RENEWAL OF PHOSPHATE IN BONE MINERALS

II. RADIOAUTOGRAPHIC STUDIES OF THE RENEWAL OF PHOSPHATE IN DIFFERENT STRUCTURES OF BONE

by

BENGT ENGFELDT, ARNE ENGSTRÖM, AND ROLF ZETTERSTRÖM

Patho-Anatomical Department and Department for Cell Research, Karolinska Institutet and Department of Metabolic Research, Wenner-Gren Institute, Stockholm (Sweden)

During the first hours after administration of radioactive phosphate there is a rapid uptake of the tracer in the skeleton (for relevant references *cf.* HEVESY[1]): further uptake, however, is very slow. MANLY, HODGE AND MANLY[2] suggested that calcified tissues are composed of two physiologically different parts, one of which rapidly establishes an equilibrium with the blood orthophosphate while the other part reaches equilibrium very slowly.

AMPRINO AND ENGSTRÖM[3], using microradiography, studied the distribution of calcium salts in thin sections of bones of different animals. It was found that calcium salts are unevenly distributed in bone. The bundles of collagen fibres are less calcified than the surrounding amorphous organic substances of bone matrix. The secondary bone, formed through reconstruction, has a lower concentration of mineral salts than primary bone. The calcification of secondary bone increases gradually but the speed seems to be higher in the beginning. The increment of calcification of secondary bone is most rapid in the parts of the matrix lying close to the vascular channels.

This paper communicates an investigation of the uptake of radioactive phosphate in different structures of bone and in the same structures but with varied content of mineral salts, (new and old bone, primary and secondary bone). The distribution of labeled phosphate in thin cross sections was investigated with radioautography and the content of calcium salts in the same sections with microradiography.

EXPERIMENTAL

Preparation of the bone specimens. Young dogs, about three months old and weighing about 7–8 kg, were injected intravenously with 10 millicuries (mc) of radioactive phosphate (^{32}P) as Na_2HPO_4, containing about 50 μg P. The animals were sacrificed after a lapse of three hours and three days, respectively.

Ground cross sections, 20–50 micra in thickness, were prepared from different parts of the long bones and from the costal ribs. The sections were ground by hand on glass plates under absolute ethanol.

Radioautography. Ground sections were placed in contact with Agfa Printon film. Filmstrips were placed on both sides of the bone specimen. By this arrangement it was possible to make sure

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that the thickness of the specimen was small enough to permit localization of a certain structure. After about three weeks exposure the films were developed. After developing, fixing, and washing the films were dried in a dust-free atmosphere. Enlargements of the radioautograms were obtained by photomicrography: Gaevert Replica high contrast plates were used.

Microradiography. A Machlett AEG 50 T X-ray tube was used as radiation source. The bone sections were pressed against the emulsion-coated side of a Lipmannfilm with extremely fine grain. At the same time and on the same plate as the microradiographic image of the specimen was recorded, a reference system was registered. The X-rays used were generated at 6000 volts, which corresponds to a wavelength maximum intensity of about 2.5–3 A. The X-rays were filtered in 1 mm Be. The wavelength band of the X-rays used corresponds to the maximum absorption of calcium on the short wave side of the K-absorption edge, thus securing maximal contrast.

Enlarged pictures of the microradiograms of the specimen and of the reference system were obtained by photomicrography. Gaevert Replica plates were used. In the photomicrographs the density between different bone structures can be compared with that of the reference system, and the data interpreted quantitatively (*cf.*[3]).

RESULTS

Fig. 1 shows a photomicrograph of a microradiogram of a cross section 30 micra thick from the metatarsus of a horse. The different distribution of calcium in different parts of the section can be clearly seen. The osteocytes appear as round or spindel-shaped areas transparent for X-rays. Some Haversian systems (osteons) are less calcified than the surrounding bone tissue.

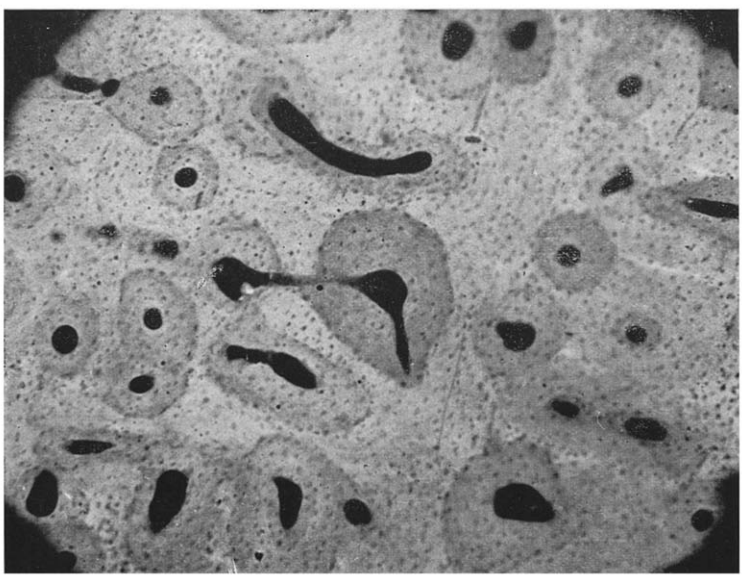

Fig. 1. Photomicrograph of a microradiogram of a thin cross section of the compacta of metatarsus from a horse. The white areas are those that have the strongest X-ray absorption. Note the difference in absorption between different Haversian systems. Magn. about 60 ×.

Some radioautograms of bone specimens of a dog killed three days after the injection of ^{32}P are shown in Fig. 2. It is quite obvious that, in compact bone, the periosteal and the endosteal layers, and certain Haversian systems have a much higher uptake of labelled phosphate than other parts of the section (Fig. 2 C and 2 E). Fig. 2 A demonstrates more in detail that the uptake of radioactive phosphate varies enormously from system

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to system. In costal ribs, *i.e.*, spongious bone, the uptake is very high, as shown in Fig. 2B. In lamellar bone, Fig. 2D, the uptake is relatively low.

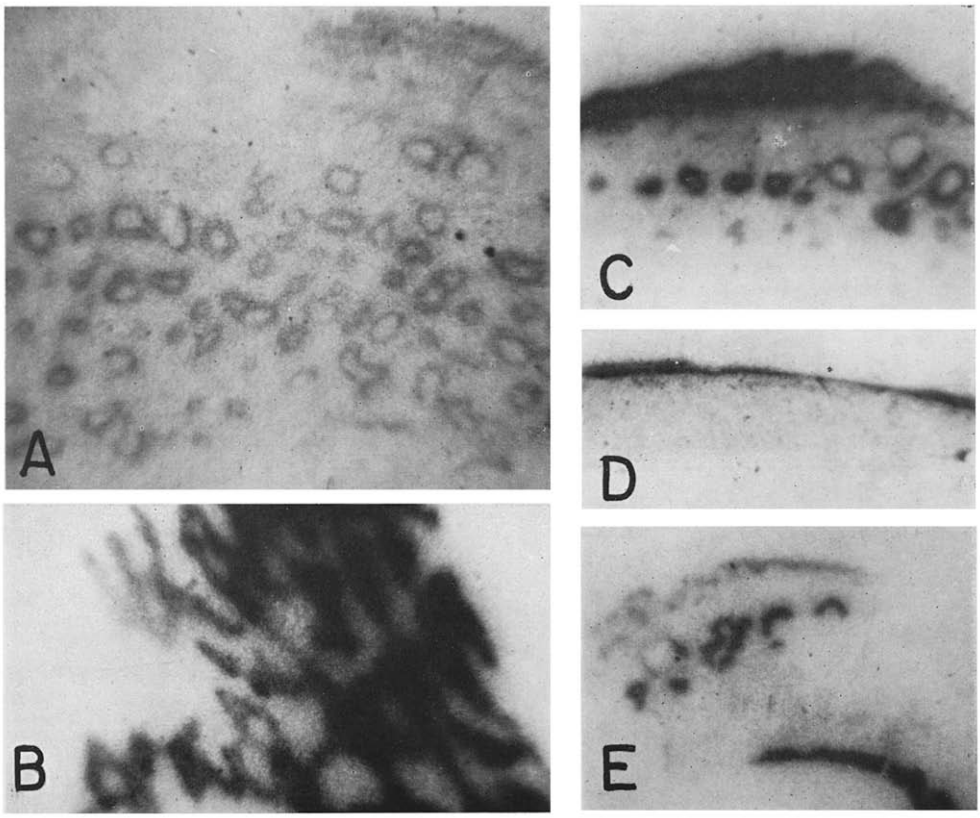

Fig. 2. Enlargements of radioautographs from cross sections of bones of a dog, given 10 mc of ^{32}P intravenously three days before sacrificing. In A, C and E the different uptake of the tracer phosphate in different Haversian systems is demonstrated. D is a part of a lamellar bone. This bone structure has a very small uptake of the labeled phosphate, except in the periosteal layer. B is a section of a rib; this spongious bone has a very high uptake of ^{32}P. Magn. about 25 ×

In radioautograms of bones of dogs killed three hours after the injection of ^{32}P the same pattern could be seen with the exception that the amount of activity in old parts of the bone tissue seemed to be less.

Fig. 3 shows a comparison between the radioautographic and microradiographic images of the same bone section. The Haversian system at 1 has a low content of mineral salts and a high uptake of labeled phosphate. The Haversian system at 2 has a high content of mineral salts and a low activity. Bone surrounding the resorption cavity at 3 has a high content of mineral salts and shows no activity. The old parts of the bone, lying between the Haversian systems, have taken up relatively small amounts of activity.

The relation between the content of radioactive phosphate, as indicated by the radioautograms, and the amount of mineral salts, as measured from the microradiograms, is demonstrated by the graph drawn in Fig. 4.

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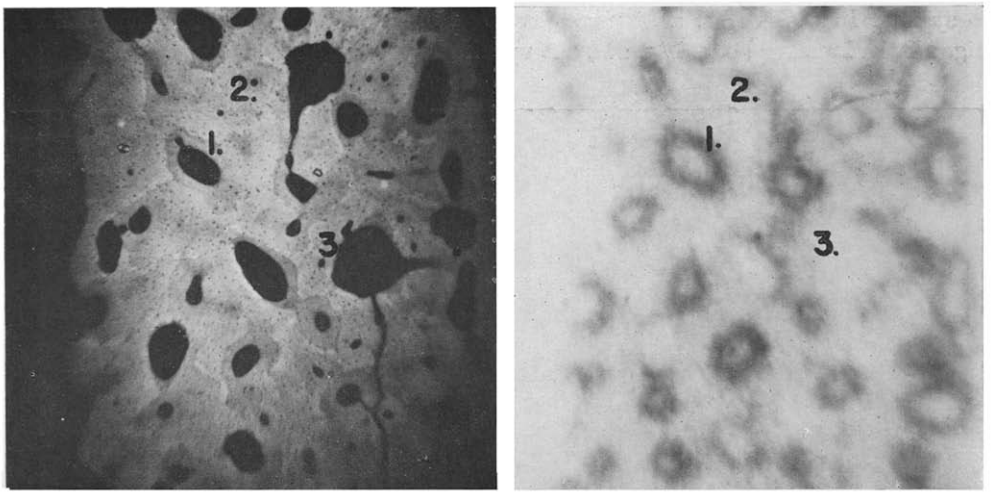

Fig. 3. To the left an enlargement of an X-ray microradiograph and to the right a radioautograph of the same section of the compacta of femur of a dog, given 10 mc of radioactive phosphate three days before sacrificing. 1. indicates a young and 2 an old Haversian system. The uptake of ^{32}P is high in 1 and low in 2. 3 is bone tissue surrounding a resorption cavity; in this part of the bone there is no activity. Magn. about 40 ×.

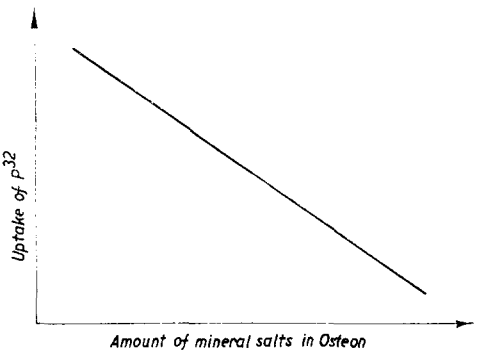

Fig. 4. Diagram showing the relation between the uptake of radioactive phosphate and the amount of mineral salts in osteons.

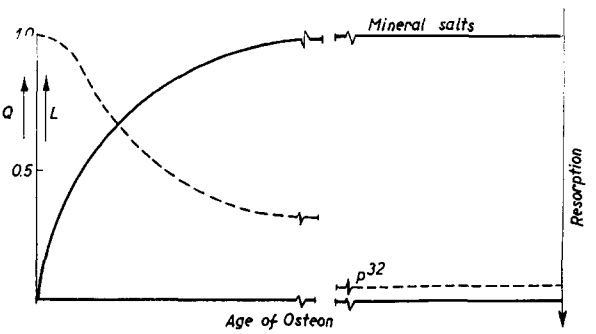

Fig. 5. Diagram showing the content of mineral salts and uptake of radioactive phosphate in different stages of the development of an osteon. Q is the quotient of mineral salts determined by quantitative microradiography between young and old osteons (the latter have full calcification, arbitrarily numbered as 1). L is the rate of renewal of phosphate.

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Fig. 5 shows schematic and hypothetic graphs of the content of mineral salts and the uptake of labelled phosphate in different stages of osteon development. The first process in the formation of an osteon seems to be relatively rapid (*cf.*[3]), since almost all Haversian system in a cross-section have a mineralization between 0.95 and 1.0; the higher value being the relative content of mineral salts in the old parts of the bone. Although no exact information exists on the length of time required for an osteon to proceed from mineralization 0.95 to 1.0, it seems reasonable that this stage in the development of the osteon requires a long period of time.

DISCUSSION

In the skeleton, the uptake of labelled phosphate is much too prompt to account for new calcification. HEVESY, LEVY AND REBBE[4] and others[5,6] have suggested that the rapid uptake is due to an ion exchange on the surfaces of the ultramicroscopic crystals. However, the radioautographic patterns of cross-sections of long bones show an uneven distribution of radioactive phosphate. The most rapid uptake of labelled phosphate is in Haversian systems with a low content of mineral salts, *i.e.*, young osteons. As these systems become older and the amount of mineral salts approaches a maximum value, the uptake of activity is very much lowered. Thus, since most of the activity is found in limited areas, the initial rapid uptake of labelled phosphate cannot be due to an ion exchange on all crystals in the bone tissue.

Furthermore, the initial uptake of radioactive phosphate in the skeleton as determined from chemical investigations[2] is too high to be accounted for solely on the basis of surface reactions in these localized areas. NEUMAN AND MULRYAN[7] have not been able to explain the uptake of labeled phosphate in fresh bone only as the result of a surface reaction. They proposed that bone minerals undergo recrystallization at a fairly rapid rate. From our experiments it seems as if the initial uptake is due exclusively to some kind of recrystallization. Thus, the recrystallization process in the skeleton cannot be a process resulting from variations in the concentrations of calcium and phosphate ions in the tissue fluids, as suggested by HEVESY[8].

The renewal of phosphate is most rapid in young Haversian systems and in the periosteal and endosteal layers, *i.e.*, in areas with newly deposited mineral salts. The differences in renewal rate of phosphate between different portions of bone minerals also seem to be dependent upon differences in solubility[9]. Therefore, since the uptake of radioactive phosphate is lowered when the osteons become older, the solubility of the bone minerals must, at the same time, be decreased. Furthermore, substitutions and alterations in the crystalline structure, which decrease the solubility of the mineral salts, must proceed parallel to the further development of the newly formed bone tissue.

The extremely low amount of radioactive phosphate taken up by the old parts of the bone tissue indicates that, in these parts, ion exchange on the surfaces of the bone crystals must be very low. Since a surface reaction undoubtedly occurs in all crystals in ashed bone powder (*cf.* FALKENHEIM, NEUMAN AND HODGE[6]), the organic constituent of fresh bone must prevent such an exchange.

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SUMMARY

1. Labelled phosphate, when injected intravenously, is unevenly distributed in bone tissue.

2. Radioautographic and microradiographic examinations have shown that young Haversian systems have the highest uptake of radioactive phosphate. When a system becomes older and the amount of mineral salts approaches a maximum value the uptake of radioactive phosphate becomes very low.

3. The main reason for the rapid initial uptake of labelled phosphate cannot be the occurrence of surface reactions throughout the whole bone tissue.

RÉSUMÉ

1. Le phosphate marqué, injecté de manière intra-veineuse, se distribue inégalement dans le tissu osseux.

2. Des examens radioautographiques et microradiographiques ont montré que les systèmes de Havers jeunes absorbent le plus de phosphate radioactif. Lorsque un système viellit et que la quantité de sels minéraux approche la valeur maximale, l'absorption de phosphore radioactif devient très faible.

3. La principale raison de la rapidité de l'absorption initiale de phosphate marqué ne doit pas être cherchée dans les réactions de surface qui ont lieu partout dans le tissu osseux.

ZUSAMMENFASSUNG

1. Intravenös injiziertes markiertes Phosphat verteilt sich unregelmässig im Knochengewebe.

2. Radioautographische und mikroradiographische Untersuchungen ergaben, dass junge Havers'sche Systeme am meisten radioaktives Phosphat aufnehmen. Wenn ein System älter wird und sich der Mineralsalzgehalt dem Höchstwerte nähert, dann wird die Aufnahme von radioaktivem Phosphor sehr schwach.

3. Der Hauptgrund für die rasche, anfängliche Aufnahme von markiertem Phosphat kann nicht das Vorkommen von Oberflächen-reaktionen überall im Knochengewebe sein.

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