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ON THE UPTAKE OF RADIOACTIVE CALCIUM AND STRONTIUM IN THE SKELETON OF NORMAL AND RACHITIC RATS

By

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Since the investigation of Chievitz and Hevesy (1) in 1937 on the uptake of radioactive phosphate in the skeleton of rats, the radioactive isotopes, especially radioactive calcium and strontium, have been applied to a great extent in the study of bone metabolism.

Different opinions have, however, been held as regards the explanation of the results. Several authors, among those Hevesy (2), Manly (3), Harrison (4) and Newman (5) have taken the view that besides the incorporation through growth, shown markedly in the incisors of the rat, reversible exchange processes seem to account for the main uptake of radioactive calcium, strontium and phosphate in the bone tissue. On the other hand Carlsson and Bauer (6) have stressed the opinion that the greater part of this uptake is determined by accretion and resorbtion processes.

This question has important bearings on the results of investigations with radioactive isotopes in bone transplants and during fracture healing as well as regards the accumulation in pathological processes of the bone tissue, recently demonstrated by Bauer and Wendeberg (7).

An interesting difference between the uptake of radioactive strontium in the bone tissue of normal and rachitic rats killed at different periods after injection of the isotope was found by Jones and Copp (8). Both in normal and rachitic animals uptake was observed, but while in the normal animals the radioactivity remained practically constant during the following day, it was shown that the activity of the bones of the

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rachitic rats decreased within 8 hours to about one half of the maximum value.

These results afford an opportunity to distinguish between different stages in the uptake of radioactive strontium in the bones, and in the present investigation the results of the uptake of radioactive calcium and strontium in normal and rachitic rats, through measurements on the living animals, give further support to such a conclusion.

The radioactive calcium isotope, Ca^{47}, was obtained from Amersham as a carrier-free solution in $CaCl_2$, it has a half life of 4.9 days and the gamma rays emitted have a spectrum with an energy maximum at 1.3 M.E.V. The radiation was measured with a scintillation counter using a bias at 0.4 M.E.V. to eliminate the radiation from Sc^{47}, which is the immediate decay product.

The beta-emitting isotope, Ca^{45}, with a half life of 160 days, used especially in autoradiography, was also obtained from Amersham as a carrier-free solution of $CaCl_2$.

The radioactive strontium isotope, Sr^{85}, was obtained from the Nuclear and Engineering Corporation, Pittsburg, as a carrier-free solution of $SrCl_2$ in HCl. This isotope has a half life of 64 days and emits gamma radiation with an energy maximum at 0.51 M.E.V. The measurements of this isotope were carried out with a scintillation counter using a canal set around the energy maximum.

The experiment animals were white rats of the Wistar strain. The standard food contained adequate amounts of calcium, phosphate and vitamins.

Rickets were produced in 4 week old rats, which were reared on Steenbock and Blach's food No. 2965 for 3 weeks without exposure to direct sunlight. The contents of Calcium in the food were 1.3 % and that of phosphorus 0.28 %. The degree of rickets was controlled by X-ray photos of the proximal tibial epiphysis.

Through an exposure of the vena femoralis during ether anæsthesia the radioactive isotope was injected intravenously in amounts of about 5–10 μc.

Autoradiographic pictures were taken through contacting the tissue sample with Ilford Nuclear Plates, G. 5, with emulsion thickness of 50 μ. Microradiographic pictures were produced with the Philips Contact Microradiographic Apparatus, Type 11990, using Kodac spectroscopic film No. 649–0. Tissue samples were prepared after imbedding in methylmetacrylate by cutting with a saw and grinding to a specimen thickness of about 50 μ.

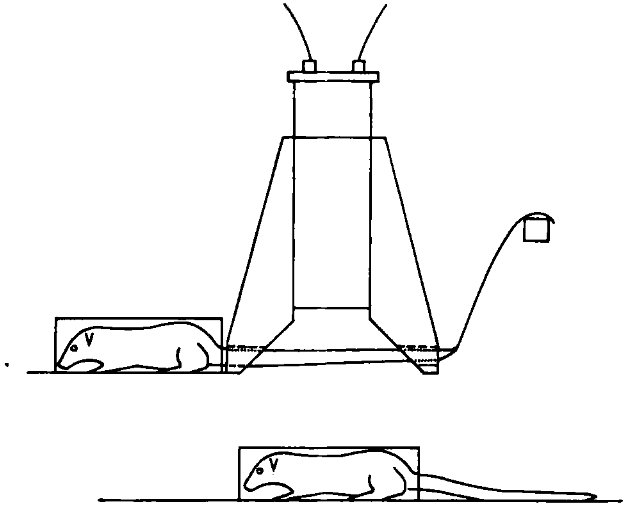

Fig. 1.
Measurement of the activity, top: of the tail, below: of the total body.

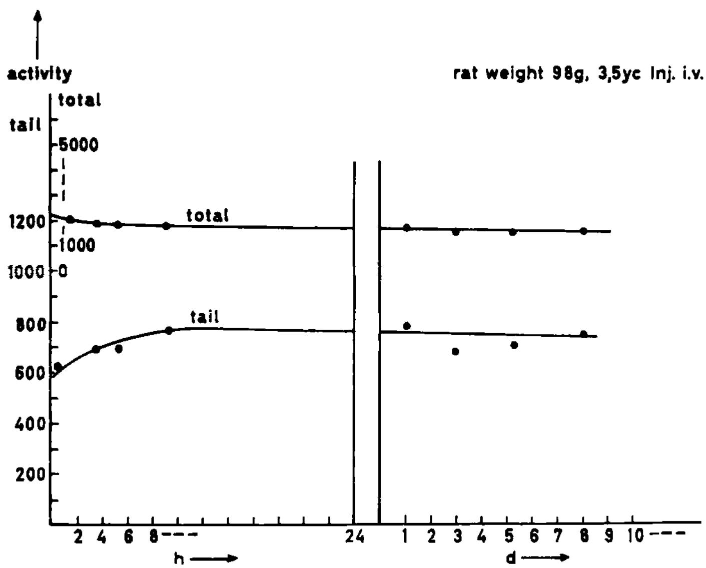

Fig. 2.
Activity of the tail and the total body of normal rats following i.v. inj. of C^{47}.

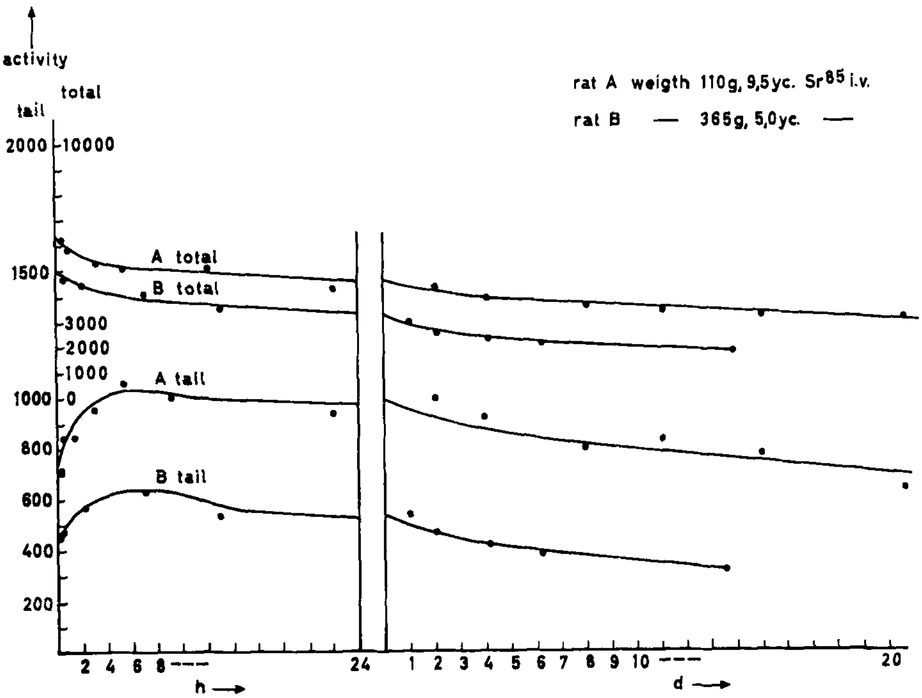

Fig. 3.
Activity of tail and the total body of normal rats following i.v. inj. of Sr^{85}.

Fig. 1 shows how the radioactive radiation from the tail was determined, when the tail was placed between openings in the lead shield and fastened with adhesive tape. The radiation from the total animal was measured when the wire cage of the animal was fixed at a certain distance from the crystal counter. These determinations were made almost continuously during the first 2–3 hours after the injection of the isotope and continued with intervals of hours through the first day and then every second or fourth day for about 10–20 days.

On Fig. 2 the results of the measurements with Ca^{47} on a normal rat weighing 90 g., are shown. The activity is given in counts pr. min., after correction for background and decay. Every determination was made with not less than 5000 counts, the counting error thus being below $\pm$ 2 %. It can be seen that while the total activity decreases a little during the first hours after the injection of the isotope, due to excretion from the rat, the activity of the tail rises until a maximum is reached about 8 hours after the injection. During the next 12 hours the activity of the tail remains constant and is only slightly reduced

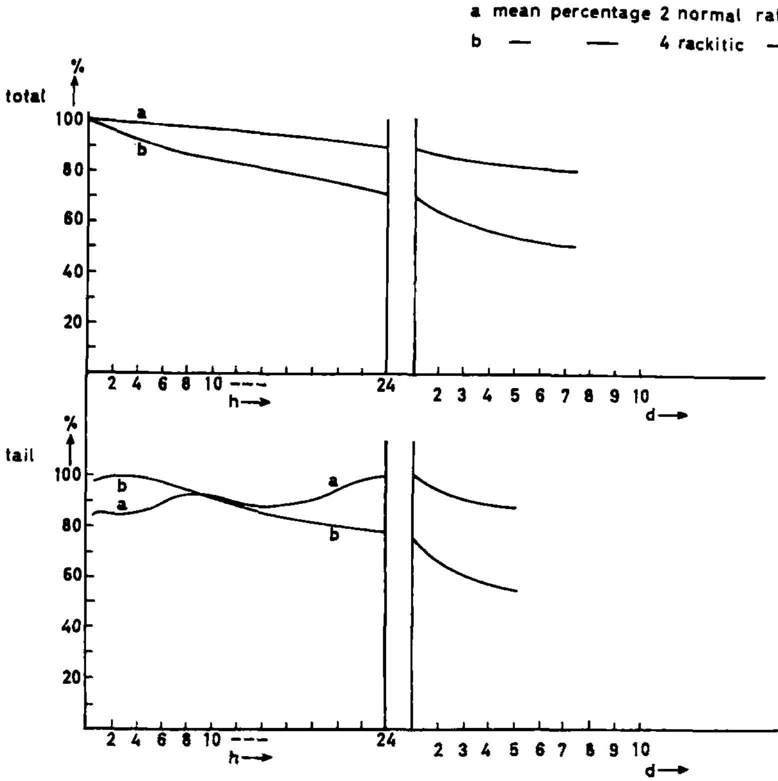

Fig. 4.
Activity in per cent of the maximal uptake measured in normal and rachitic rats following i.v. inj. of Ca^{47}.

during the following days. Fig. 3 shows that the results with Sr^{85} are practically the same except that the activities of the total animal and the tail decrease at a somewhat faster rate after the lapse of 24 hours than in the case of Ca^{47}. In the adult rat, B, weighing 365 g., the results are the same as in the young rat, A, weighing only 110 g. The reduced activities in B are due to a correspondingly smaller amount of radioactive isotope injected into this animal.

The measurements on the rachitic rats are shown on Figs. 4 and 5, where the results are given as percentage deviation from the maximal activities obtained. In the rachitic animals the total activity as well as the activity of the tail decrease from about 4 hours after the injection of the isotope. In the case of Sr^{85}, where the measurements were continued for a longer period, the activities of the total body and the tail were

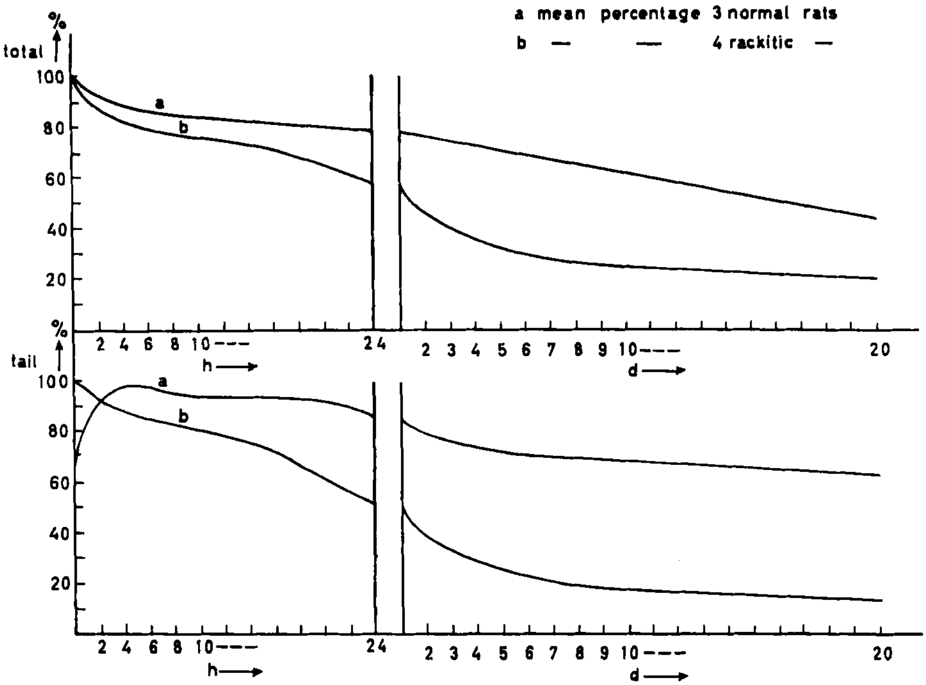

Fig. 5.

Activity in per cent of the maximal uptake measured in normal and rachitic rats following i.v. inj. of Sr^{85}.

reduced to 1/5 as compared with the normal animals in the course of 10 days. Tables 1 and 2 show that the amount of radioactive calcium and strontium taken up in the rachitic rats during the first hours after the injection of the isotope is just as large as in the normal rats. This corresponds well with the results seen on Fig. 6, which show that the amount of radioactive calcium in the blood during the first two hours following an intravenous injection of Ca^{45}, decreases along almost identical curves in normal and rachitic rats. The activity of the blood is measured every 5 min. pr. 0.025 ml. whole blood with a Geiger Müller counter arrangement and the results given as a percentage of the activity in blood calculated at the moment of the injection.

Fig. 7 shows an autoradiographic picture of a longitudinal section of the tail of a rachitic rat, 2 hours after an intravenous injection of Ca^{45}, where it is seen that the radioactive calcium is located mainly in the bone. The activity of the tail therefore closely corresponds to the amount of radioactive isotope already taken up in the bone tissue 2 hours after the injection.

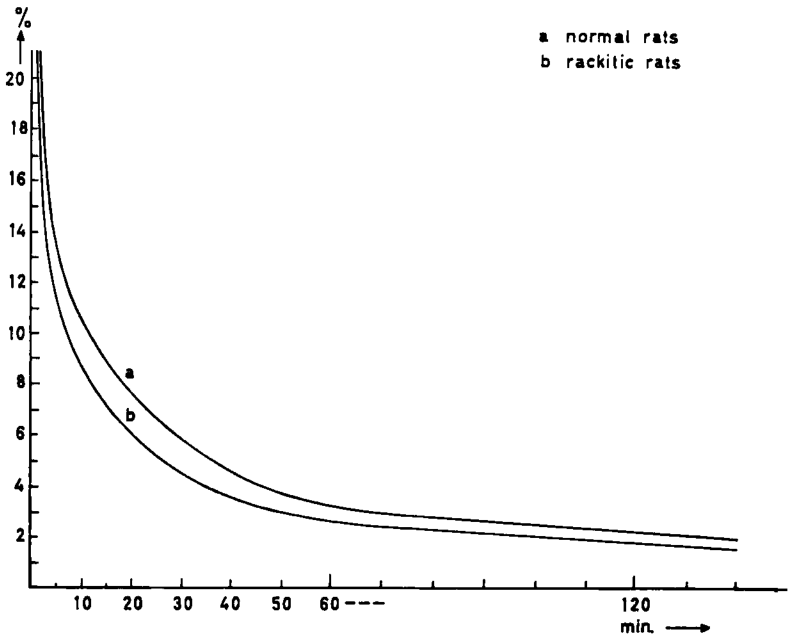

Fig. 6.
Activity pr. ml. blood in per cent of the total activity following i.v. inj. of Ca^{45}.

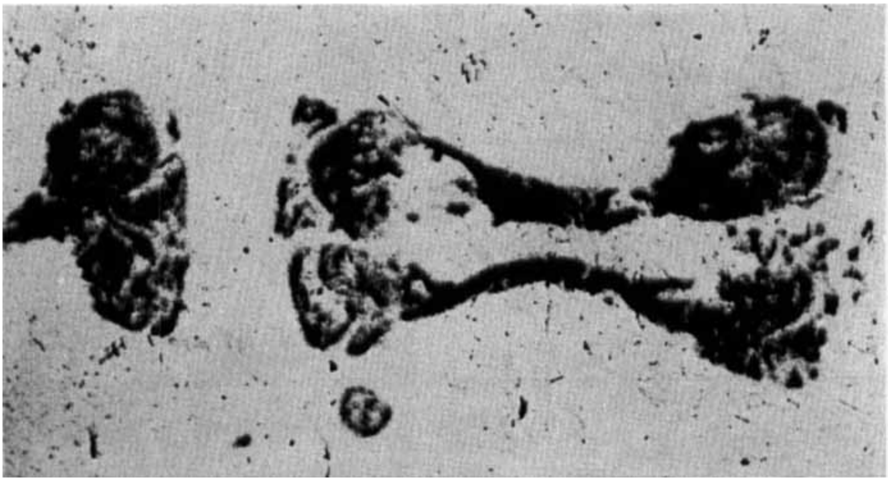

Fig. 7.
Autoradiography of a longitudinal section of the tail of a rachitic rat 2 hours after i.v. inj. of Ca^{45}.

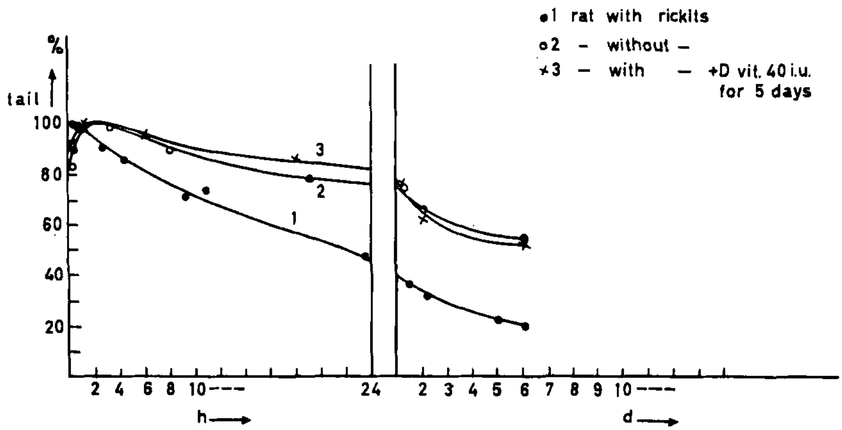

Fig. 8.
Activity in per cent of the maximal uptake measured in rats on rachitogenic food after i.v. inj. of Sr^{85}.

TABLE 1
Relative activities of the tail and the total body of normal and rachitic rats following i.v. inj. of Ca^{47}.

		Mean value c/m 1/2 hour after inj.	Mean value c/m maximal activity
Rachitic rats	tail	901	910
	total		2393
Normal rats	tail	865	1005
	total		2560

TABLE 2
Relative activities of the tail and the total body of normal and rachitic rats following i.v. inj. of Sr^{85}.

		Mean value c/m 1/2 hour after inj.	Mean value c/m maximal activity
Rachitic rats	tail	1561	1616
	total		7700
Normal rats	tail	1203	1493
	total		7400

Fig. 8 shows the results of the activity measurements after injection of Sr^{85} into 3 young rats of almost the same weight, all of which had been treated with the rachitogenic food of Steenbock and Black and under the same conditions. Rat No. 1 showed well developed rickets on X-ray photos before the injection of radioactive strontium. No. 2 did not show signs of rickets and in No. 3 rickets produced and verified on X-ray photos were cured with the addition of 40 I.U. D-vitamin daily for 5 days to the rachitogenic food. The different curves demonstrate how the rachitic state of the bones can be shown through the decrease in the activity measured.

DISCUSSION AND CONCLUSION

It has been shown by several authors, among these Leblond and Greulich (9), Comar, Gotz and Boyd (10), Kidman, Rayner, Tutt and Waughan (11) and Engstrøm, Bjørnerstedt, Clemedson and Nelson (12) that the radioactive calcium and strontium injected into the animal in the course of a few minutes rapidly accumulates in the skeleton, the main part being taken up already within the lapse of 1 hour.

As the bone tissue occupies a greater part of the tail than of the rest of the body it is to be expected that a rise in the activity of the tail should take place during the first hours following the injection of the isotope, in agreement with the results shown in the present investigation.

The measurements on the rachitic animals have confirmed the observations of Jones and Copp (8) which show that the uptake of radioactive calcium and strontium during the first hour following the injection of the isotope is almost equal in the bones of normal and rachitic animals, but that the amount of radioactive isotope in the rachitic bone then decreases to about one half during the next 24 hours, while it remains almost constant in the normal bones.

Furthermore, the present investigations, in agreement with the results of Norris and Kisieleski (13) and Bauer, Carlsson and Lindquist (14) have shown that strontium, at least in tracer doses follows calcium both as regards the uptake in the bone and the intermediate metabolism in bone tissue but that strontium is excreted at a faster rate than calcium from the organism.

In Figs. 9 and 10 the distribution of the radioactive calcium taken up in the proximal part of the tibia bone 2 hours after an intravenous injection of Ca^{45} into a normal and rachitic rat, can be seen through

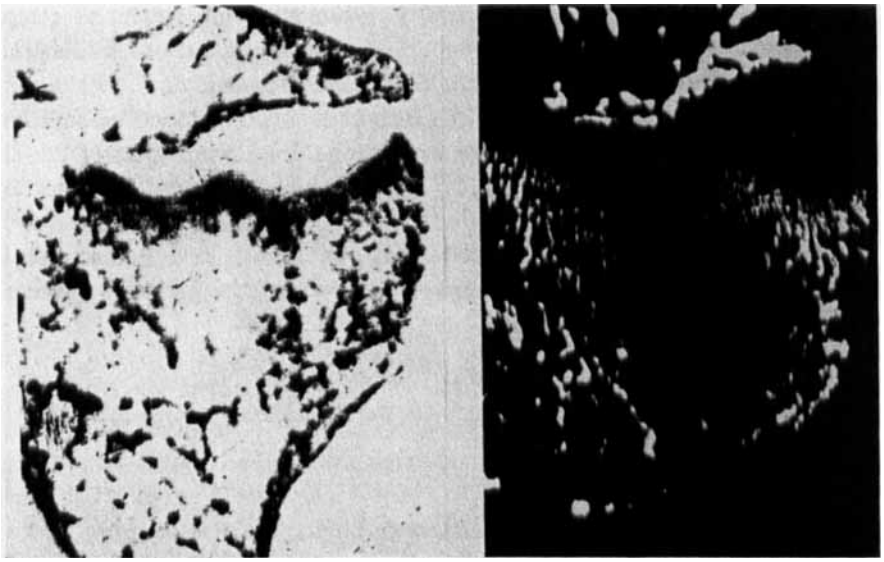

Fig. 9.
Autoradiography (left) and microradiography (right) of the proximal end of the tibia from a rachitic rat 2 hours after i.v. inj. of Ca^{45}.

comparison of an autoradiographic and a microradiographic picture. It is shown that besides the more diffuse uptake in the bone tissue itself, demonstrated in both the normal and the rachitic bone, a heavy accumulation takes place on the metaphyseal side of the epiphyseal line in the normal bone, which has almost no corresponding phenomenon in the rachitic bone.

From the above-mentioned results it can be presumed that the immediate uptake of radioactive calcium and strontium in the bone, as seen during the first hours in both normal and rachitic animals, is due mainly to a reversible exchange process shown by the decrease in the activity of the rachitic animals during the succeeding hours. And only that part of the activity which is taken up at the epiphyseal line has a possibility of proceeding deeper into the skeleton by accretion processes.

Such a distinction between the primary uptake due to exchange processes and a secondary slower uptake due to irreversible accretion processes is illustrated by the results obtained under different experimental conditions.

Through autoradiographic studies it was thus shown by Engström and coll. (12) that the activity taken up in the skeleton during

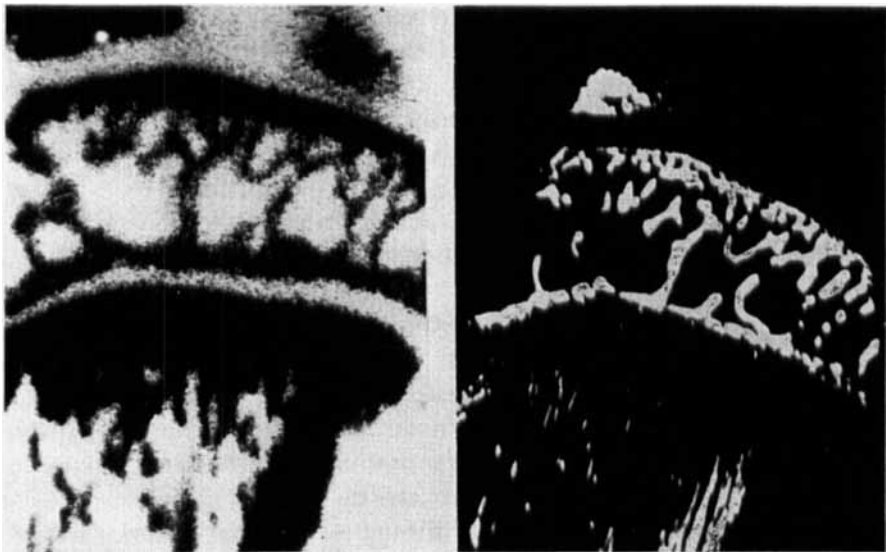

Fig. 10.
Autoradiography (left) and microradiography (right) of the proximal end of the tibia from a normal rat 2 hours after i.v. inj. of Ca^{45}.

the first hours after an intraperitoneal injection of radioactive strontium into rats almost entirely disappeared during the following five months. Hevesy (15) on the other hand has shown that about one half of the radioactive calcium taken up in the skeleton of mice during the foetal stage through the mother organism, is retained throughout the life of the animal.

If we want to evaluate the intensity of the accretion processes, determining the degree of growth or remodelling of the bones through measurements of the uptake of radioactive calcium or strontium it seems necessary, according to the conclusions drawn here, to follow such activity over longer periods, periods in the case of growing rats likely to be at least 10 days.

It is hoped by continued investigation into these questions to obtain further experimental evidence regarding the nature of calcium metabolism under normal and pathological conditions.

SUMMARY

The uptake of radiocalcium, Ca^{47}, and radiostrontium, Sr^{85}, given through intravenous injection to normal and rachitic rats is followed

through measurements of the activity of the tail and the total body of the living animals. During the first hours after the injection the activity of the total body decreases a little due to excretion of the radioactive isotope from the animals while the activity of the tail rises on account of accumulation of radioactive calcium and strontium in the bone. The uptake in the bone during the first hour after the injection is the same in the normal and the rachitic rats, but while the activity remains practically constant during the following 24 hours in the normal animals it is shown that the activity in the rachitic rats decreases within 8 hours to about one half of the maximal value.

These results, which confirm the previous investigations by Jones and Copp, afford an opportunity to distinguish between different stages in the uptake of radioactive calcium and strontium in the bone. It thus seems that the immediate, primary uptake must be considered due mainly to a reversible exchange between the blood and the bone tissue, and that accretion takes place only through a secondary, slower uptake.

RESUME

L'assimilation de radiocalcium, Ca^{47}, et de radiostrontium, Sr^{85}, administrés par injection intraveineuse à des rats normaux et rachitiques est suivie par des mensurations de l'activité de la queue et du corps entier chez les animaux vivants. Durant les premières heures après l'injection, l'activité du corps entier tombe un peu en raison de l'excrétion par les animaux d'isotopes radioactifs, alors que l'activité de la queue augmente par suite de l'accumulation de calcium et de strontium radioactifs dans les os. L'assimilation dans les os durant les premières heures après l'injection est la même chez les rats normaux et rachitiques, mais alors que l'activité reste pratiquement constante durant les 24 heures suivantes chez les animaux normaux, on constate que l'activité tombe chez les rats rachitiques dans l'espace de 8 heures à la moitié de la valeur maximum.

Ces résultats, qui confirment les recherches antérieures de Jones et Copp, fournissent l'occasion de faire une distinction entre les différents stages de l'assimilation du calcium et du strontium radioactifs dans les os. D'après cela, il semble que l'absorption immédiate, primaire, doit être considérée comme principalement due à un échange réversible entre le sang et les tissus osseux et que l'accrétion n'a lieu que par une assimilation secondaire plus lente.

ZUSAMMENFASSUNG

Die Aufnahme von Radiumcalcium, Ca^{47}, und Radiostrontium, Sr^{85}, das mittels intravenöser Injektion an normale und rachitische Ratten verabreicht worden war, wurde mit Hilfe von Messungen der Aktivitet des Schwanzes und des gesamten Körpers am lebenden Tiere verfolgt. Während der ersten Stunden nach der Injektion fällt die Aktivitet des Gesamtkörpers ein wenig wegen der Ausscheidung von radioaktiven Isotopen durch das Tier, während die Aktivitet des Schwanzes auf Grund der Anhäufung von radioaktivem Calcium und Strontium im Knochen, ansteigt. Während der ersten Stunde nach der Injektion ist die Aufnahme im Knochen dieselbe bei normalen und rachitischen Ratten. Während jedoch die Aktivitet innerhalb der nächsten 24 Stunden bei normalen Tieren konstant verbleibt, zeigt es sich, dass die Aktivitet bei rachitischen Ratten innerhalb von 8 Stunden zur Hälfte der grössten Werte herabsinkt.

Diese Ergebnisse, die die vorausgegangenen Untersuchungen von Jones und Copp bestätigen, geben die Möglichkeit zwischen verschiedenen Stadien der Aufnahme von radioaktivem Calcium und Strontium in den Knochen zu unterscheiden. Es scheint daher, dass die unmittelbare, primäre Aufnahme hauptsächlich als ein reversibler Austausch zwischen Blut und Knochengewebe angesehen werden muss, und dass der Zuwachs nur infolge einer sekundären, langsameren Aufnahme vor sich geht.

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