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LONG TERM REDISTRIBUTION OF BONE SODIUM STUDIED IN RATS BY MEANS OF Na^{22} RADIO-AUTOGRAPHY

By

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Sodium is present in bone in principally 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 60 % of this excess sodium does not enter into exchange with the serum sodium. In an earlier paper (*Bauer* (1954 c)), it was shown that this non-exchangeable sodium is deposited with the formation of bone tissue. Following the administration of Na^{22} to growing rats, a comparison of the skeletal and incisor teeth sodium activities with the serum sodium activity made possible a computation of the rate of incorporation of sodium into this stable bone sodium.

The following autograms show how the radiosodium incorporated in the humerus of the rat is not released until the corresponding part of the bone is reached by a resorption process.

EXPERIMENT

In an earlier experiment (*Bauer*, l. c.) about 90 μC Na^{22} were injected subcutaneously into twenty-one six-week-old rats. The rats were killed in groups of three at varying intervals after injection. In each animal a serum sample, both femurs and tibias, one humerus and

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the incisor teeth were used for quantitative measurements, described in the earlier paper.

For the present investigation, one humerus in each animal of the 1 day–25 day groups was removed immediately after death of the animals, dissected free from adhering soft tissues and dried at room temperature for a few days.

A thin longitudinal section of each humerus was then prepared by grinding under water on a stone and with fine sand. When about 40 μ thick, the section was mounted on a thin glass plate with plast cement and placed in close contact to a photographic plate (Gaevert Superchrom 32°) and held there by means of tape.

After sufficient exposure time (in this series two-four weeks) the radio-autograms were developed (using as a developer Promicrol M & B), fixed, washed, and dried.

The technique followed in general was that described as the contact autography by *Leblond et al.* (1950).

RESULTS

The rats grew in size during the experiment. The body weight increased from 119 g at the start of the experiment to 136 g twenty-five days later. During this period of time, the dry weight of the humerus increased from 86.6 mg to 99.3 mg. From a comparison of the autograms (plate 1) it is seen how the humerus increased in length.

In the autograms from the animals which were killed a short time (1–4 days) after the administration of the radiosodium, both shaft and ends of the bone are clearly visible (plate 1, A). A long time after the administration of the isotope (16–25 days), a clear image is still to be seen over the shaft of the humerus (plate 1, C and D). The proximal metaphyseal portion of the bone is at this time hardly visible.

By comparing the 4, 8, 16, and 25 day autograms it is seen that this difference gradually develops. The distal metaphyseal portion of the bone is clearly visible at all intervals investigated.

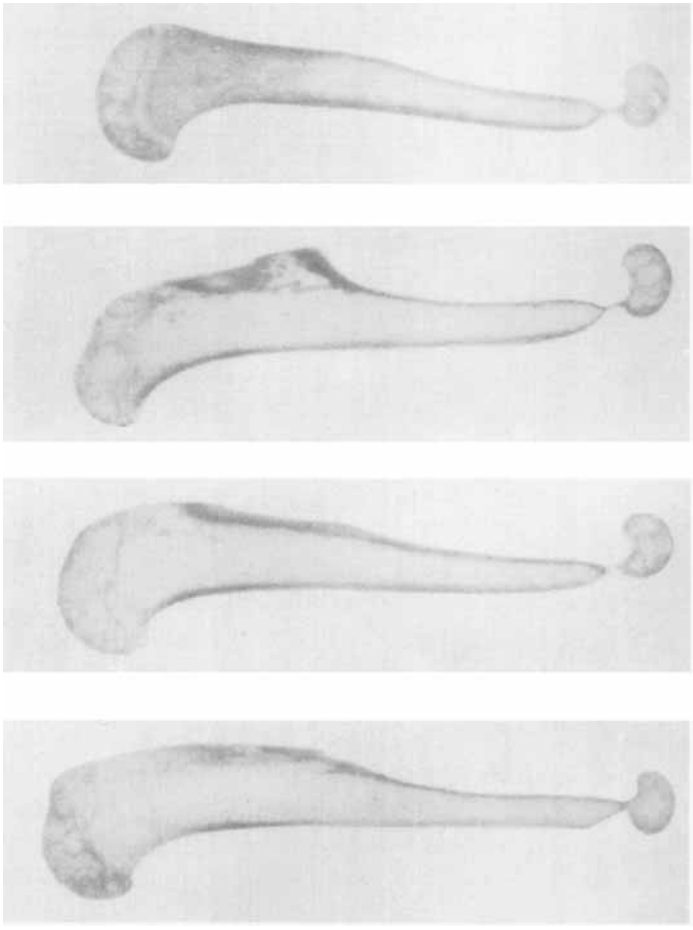
DISCUSSION

Immediately after the administration of the radiosodium, the specific activity of the serum sodium was high. This value decreased to a sixteen-day value of less than one tenth of the one-day value. At day twenty-five, practically no radiosodium was left in the serum.

During this twenty-five day period, a considerable part of the stable

PLATE 1

Humerus Radio-Autograms From Rats Killed 4–25 Days Following the Administration of Na^{22}.



A. Killed 4 days after isotope injection

B. Killed 8 days after isotope injection

C. Killed 16 days after isotope injection

D. Killed 25 days after isotope injection

Note: The autograms of this plate have been magnified 4 times their original size.

bone sodium had been deposited from the serum. A long time after the administration of the radiosodium, the sodium specific activity of the bone was therefore higher than that of the serum.

However, growth of a long bone implies not only formation but also resorption of bone tissue as the marrow cavity widens and the bone ends are remodeled. In the bone shaft the interval of time between formation and resorption of bone tissue is longer than is this interval in the metaphyseal part of the bone. There is furthermore a difference between the ends so that the rate of bone growth is higher in the

proximal than in the distal metaphysis of the humerus. (For a survey of the morphological events during bone growth, see *Lacroix* (1951)).

The Na^{22} autograms reflect this difference in the length of time between formation and resorption of the different parts of the humerus. Thus an image of the shaft is seen even twenty-five days after the administration of the isotope, presumably because the radiosodium deposited in the subperiosteal zone of bone growth has not yet been reached by the endosteal zone of bone resorption.

The initial clearly visible image of the metaphyseal area is, however, hardly visible at day twenty-five. This loss of radiosodium is probably due to the relatively short interval of time between formation and resorption of the bone trabeculae of the actively growing metaphysis.

In the distal metaphysis no such heavy loss of radiosodium is observed: an image of this area is still to be seen at day twenty-five. This finding corresponds with the above statement that practically all longitudinal growth occurs in the proximal metaphysis of the humerus.

The redistribution of bone salt in the growing skeleton has been studied radio-autographically by *Leblond et al.* (l. c.) (P^{32} in cats and rats), *Comar et al.* (1952) (Ca^{45} in pigs), *Kidman et al.* (1952) (Sr^{89} in rabbits), *Skipper et al.* (1951) (C^{14} in rats). These investigations show that a great part of the bone salt crystals formed in the zones of bone growth, lie unchanged until they are ultimately reached by the process of resorption of the bone part in question (see *Bauer* (1954 a and b)). The present investigation seems to confirm this opinion. This confirmation is even more interesting as it is not known for certain whether or not sodium is part of the bone salt crystal structure or bound to the surfaces of the crystals. It suggests that at leasf 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)).

SUMMARY

Growing rats were killed one to twenty-five days after the administration of Na^{22}. A radio-autogram was prepared from one humerus in each rat. A comparison of the autograms warrants the following conclusions:

1) During bone growth sodium is incorporated in the stable (non-exchangeable) part of the bone sodium.

2) This stable bone sodium is not removed until the bone structure is reached by resorption.

3) The difference in mode and rate of growth within a bone is accordingly visualized by the Na^{22} radio-autograms.

RESUME

Des rats ont été sacrifiés durant la période de croissance à des intervalles variant entre un et 25 jours après l'administration de Na^{22}. Un radio-autogramme d'un humérus de chaque rat a été préparé. La comparaison de ces autogrammes permet de formuler les conclusions suivantes:

1) Durant la croissance de l'os, le sodium est incorporé dans la partie stable — non-interchangeable du sodium de l'os.

2) Ce sodium stable de l'os n'est pas éliminé avant que la structure de l'os soit atteinte par la résorption.

3) La différence dans le mode et le degré de croissance à l'intérieur d'un os est démontrée en conséquence par les radio-autogrammes Na^{22}.

ZUSAMMENFASSUNG

Heranwachsende Ratten wurde einen bis fünfundzwanzig Tage nach der Verabreichung von Na^{22} getötet. Ein Radio-Autogramm wurde von einem Humerus jeder Ratte ausgeführt. Ein Vergleich der Autogramme ergibt die folgenden Schlussfolgerungen.

1) Während des Knochenwachstums wird Natrium in den stabilen (nicht auswechselbaren) Teil des Knochennatriums einverleibt.

2) Dieses stabile Knochennatrium wird nicht weggeschafft ehe der Knochen vom Aufsaugungsprozess erreicht wird.

3) Der Unterschied in der Art und Geschwindigkeit des Wachstums innerhalb des Knochens wird daher durch die Radio-Autogramme anschaulich gemacht.

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