside the macroaggregates.

Validity of the radiopharmaceutical. A chromatogram on Whatmann No. 1 paper with methanol established the quality of the radiopharmaceutical. Furthermore, the biologic behavior of the preparations was tested by means of macroautoradiograms (Fig. 2A). A line of increased activity at the junction of the epiphyseal disk and metaphysis is noticeable. Moreover, the tracer uptake is far greater in the metaphysis than in the epiphysis. These results are in good agreement with those reported by Genant et al. (*4*).

Microautoradiograph results. The blackening of the photographic emulsion reflects the local distribution of the tracer. It presented some salient features (Figs. 2, 3, and 4). First, there was no accumulation of the labeled pyrophosphate in the different zones of the cartilage plate, whether in the resting zone or in the proliferating, maturing, or calcified cartilage. On the other hand, uptake was intense at the extremities of the vascular buds, so that the junction between the epiphyseal disk and the metaphysis was, in fact, a discontinuous line (Figs. 2C and 3).

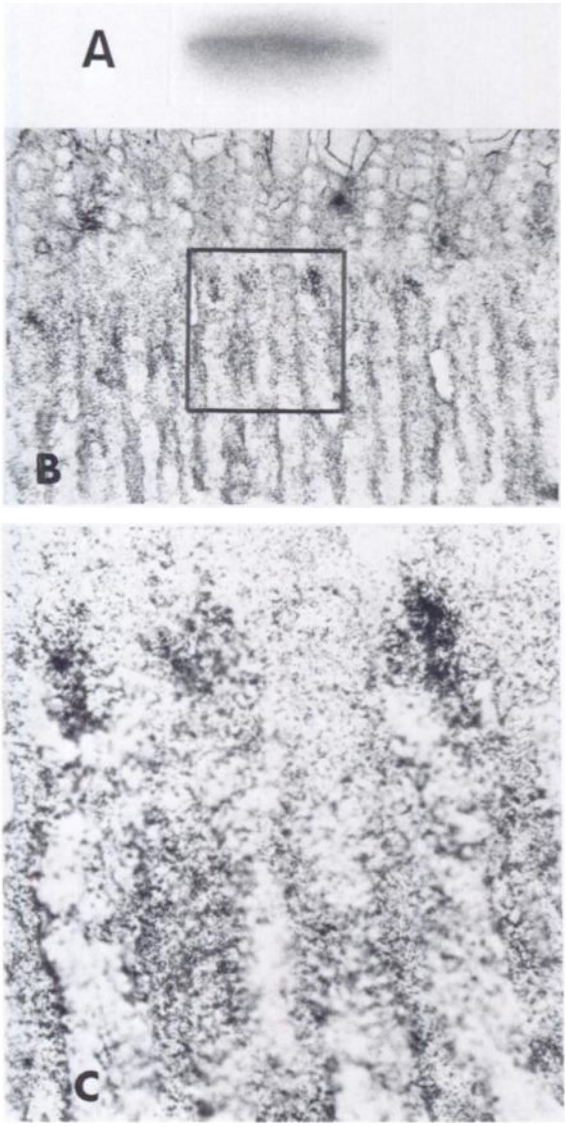

FIG. 2. Autoradiograms of the rabbit's epiphyseal plate after administration of technetium stannous pyrophosphate. (A) Crude distribution of the tracer obtained from tissue blocks. (B) Microautoradiogram of an undecalcified longitudinal section through cartilage plate (toluidine blue). (C) Framed area in (B) under higher magnification (unstained), to show calcified cartilage and zone of capillary invasion.

In the metaphysis, relatively linear deposits were following the lamellar bone (Fig. 3). Essentially no label could be observed in the centers of the osseous trabeculae; in fact, the label was distributed over a part of the bone tissue fluids with a much higher concentration at the calcified front.

Moreover, an important tracer incorporation could be seen over giant cells abutted on bone surfaces (Fig. 3). To identify such cells, a Masson's trichrome stain was performed and it established (Fig. 4) their multinucleated nature, suggesting strongly that they are osteoclasts.

DISCUSSION

Compared with the remodelling of mature bone, endochondral ossification is a process involving high turnover and more complex metabolic pathways. This model has been widely used for autoradiographic investigations of the distribution and movement of bone-seeking agents. Broadly speaking, the uptake of labeled pyrophosphate in the growing long bones follows the same pattern as the one already described for phosphates (*12*), fluoride (*4*), or alkaline earths such as calcium (*13,14*), or strontium (*4,13*): shortly after injection, these radionuclides are deposited in the new osseous trabeculae that are forming and calcifying on the diaphyseal side of the epiphyseal disks.

Previous investigations have already suggested that the localization of labeled phosphorus compounds is governed mainly by exchanges at highly vascularized bone surfaces (*4,6*). Results obtained in this study confirm the importance of the blood supply; indeed the crude repartition of the tagged material is strongly correlated with the general arrangement of the vessels in the epiphyseal growth plate (*15*). It is obvious that blood flow must play a significant part in the short-term uptake by delivering the radiopharmaceutical. Accordingly, a simple explanation for the failure of uptake by the calcified cartilage could be the absence of capillary vessels in such an area. On the other hand, the intense uptake at the extremities of the vascular buds with an interspersed pattern could well illustrate the local abundance of the capillary network.

Areas of increased blood flow such as the metaphyseal region, however, are also sites of rapid bone formation. Osteogenesis involves the availability of a large surface for ionic exchanges, high enzyme activities including alkaline phosphatase and pyrophosphatase, and increased matrix formation. Because of the great affinity of pyrophosphate both for hydroxyapatite (*7*), enzymatic systems (*8*), and immature collagen (*9*), an assessment of the precise mechanism responsible for bone localization is needed. It has already been demonstrated by histochemical methods (*16*) that osteoblasts invading the diaphyseal side of the plate produce phosphatase, whereas the zone of calcified cartilage lacks this enzyme. Nevertheless, beneath the epiphyseal disk, the band of intense phosphatase activity is quite large and could hardly account for the thin and discontinuous line of intense pyrophosphate accumulation. Moreover, in the metaphysis, the osteoblasts are characterized by their extremely high content of alkaline phosphatase, while the neighboring osteoid tissue is rich in immature collagen (*13*). Clearly the linear deposits of tagged pyrophosphate, which are

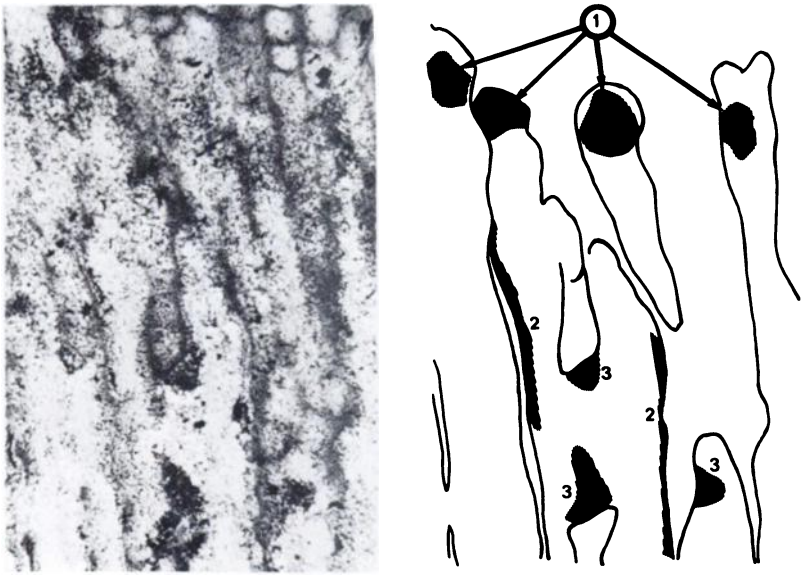

FIG. 3. Longitudinal section through distal epiphyseal cartilage plate and metaphysis (toluidine blue) to show (see diagram "B"): (1) intense labeling of extremities of vascular buds; (2) linear patterns underlining zones of appositions; and (3) high grain densities over giant cells.

observed essentially at the calcified front of the osseous trabeculae, do not reflect the local repartition of either phosphatase activity or immature collagen.

In the metaphysis, there is a great abundance of linear deposits of pyrophosphate (Fig. 2B), which indicates the formation of many new bone spicules and trabeculae. This pattern is in good agreement with the localizations of other phosphorus compounds in cancellous bone reported by Tilden et al. (*17*) and Jones et al. (*3*). As already observed by Tilden's group, this study shows that 3 hr after injection there is enhanced uptake at the calcified front (Fig. 3), although there is still scattered radioactivity over the osteoid matrix (Fig. 4). Twenty-four hours after administration, however, Jones et al. (*3*) found deposits of silver grains only at the calcified front below the osteoid layer. From these results, one might speculate that in the young and incompletely calcified trabeculae with enriched blood supply, the phosphorus compounds might be involved in exchanges between blood and the labile fraction of the bone mineral. After diffusion, the tagged material might precipitate and accumulate on bone-crystal nuclei. Additional microautoradiographic studies, at various times after injection, must be done to confirm this hypothesis. The display of long-term uptake of pyrophosphate in mature bone demands long-lived isotopes of technetium such as Tc-95m and Tc-96. Such kinetic studies are currently progressing in our laboratory.

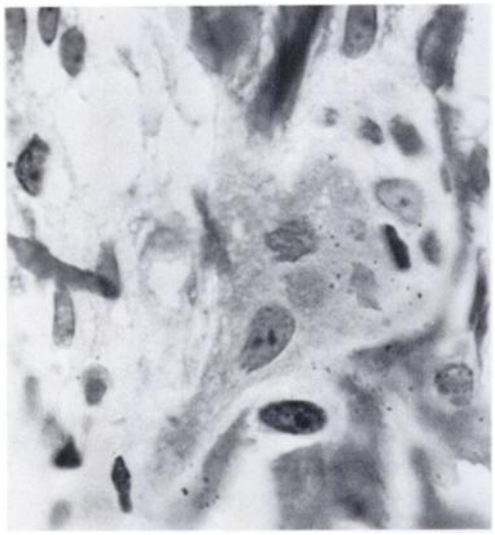

FIG. 4. Detail of metaphysis (Masson's trichrome). One multinucleated cell, identified as an osteoclast, is well labeled. A more diffuse uptake is noticeable facing the neighboring osteoid matrix stained light green.

Beside apposition, osteoclasts also contribute to the skeletal accumulation of pyrophosphate (Fig. 4). This result, however, would not guarantee a positive bone scan in the presence of an osteolytic lesion. When osteolysis results from bone resorption actually due to active osteoclasts, one might expect a locally increased uptake mediated by both cellular and extracellular components. But when osteolysis is due to other processes—such as destruction by other giant cells (*18*)—no firm result can be expected.

Finally, this study emphasizes the great interest of the 95m and 96 isotopes of technetium for animal experiments. In recent years, the major contribution of Tc-99m to the development of nuclear

medicine necessitates careful investigations of the biologic behavior of the radiopharmaceuticals labeled with this nuclide. Long-lived isotopes of technetium are essential to the progress of our knowledge in this field.

FOOTNOTES

* K2 or K5, Ilford.
† Kodak.
‡ Ilford.
|| PE 4006, Kodak.

ACKNOWLEDGMENT

The authors express their gratitude to A. Bardy and M. Hegesippe of the Commissariat à l'Energie Atomique, Centre d'Etudes Nucléaires de Saclay, Gif-sur-Yvette, for their helpful advice; also to J. C. Allard of the Laboratoire d'Anatomie Pathologique, Faculté de Médecine, Tours, for the preparation of the histologic material. We are grateful to Miss A. Jouhanneau for secretarial assistance.

This work was supported in part by Research Grant No. CA 424 from the Institut National de la Santé et de la Recherche Médicale.

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