FROM THE ORTHOPAEDIC CLINIC, MALMÖ (CHIEF: S. VON ROSEN, M.D.) AND THE PHARMACOLOGIC INSTITUTE (CHIEF: G. AHLGREN, M.D.) OF THE UNIVERSITY OF LUND, SWEDEN

BONE SALT METABOLISM IN BONE GROWTH AND BONE REPAIR STUDIED IN RATS BY MEANS OF Ca^{45}, P^{32}, AND Na^{22}

By

GÖRAN C. H. BAUER

In 1951 there was universal agreement that the bone salt of the skeleton is in a state of rapid equilibrium with the body fluids, and that it is therefore impossible to use radioactive isotopes as an index of the amount of calcium and phosphorus being laid down in bone.

In controversy to this opinion, *Carlsson* (1951 and 1952) demonstrated that the deposition rate of bone salt can be determined by means of the isotope technique.

The difference between these two opinions is of fundamental importance for the understanding of the metabolism of the inorganic part of the skeleton. In the present paper the main issue of this controversy will be discussed.

Some papers by the author (*Bauer* 1954 a–f) on this subject will be dealt with in their connection with the main problem.

When radiocalcium or radiophosphorus is administered to rats, the initial activity uptake is greater in the epiphyseal-metaphyseal ends than in the diaphyseal portions of the long bones. Thus, twenty-four hours after the administration of radiocalcium to young rats, *Harrison* and *Harrison* (1950) found about three times more activity per unit weight of calcium (ash) in the ends than in the shafts of the long bones. Fourteen days after the administration of the isotope, the same specific activity was found in the ends as in the shafts of the long bones. *Harrison* and *Harrison* stated that the specific activity of all calcium in the skeleton would then be uniform and equal to that of the body fluid calcium.

This interpretation implies that during these fourteen days, all cal-

cium of the entire skeleton had been renewed. According to *Harrison* and *Harrison* this had been brought about through a rapid, reversible process in which ions of the bone salt crystals exchange with ions of the surrounding fluid.

Eighteen days after the administration of P^{32} to young rats, *Neuman* and *Mulryan* (1952) found the specific activities of the epiphyseal and diaphyseal portions of the long bones to be equal. *Neuman* and *Mulryan* believed that the specific activity of all inorganic phosphorus of the skeleton was then equal and uniform to that of the body fluid inorganic phosphorus, i.e. an equilibrium with respect to the distribution of the isotope had been reached.

Neuman and *Mulryan* assumed that the calcium-phosphorus-salt of the skeleton is in a state of continual "recrystallization" and that this mechanism explains the supposed rapid equilibrium between bone salt and body fluid. (No proper definition of the term recrystallization seems available. In a recent review *Neuman* and *Neuman* (1953) seem to mean by recrystallization both "surface exchange" and dissolution-reprecipitation of the bone salt crystals.)

The experiments of *Harrison* and *Harrison* and *Neuman* and *Mulryan* have lent support to the theory that all the inorganic salt of the entire skeleton is readily available for ionic exchange with ions of the body fluids. According to this theory, bone growth (which implies bone salt deposition and bone salt resorption together with the organic matrix, and is generally a slower process than the supposed exchange processes) is of minor importance for the redistribution of administered radiocalcium and radiophosphorus in the skeleton.

In 1951 this interpretation of skeletal tracer-data had been universally accepted and is still the basis of the prevalent opinion (at least in the USA) as evidenced by *inter al. Sacks* (1953, page 287) who stated that "... the occurrence of chemical exchange reactions between bone and the ions it yields to solution ... have made it impossible to use isotopes as an index of the amount of calcium and phosphorus being laid down in bone."

Carlsson (1951), on the other hand, demonstrated that eighteen hours after the administration of the isotope to growing rats *the distribution of radiocalcium was closely related to the growth and calcification of the different parts of the skeleton.*

Lindquist (1952) made similar observations. *Carlsson* (1952) also showed that the uptake of activity in the incisor teeth was an irreversible process (*vide infra*) and expressed the view that even in the skeleton, exchange processes were of minor importance.

In absolute contrast to *Carlsson*'s opinion, *Neuman* and *Neuman*

in their recent review still maintained that the bone salt of the skeleton is in a state of "dynamic equilibrium" with the body fluids.

It should be realized that the difference between these opinions is of fundamental importance for the interpretation of skeletal tracer-data.

The remark should be made that, concerning the redistribution of isotope in the long bones, there is a flaw in the interpretation of *Harrison* and *Harrison* and *Neuman* and *Mulryan*: they have not shown that the specific activity ratio epiphyseal/diaphyseal bone, once it had reached unity, really remained at this level. This condition is, however, essential to make their theory plausible (*Bauer* (1954 a)). The following experiment, therefore, was made to find out whether or not the specific activities remain equal in different bone locations:

Young rats were killed eighteen hours—sixty-seven days following the administration of Ca^{45}. It was found that the specific activity ratio diaphyseal/epiphyseal bone rose from a value below unity to a value far above this level. This rise was continuous during a period of sixty-seven days following the administration of the isotope. It was further shown (*Bauer* 1954 a and b) that the redistribution of radiocalcium and radiophosphorus in different parts of the skeleton varied with the rate of growth of these parts.

The consequence of this finding is that rapid, reversible processes seem to be of minor importance compared to skeletal growth for the redistribution of radiocalcium and radiophosphorus in the skeleton: Isotope, once deposited in the bone salt, is only released to the circulation when the bone salt, containing the isotope, is reached by a resorption process which removes (dissolves) the bone salt together with the organic matrix. Such a resorption process is a normal feature both in growing bone (see *Lacroix* (1951)) and in mature bone (see *Jowsey et al.* (1953)).

Bone salt (calcium), once deposited in the organic matrix of the skeleton (teeth) is thus relatively unavailable for the supposed exchange processes.

The importance of this conclusion was demonstrated by *Carlsson* (1952) when from tracer-data he made a numerical computation of the deposition rate of calcium in the incisor teeth of rats:

Supposing the calcium deposition (formation of bone salt) to be an irreversible process occurring at a certain, constant rate, the blood calcium activity curve gives the amount of activity which would accumulate in the calcified tissues at varying intervals following the administration of the isotope. A comparison with this amount of activity eight hours after the administration of radiocalcium to rats revealed

that the amount of activity recovered from the incisor teeth corresponded to a deposition rate of about 0.10 mg calcium per hour in the four incisors. Eighteen hours after the administration of the isotope, the value computed for this rate was found to be the same as the eight hour value. This value for the rate of accretion of calcium in the incisor teeth was found to be in agreement with values arrived at by means of earlier (morphological) methods.

Carlsson drew the conclusion that an irreversible process accounts for the uptake of activity in the incisors.

However, during the initial hours following the administration of the isotope, the values for the deposition rate of calcium, arrived at with the above method, were found to be higher than the steady value of the eight to eighteen hour interval. A reversible process might thus account for part of the activity recovered from the incisors during the early intervals. The following radiosodium experiment (*Bauer* 1954 c) demonstrates the influence of a reversible process on the above computation.

Sodium is present in bone principally in two fractions:

1) The fraction that can be accounted for on the basis of the extracellular water present in bone.

2) The fraction that cannot be accounted for on this basis ("excess sodium").

About sixty per cent of this excess sodium does not enter into exchange with the serum sodium as demonstrated by *in vivo* experiments with radiosodium.

Following the administration of Na^{22} to young rats, it was found that the non-exchangeable bone sodium is deposited with the formation of bone tissue (*Bauer* 1954 c and d). The Na^{22} incorporated in the non-exchangeable bone sodium is not removed until the bone structure is reached by resorption (*Bauer* 1954 e).

The accretion rate of sodium into the non-exchangeable part of the incisor sodium was computed according to the same principle as described above. It was found that this computation does not give a true value for this rate until the amount of activity due to exchangeable sodium in the incisors was low compared to the amount of activity due to the non-exchangeable fraction of the incisor sodium.

The period of time following the administration of the isotope during which the above computation is influenced by reversible activity uptake is thus determined by the ratio of the exchangeable/non-exchangeable fractions of the total activity uptake in a certain specimen of bone or teeth. The following factors make this period of time shorter in a radiocalcium experiment than in a radiosodium experiment. With radiocalcium there is:

a) a more rapid fall in serum specific activity,

b) a lower ratio of the exchangeable fraction (including the water phase fraction) to the non-exchangeable fraction.

A knowledge of these relations permits a computation of the rate of formation of tissues from tracer-data (probably not only in the calcified tissues).

The difference in mode and rate of growth within a bone is visualized by a series of Na^{22} radio-autograms (*Bauer* 1954 e). The autograms serve to confirm the opinion that sodium, incorporated within the stable (non-exchangeable) part of the bone sodium, is not removed until the corresponding bone structure is reached by resorption. This finding suggests that at least part of the bone sodium is located in the bone salt crystals (as is the opinion of *Engström* (1953) and not only on the surfaces of these crystals (as was maintained by *Hendricks* (1952)).

Earlier methods for the determination of the dynamics of bone growth and bone repair (chiefly histological methods including vital staining and X-ray investigations) can seldom estimate the relative or absolute rate of bone salt formation and bone salt resorption. The radiotracer method was applied to this problem:

Ca^{45} was administered to rats with a seven-day-old fracture of one femur shaft (*Bauer* (1954 f)). *It was found that the isotope technique permitted a computation of the rate of bone salt formation both in the intact and in the fractured shaft.* The rate of calcium deposition in the fracture callus was determined even during the period of rapid increase in this rate. It was further found that the bone salt of the fracture callus stays unremoved by any resorption process at least during the initial four to five days following its formation.

In the metaphyseal area adjacent to the fractured shaft, the rate of bone salt deposition seemed to be relatively unaffected by the trauma whereas the rate of bone salt resorption was enhanced.

These interpretations conflict with the opinions of earlier investigators (*Bohr* and *Sørensen* (1950), *Wilkinson* and *Leblond* (1953)), presumably because these have expressed the uptake of activity in terms of activity per unit weight of calcium (ash) instead of in terms of absolute activity uptake. With a normal deposition rate of bone salt, a higher initial specific activity will be reached if the removal rate of bone salt is increased than if the removal rate of bone salt is normal. (It seems possible that this explains why *Wilkinson* and *Leblond* found a higher specific activity in the metaphyseal bone of the fractured femur than in that of the intact femur in spite of the fact that in their radio-autograms they found the same degree of blackening.)

Even in the tibia of the fractured side a distinct effect on the deposition and removal of activity was caused by the femur fracture.

In this paper (*Bauer* 1954 f) it was pointed out how, *under certain conditions, the activity ratio of any two specimens of the calcified tissues will give the ratio of their respective rates of bone salt deposition.*

This method of computing the deposition rate of bone salt in the skeleton is easier and more accurate than earlier methods. It is applicable to problems which have as yet been impossible to solve: The accretion rate of calcium in a healing fracture has thus not been possible to determine with earlier methods. Preliminary data suggest that this method will give a better understanding of the influence of various factors on the normal process of bone growth and fracture healing.

REFERENCES

Bauer, G. C. H.: The Importance of Bone Growth as a Factor in the Redistribution of Bone Salt. I. Redistribution of Radio-Active Calcium in the Skeleton of Rats. J. Bone and Joint Surg. *36* A: 375–380, 1954 a.

– The Importance of Bone Growth as a Factor in the Redistribution of Bone Salt. II. Redistribution of Radio-Active Phosphorus in the Skeleton of Rats. Ibidem. *36* A: 381–386, 1954 b.

– Metabolism of Bone Sodium in Rats Investigated with Na^{22}. Acta Physiol. Scand. (in press), 1954 c.

– A Rapid Method for the Simultaneous Determination of Calcium and Sodium in Bone. Ibidem (in press), 1954 d.

– Long Term Redistribution of Bone Sodium Studied in Rats by Means of Na^{22} Radio-Autography. Acta Orthopaed. Scand. *23*: 192–197, 1954 e.

– Rate of Bone Salt Formation in a Healing Fracture Determined in Rats by Means of Radiocalcium. Ibidem *23*: 169–191, 1954 f.

Bohr, H. and *Sørensen, A. H.:* Study of Fracture Healing by Means of Radioactive Tracers. J. Bone and Joint Surg. *32* A: 567–574, 1950.

Carlsson, A.: Metabolism of Radiocalcium in Relation to Calcium Intake in Young Rats. Acta Pharm. et Toxicol. *7*: Suppl. 1, 1951.

– On the Mechanism of the Skeletal Turnover of Lime Salts. Acta Physiol. Scand. *26*: 200–211, 1952.

Engström, Arne: Personal communication, 1953.

Harrison, H. E. and *Harrison, H. C.:* The Uptake of Radiocalcium by the Skeleton: The Effect of Vitamin D and Calcium Intake. J. Biol. Chem. *185*: 857–867, 1950.

Hendricks, S. B.: Trans. Macy Conference on Metabolic Interrelations, *4*: page 246, 1952.

Jowsey, J., Owen, M. and *Vaughan, J.:* Microradiographs and Autoradiographs of Cortical Bone From Monkeys Injected with ^{90}Sr. Brit. J. Exp. Path. *34*: 661–667, 1953.

Lacroix, P.: The Organization of Bones. J. & A. Churchill Ltd, London, 1951.

Lindquist, B.: Effect of Vitamin D on the Metabolism of Radiocalcium in Rachitic Rats. Acta Paediatrica. Vol. 41. Suppl. 86. 1952.

Neuman, W. F. and *Mulryan, B. J.:* The Surface Chemistry of Bone. VI. Recrystallization in Vivo. J. Biol. Chem. *195*: 843–848, 1952.

Neuman, W. F. and *Neuman, M. W.:* The Nature of the Mineral Phase of Bone. Chemical Reviews. *53*: 1–45, 1953.

Sacks, J.: Isotopic Tracers in Biochemistry and Physiology, ed. 1, McGraw-Hill Book Company, York 1953, page 287.

Wilkinson, G. W. and *Leblond, C. P.:* The Deposition of Radiophosphorus in Fractured Bones in Rats. Surg. Gyn. and Obstr. *97*: 143–150, 1953.