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

RATE OF BONE SALT FORMATION IN A HEALING FRACTURE DETERMINED IN RATS BY MEANS OF RADIOCALCIUM

By

GÖRAN C. H. BAUER

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 rarely estimate the relative or absolute rate of bone salt formation and bone salt resorption.

It is therefore natural that the radiotracer technique when available was applied to problems of this nature. The results have thus far been disappointing (see *Sacks* (1953)), presumably because problems of a basic nature remained to be solved before a correct interpretation of the experimental data could be made.

However, *Leblond et al.* (1950) showed that morphological data concerning the mode of bone growth could be reached by their radio-autography technique, and *Carlsson* (1951) was able to differentiate between the building up and resorption of bone salt by means of tracer data.

The first attempt to obtain an exact figure for the formation rate of lime salt was made by *Carlsson* (1952) when he showed that the rate of growth of the incisor teeth in rats could be determined from tracer data.

The present investigation shows that it is possible to correlate the amount of activity recovered from different portions of bones in rats to the amount of bone salt formed in these bones in the course of bone growth and bone repair.

EXPERIMENT

The experiment was carried out on three series, comprising altogether seventy-seven female mature rats of an inbred strain. The rats weighed about 170 g at the start of the experiment and they increased insignificantly in weight during the experimental time. They received a normal diet before and during the experiment.

In *series I* each rat was subjected to a fracture of the right femur shaft. The fractures were made under ether anaesthesia by manually bending the bone. No attempt was made to immobilize the fractured leg. One week later, each rat was injected with a solution of calcium

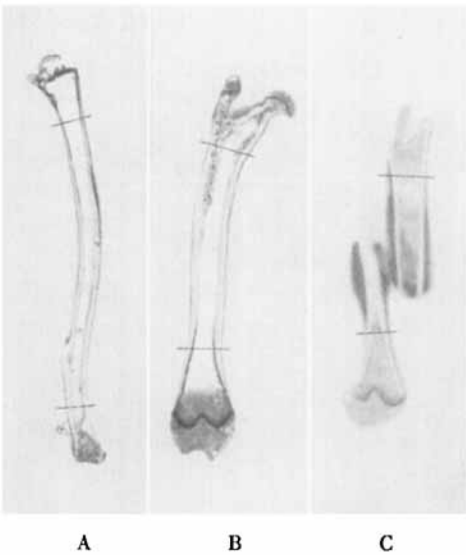


Plate 1.

Radio-autograms of an uninjured tibia (A), an uninjured femur (B), and a fractured femur (C). The animal was killed 24 hours after the administration of a few μC of Ca^{45}. The radio-autograms have been prepared according to the technique described by *Bauer* (1954 d).

In the present investigation the bones were divided in ends and shafts. The levels of division have been indicated in the radio-autograms.

lactate, containing 10 mg of a low specific activity Ca^{45}. The dose was a few μC. The injections were made in the loose subcutaneous tissue of the back.

The rats were killed with ether in six groups of five rats at intervals ranging from one to thirty-two days after the administration of the radiocalcium.

Immediately after death of the animals, the tibias, the femurs, and the incisor teeth were removed and dissected free from adhering soft tissues. By means of a circular saw, the ends of the tibias and the femurs were divided from the shafts. Care was taken, always to make these divisions through constant landmarks in the respective bones. In plate 1 the levels of division have been indicated on radioautographic images of a tibia and an intact and a fractured femur. (The technique used for these radio-autograms will be published elsewhere).

In each animal the following samples were thus obtained:

right femur metaphyseal ends,
left femur metaphyseal ends,
right femur shaft,
left femur shaft,
right tibia metaphyseal ends,
left tibia metaphyseal ends,
right tibia shaft,
left tibia shaft,
incisor teeth.

Each sample was ashed in an electric muffle. The ash was weighed and the activity was determined on a nitric acid solution of the ash. The technique followed was that of *Carlsson* (1951), with the exception that a rate-meter was used instead of a scaler, see *Bauer* (1954 a).

The standard deviation of the activity measurements was less than 5 % of the counting rates encountered. As a reference was used a solution containing the dose of Ca^{45} in 100 ml of nitric acid. This solution gave a counting rate of 42 c.p.s. The background was always less than 1 c.p.s.

In this paper the amounts of activity recovered from the different samples have been expressed in per cent of the dose administered. Unless expressly stated, no use has been made of the term "specific activity".

In *series II* alternatively the right or the left femur was fractured in each animal. One week after the fractures, the rats received a few μC Ca^{45} by means of a subcutaneous injection of calcium gluconate containing 20 mg of Ca.

The rats were killed in groups of two or three by bleeding under ether at intervals ranging from 1 to 120 hours after the administration of the radiocalcium. The blood was collected and the serum Ca was determined according to *Clark* and *Collip* (1925). The activity measurements were made on the solutions of Ca obtained in this way after they had been titrated with $KMnO_4$.

The tibias and the femurs were treated in the same way as those of series I. The incisors of each group were pooled. The activity measurements were made as those of series I.

In *series III* alternatively the right or the left femur shaft was fractured in each animal. 2–12 days after the fractures, the animals

TABLE 1

Activity Values of the Different Bone Samples and the Incisors at Varying Intervals of Time after the Administration of Radiocalcium to Mature Rats.

Days after the administration of the radiocalcium	Number of animals	Average weight of the animals	Average amount of activity[1] (expressed in per cent of dose) recovered from								
			femur ends[2]		femur shafts		tibia ends		tibia shafts		incisor teeth
			fract.[3]	uninj.	fract.	uninj.	fract. side	uninj. side	fract. side	uninj. side	
1	5	170 g	1.57	1.54	2.80	0.498	1.20	1.45	0.336	0.386	2.51
2	5	168 „	1.33	1.38	2.90	0.497	1.06	1.27	0.377	0.451	3.62
4	5	166 „	1.21	1.46	3.30	0.582	0.974	1.22	0.440	0.512	4.17
8	5	173 „	0.948	1.24	2.84	0.574	0.832	1.13	0.401	0.521	5.07
16	5	171 „	0.720	1.12	2.05	0.700	0.537	0.894	0.497	0.607	5.51
32	5	173 „	0.661	0.954	1.70	0.769	0.470	0.681	0.497	0.656	5.71

1 The S.D. of the activity measurements has been kept below 5 % of the counting rates encountered.

2 Consult plate 1 for an explanation of how the different bones were divided in ends and shafts.

3 One femur in each animal was fractured manually one week before the administration of the radiocalcium.

TABLE 2

Ash Weights of the Different Bone Samples and the Incisors at Varying Intervals of Time After the Administration of Radiocalcium to Mature Rats.

Days after the administration of the radiocalcium	Average ash weights (expressed in mg) of								
	femur ends[1]		femur shafts		tibia ends		tibia shafts		incisor teeth
	fract.[2]	uninj.	fract.	uninj.	fract. side	uninj. side	fract. side	uninj. side	
1	85.4	92.0	101.6	84.2	62.0	66.8	74.2	77.2	205.6
2	79.8	86.0	99.8	82.6	54.0	58.2	76.6	78.6	206.2
4	72.6	85.6	107.0	81.4	52.8	58.6	76.4	78.2	215.6
8	74.6	92.0	122.6	89.2	57.8	85.2	77.2	81.8	209.0
16	62.4	88.6	125.2	94.0	53.8	71.6	71.0	85.0	219.2
32	62.6	84.4	124.4	97.2	53.4	58.8	75.8	88.0	225.4

[1] Consult plate 1 for an explanation of how the different bones were divided in ends and shafts.

[2] One femur in each animal was fractured manually one week before the administration of the radiocalcium.

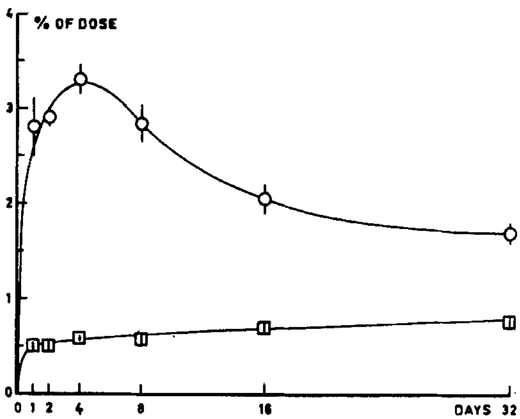
received a few μC Ca^{45} by means of a subcutaneous injection of calcium gluconate containing less than 10 mg of Ca.

The rats were killed in groups of six, twenty-four hours after the administration of the radiocalcium. The tibias and the femurs were treated the same as those of series I and II. The activity measurements were made as described above. However, the bones of one animal in each group were kept for radio-autography and therefore activity measurements were made on five animals in each group.

RESULTS

Series I. (The rats received Ca^{45} one week after the femur fracture and were killed in groups of five, one to thirty-two days later).

In table 1 and table 2 are recorded the average activities and ash weights of all the samples of the different groups. It is seen in these tables that there is a continuous loss of activity from the tibia and femur metaphyseal ends of both the fractured and the non-injured side from day 1 through all groups of the investigation. However, the



GRAPH 1

Activity Values[1] *of the Shafts*[2] *of the Femurs at Varying Intervals of Time After the Administration of Radiocalcium to Mature Rats.*

Explanation of symbols:

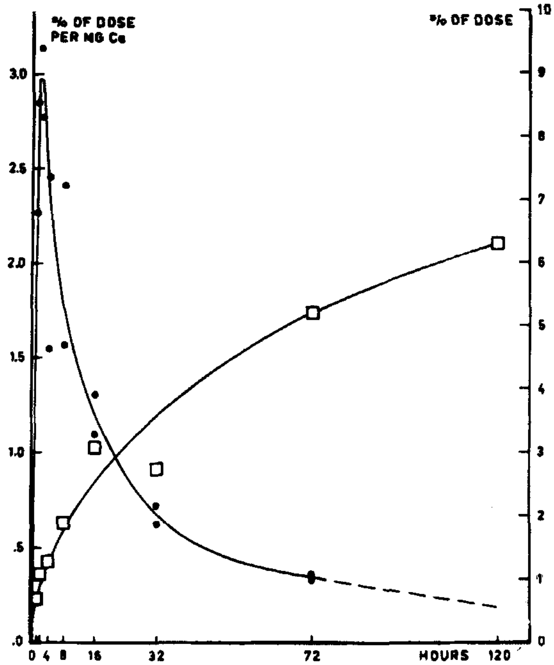
○ The shaft of the fractured[3] femur

□ „ „ „ „ uninjured „

[1] The activity values have been expressed in per cent of dose. The mean values are indicated in the graph ± the standard error. Five animals in each group.

[2] Consult plate 1 for an explanation of how the different bones were divided in ends and shafts.

[3] One femur in each animal was fractured manually one week before the administration of the radiocalcium.



GRAPH 2

Specific Activity of the Serum Ca and the Activity Values of the Incisor Teeth at Varying Intervals of Time After the Administration of Radiocalcium to Mature Rats.

Explanation of symbols:

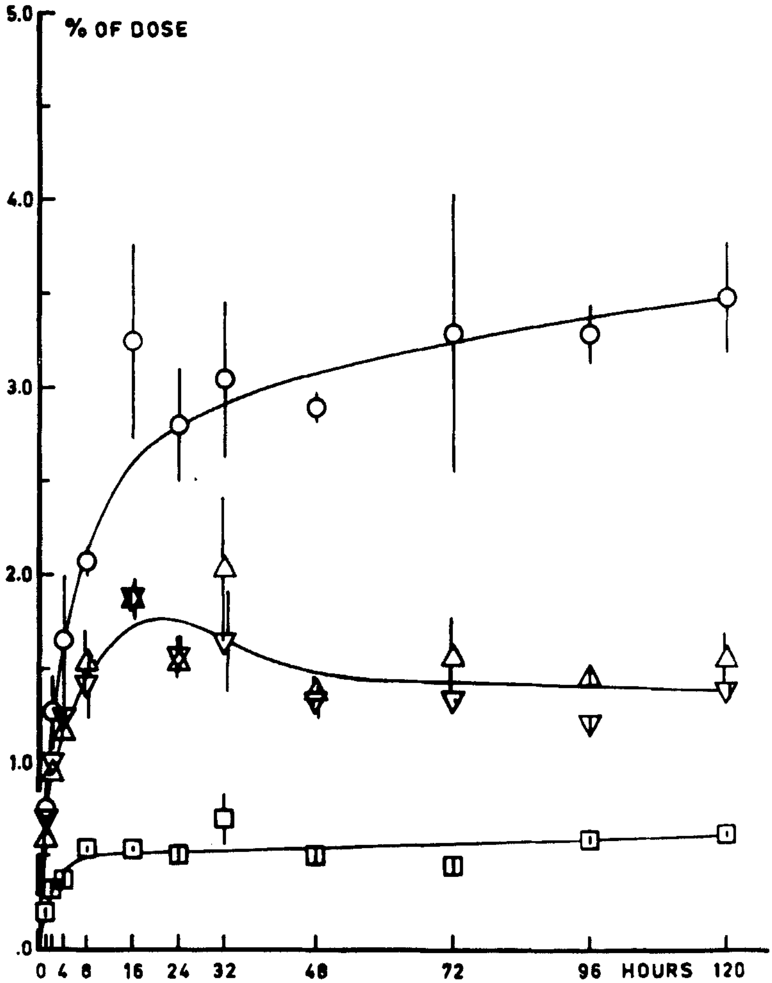
● Specific activity of the serum Ca (plotted according to scale to the left of the graph). The individual values have been indicated in the graph.

□ Activity values (expressed in per cent of dose) of the incisor teeth. The incisors of each group have been pooled. The scale is indicated to the right of the graph.

activity loss seems to be less in the ends obtained from the non-injured than in those from the fractured side.

The activity of the tibia shafts and that of the intact femur shaft slowly rise during the period of time covered by the investigation. The same tendency is exhibited by the incisor teeth. The amount of activity recovered from the intact side tibia shaft is always higher than that of the fracture side tibia shaft of the same group.

The amount of activity recovered from the femur shafts of the different groups is shown both in table 1 and in graph 1. It is seen that the amount of activity recovered from the fractured femur shaft on day 1 is more than five times that of the uninjured femur shaft. The activity of the fractured femur shaft then rises from day to day, to reach a peak value at day 4. From then on, the activity declines to reach a value at day 32 which amounts to about half the peak value.



GRAPH 3

Activity Values[1] of the Ends[2] and Shafts of the Femurs at Varying Intervals of Time After the Administration of Radiocalcium to Mature Rats.

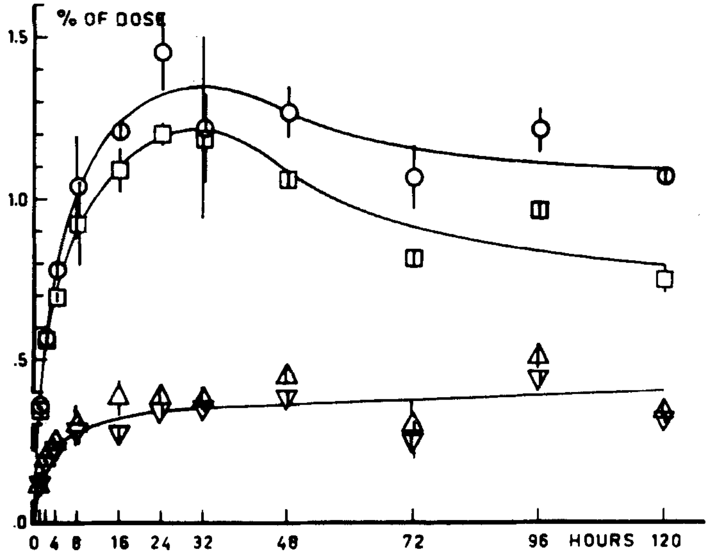
Explanation of symbols:

▽ The ends of the fractured[3] femur
○ „ shaft „ „ „ „
△ „ ends „ „ uninjured „
□ „ shaft „ „ „ „

[1] The activity values have been expressed in per cent of dose. The mean values are indicated in the graph ± the standard error. Five animals in each of the 24, 72 and 96 hour groups. Three animals in the 120 hour group. Two animals in all other groups.

[2] Consult plate 1 for an explanation of how the different bones were divided in ends and shafts.

[3] One femur in each animal was fractured manually one week before the administration of the radiocalcium.



GRAPH 4

Activity Values[1] of the Ends[2] and Shafts of the Tibias at Varying Intervals of Time After the Administration of Radiocalcium to Mature Rats.

Explanation of symbols:

□ The ends of the tibia of the side of the fractured femur[3]
▽ „ shaft „ „ „ „ „ „ „ „ „ „
○ „ ends „ „ „ „ „ „ „ „ uninjured „
△ „ shaft „ „ „ „ „ „ „ „ „ „

1 The activity values have been expressed in per cent of dose. The mean values are indicated in the graph ± the standard error. Five animals in each of the 24, 48 and 96 hour groups. Three animals in the 120 hour group. Two animals in all other groups.

2 Consult plate 1 for an explanation of how the different bones were divided in ends and shafts.

3 One femur in each animal was fractured manually one week before the administration of the radiocalcium.

Series II. (The rats received Ca^{45} one week after the femur fractures and were killed in groups of two or three, 1–120 hours later.)

In graph 2 is recorded the specific activity of the serum Ca (per cent of dose per mg Ca) at the end of the different intervals of time after the administration of the radiocalcium. In this graph are also recorded the amounts of activity recovered from the incisor teeth of the different groups of the investigation.

In graph 3 are recorded the amounts of activity recovered from the various portions of the femur ends and shafts. The activity of the fractured femur shaft rises through all intervals to reach the highest value at the last interval investigated in this series (120 hours after the administration of the radiocalcium). The activity of the femur ends drops after a peak value recorded sixteen to twenty-four hours after the administration of the radiocalcium. The activity of the uninjured femur shaft slowly rises during the investigation.

The activities of the tibia ends and shafts are recorded in graph 4. The values derived from the tibias of the side of the fractured femur have a tendency to lie below those of the non-injured side. The same tendency may be seen in the values of the metaphyseal ends of the fractured femur compared to those of the uninjured femur.

Series III. (The rats received Ca^{45} in groups of five, two to twelve days after the femur fractures and were killed twenty-four hours after the administration of the radiocalcium.)

In this series the interval between the administration of the radiocalcium and death of the animals was thus kept constant. Instead the interval of time between the fractures and the administration of the radiocalcium varied.

In graph 8 are recorded the amounts of activity recovered from the femur shafts. It is seen that while this activity uptake is constant for the intact bone, there is a sharp rise in this value recorded for the fractured shafts. A peak is reached for the ten day old fractures and the twelve day values show no further increase. This finding is in accordance with previous investigators (see *inter al. Bohr* and *Sørensen* (1950): The activity uptake in a fracture (as recorded when a constant interval of time between the administration of the activity and death of the animals is employed) shows a rise during the early intervals of time after fracture, to reach a plateau or a peak after about ten days.

In table 5 the average ash weights of the femur shafts of this series are recorded. It is seen from this table that the ash weights of the fractured femur shafts are higher than those of the intact shafts. This increase in ash weight develops gradually and at day 12 after fracture, it amounts to more than fifteen per cent of the ash weight of the intact femur shaft.

DISCUSSION

The pattern of the uptake and of the redistribution of radiocalcium and radiophosphorus in the skeleton is closely related to the time table of building up and destruction of bone tissue in the various parts of the skeleton and in the incisor teeth (see *Carlsson* (1951) and *Bauer* (1954 a and b)).

Thus the rise in activity in the incisor teeth and in the shafts of the long bones during a period of thirty-two days following the administration of radiocalcium to mature rats, is explained by the fact that the newly built-in bone salt of a high activity is not subjected to removal during this period of time.

In the ends, however, the bone trabeculae are subject to destruction shortly after their formation. This explains the continuous loss of activity from the ends of the femurs and the tibias through all groups of the investigation after the initial twenty-four hour interval.

So far the findings of the present experiment are in accordance with previous investigations.

It is known from investigations by means of ordinary histological methods that the calcification rate is high in a seven day old fracture in rats (cf. *Urist* and *McLean* (1941)). The amount of bone salt laid down in the callus can thus be assumed to be large compared to the amount of bone salt laid down in a normal shaft in the course of bone growth in the mature rats of the present investigation.

During the initial twenty-four hours after the administration of the radiocalcium, the fractured femur shaft of series I and II took up more than five times as much activity as did the uninjured femur shaft.

Whether or not all the activity of the callus can be accounted for as the result of formation of new bone is a matter of dispute. Thus *Bohr* and *Sørensen* (1950) are of the opinion that the increased uptake of activity in the fractured bone shaft (as measured five days after the administration of P^{32} to rats) is the result of an increased exchange of the radioactive atoms with non-radioactive atoms, presumably bound to the surfaces of the pre-existing bone salt crystals. The opinion of these authors is in accordance with a generally held belief that the uptake of labelled bone seekers (e.g. Ca^{45}, P^{32}, Sr^{89}) only to a small degree is the result of formation of new bone salt and to a major degree is the result of some physico-chemical reaction such as "surface exchange" or "recrystalization" (see *Bauer* (1954 a, b and c) and *Bauer* and *Carlsson* (1954) for a further discussion and criticism of this view).

Carlsson (1952) showed that this earlier concept is wrong concerning the incisor teeth: his results showed that the major fraction of the Ca^{45} recovered from the incisor teeth eight hours after the administration of the isotope to 160 g rats had been deposited by an irreversible process. By comparing the uptake with the established knowledge of the rate of the calcification of the incisor teeth, *Carlsson* was able to demonstrate that this irreversible process is indeed deposition of lime salt during growth of the incisors.

In the present investigation the deposition rate of calcium salt in the incisors has been determined in two different ways:

a) In series II the rate of Ca deposition has been calculated according to the following formula (see *Carlsson* (1952)).

$U = \frac{C}{T \times S}$. In this formula

U = Ca uptake in mg per hour,

C = the percentage of the dose of Ca^{45} which was recovered from the calcified tissue in question, T hours after the administration of the isotope,

S = the average specific activity of the serum Ca, expressed in per cent of the dose per mg Ca during the interval of time in question (O – T hours).

TABLE 3

Rate of Lime Salt Formation[1] *in the Incisors of Mature Rats as Calculated at Varying Intervals of Time After the Administration of Radiocalcium.*

Interval of time after the administration of the radiocalcium	Average specific activity of the serum Ca (per cent of dose per mg Ca)[2]	Ca deposition[3] (mg per hour) in the incisors
0– 1 hours	2.76	0.25
0– 2 „	2.82	0.15
0– 4 „	2.68	0.12
0– 8 „	2.21	0.099
0– 16 „	1.88	0.083
0– 32 „	1.42	0.078
0– 72 „	0.90	0.080
0–120 „	0.62	0.085

[1] Rate of lime salt formation has been expressed in terms of Ca deposition per hour.

[2] The average specific activity of the serum Ca has been calculated from the values of graph 2.

[3] The Ca deposition has been calculated from the specific activity values of the serum Ca in this table and the incisor activity values of graph 2, by means of the U-formula (page 180).

It is seen from table 3 that the incisors of series II show a constant value of U from sixteen hours after the administration of the radiocalcium. This steady value represents a deposition rate of Ca in the incisors of *0.081 mg per hour.*

b) In series I the average ash weight of the four incisors of each animal was 213.5 mg (table 2). The incisors have a life span of fourty-fifty days in adult rats (*Schour* and *Massler* (1942)). A life span of fourty-five days means that the equivalent of 4.8 mg of ash has to be deposited every day to make up for the daily attrition. From the data in table 2 it can further be calculated that the four incisors gained on the average 0.6 mg of ash weight per day. With a deposition rate of

4.8 + 0.6 mg of ash every day, the rate of deposition of Ca in the teeth of series I thus was *0.080 mg Ca per hour.*

The agreement of these independent sets of data serves to confirm Carlsson's work.

In the present experimental series I, the ratio of the activity of the intact femur shaft to the activity of the incisors stays practically unchanged from two days after the administration of the isotope (see table 4).

TABLE 4

The Ratio of the Activity[1] of the Uninjured[2] Femur Shaft[3] to That of the Incisor Teeth at Varying Intervals of Time After the Administration of Radiocalcium to Mature Rats.

Time after the administration of the radiocalcium	Activity ratio (± SE of the mean)
1 day	0.207±0.031
2 days	0.139±0.016
4 „	0.143±0.015
8 „	0.116±0.013
16 „	0.127±0.007
32 „	0.135±0.013

Mean value of the 2–32 day groups: 0.133±0.006.

P-value of the difference between the 4 and 8 day values > 10 %.

1 The activity values have been expressed in per cent of dose when used for this calculation. In this table the means of the five ratios in each group are recorded.

2 One femur in each animal was fractured manually one week before the administration of the radiocalcium.

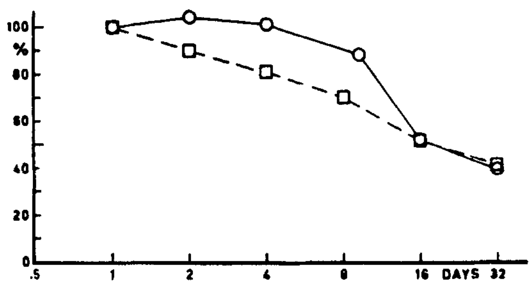
3 Consult plate 1 for an explanation of how the bones were divided in ends and shafts.

This means that even in the intact shafts an irreversible process accounts for the activity recovered from day two on.

However, during intervals shorter than two days after the administration of the radiocalcium, a reversible process seems to account for a relatively greater proportion of the radioactivity recovered from the shafts in these old rats as the shaft/incisor activity ratio has a higher value at day one than the steady value encountered through all later intervals in the series (table 4).

In graph 5 is plotted the variation with time of the activity ratios of the fractured femur shaft and of the ends of the intact femur to the shaft of the intact femur. While the ratio obtained for the ends drops through all groups of series I, the ratio obtained for the fractured shaft lies unchanged during the early intervals (one to four days after the administration of the Ca^{45}) and shows a drop only at day eight. From then on, the ratio drops continuously.

It is thus evident that up to day four, the activity of the fractured shaft can be accounted for by the same process as that governing the activity in the intact shaft. This means that essentially all the new bone salt in the callus, labelled by Ca^{45}, lies unremoved by any resorption process at least up to day four and that the activity of this portion is then removed. By comparison of this mechanism with that governing calcification in the metaphyseal ends of the long bones, it is clear



GRAPH 5

Activity Ratios of the Fractured[1] Femur Shaft[2] and the Ends of the Uninjured Femur to the Shaft of the Uninjured Femur at Varying Intervals of Time After the Administration of Radiocalcium to Mature Rats.

The activity ratios have been plotted in the graph as per cent of the 1 day value.

Explanation of symbols:

○ Ratio of the activity[3] of the fractured femur shaft to that of the uninjured femur shaft.

□ Ratio of the activity of the ends of the uninjured femur to that of the uninjured femur shaft.

[1] One femur in each animal was fractured manually one week before the administration of the radiocalcium.

[2] Consult plate 1 for an explanation of how the different bones were divided in ends and shafts.

[3] The activity values used for this calculation have been expressed in per cent of dose. They can be found in table 1.

Note: A logarithmic time scale has been used for this graph.

that there is a difference only in the time table for building up and removal of the new bone salt. The interval of time between these two processes is short for a great part of the calcification in the metaphyseal ends, while in the fracture callus this interval must be assumed to be at least four-five days in the seven-day-old fracture of the present experiment.

This finding is in accordance with the opinion reached by means of ordinary histological methods. Thus *McLean* and *Urist* (1951) state "The process of calcification of bone matrix in a healing fracture also closely resembles that of calcification of growing bone".

A comparison of the curves in graph 5 (see also tables 1 and 2) seems further to rule out the possibility that the "exchange" processes in any important degree could affect the activity values as recorded at day two and onwards. *The specific activity* (per cent dose per mg ash) of the fractured shaft is far higher than the activity of the ends already at day one. The ratio of the respective specific activities nevertheless rises during the early intervals. Even at the last interval investigated (thirty-two days after the administration of the radiocalcium) this ratio is above one.

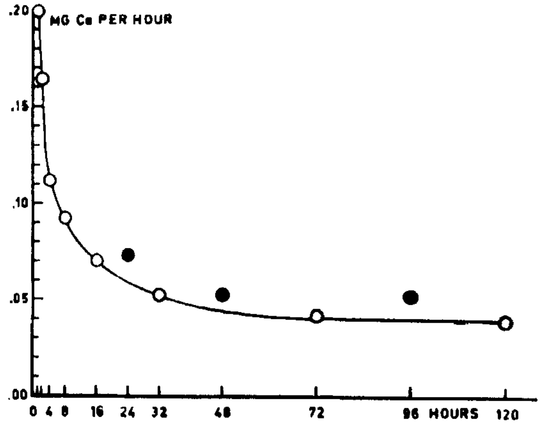
By means of the aforementioned "U-formula" it is possible to calculate the absolute rate of deposition of bone salt in the fracture callus[1]:

The activity of the callus has been calculated from graph 3 by subtracting the values of the curve obtained for the intact femur shaft from the corresponding values of the curve of the fractured femur shaft. The U-values of the callus obtained in the same manner as those of the incisors have been plotted in graph 6. It is seen that after an initial heavy drop, these values stay practically equal during an interval of time ranging from 48 hours to 120 hours after the administration of the radiocalcium. This steady value represents a deposition rate of about 0.04 mg Ca per hour.

If calculated for longer intervals of time after the administration of the radiocalcium, the U-values would drop. This statement is made from the fact that the ratio of the activity of the fractured shaft to that of the intact shaft starts to drop at intervals of time longer than four or five days after the administration of the radiocalcium, i.e. the newly formed bone salt is reached by the process of bone resorption.

It has been pointed out above that the U-values will reach a plateau if the deposition rate of calcium is constant. From graph 8 it is seen that the activity uptake of the fractured femur shaft rises during early intervals after fracture (as recorded when constant intervals of time between the administration of the radiocalcium and the death of the animals have been employed). It is seen in graph 6, however,

[1] "Callus" is used in this paper to designate the bone formed in and around the broken shaft in excess of that formed in the intact shaft. The term callus is thus not restricted to the bone formed in the immediate vicinity of the fracture. The validity of the use of the term in this sense is apparent from the fracture radioautograms (see *Bauer* (1954 d)).



GRAPH 6

Rate of Bone Salt Formation[1] *in the Callus*[2] *of the Fractured*[3] *Femur as Calculated at Varying Intervals of Time After the Administration of Radiocalcium to Mature Rats.*

Explanation of symbols:

○ Rate of bone salt formation calculated by means of the U-formula from the specific activity of the serum Ca (see graph 2, table 3 and page 180).

● Rate of bone salt formation calculated by means of the "simplified U-formula" from the activity of the incisors of series I (see page 186).

1 Rate of bone salt formation has been expressed in terms of Ca deposition per hour.

2 The activity of the callus has been calculated by subtracting the activity of the uninjured femur shaft from that of the fractured femur shaft. These values appear in graph 3.

3 One femur in each animal was fractured manually one week before the administration of the radiocalcium.

that the plateau for the U-values was not reached until 24–48 hours after the administration of the radiocalcium or eight-nine days after the fractures. It is seen in graph 8 that the rate of activity uptake shows but a small change during the interval of nine-twelve days after the fractures. The plateau for the U-values encountered during the period of time of 48–120 hours after the administration of the isotope can thus not be an effect of a rise in deposition rate in the fracture callus.

In graph 8 the serum specific activity of series II has been superimposed. It can be calculated from this curve that 80 % of the activity recovered from the fractured shafts at day five after the administration of the radiocalcium has been deposited during the initial three days. Even if the rate of deposition of bone salt in the fracture callus had not reached a constant value at day ten after fracture, a further rise

TABLE 5

Ash Weights of the Fractured and Intact Femur Shafts at Different Intervals of Time After a Femur Fracture in Mature Rats.

Days after fracture	Average wt. of animals at death	Ash weight in mg of		Weight difference in mg	P-value of the weight difference
		fractured shaft	intact shaft		
3 days	179 g	86.2	83.2	3.0±1.73	<25
5 "	183 "	96.6	91.4	5.2±2.24	<10
7 "	180 "	96.8	91.2	5.6±0.975	<1
9 "	180 "	102.4	89.4	13.0±2.39	<1
11 "	183 "	105.0	89.8	15.2±3.66	<2.5
13 "	180 "	111.1	93.2	18.2±1.52	<0.1

Note: Five animals in each group.

in this deposition rate would not appreciably interfere with the U-values encountered.

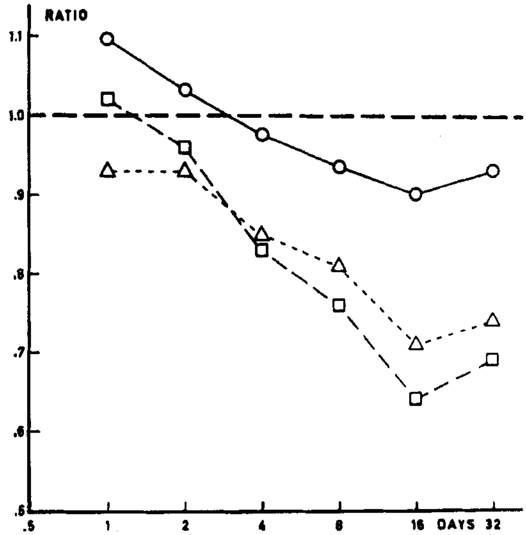
The figure for the deposition rate of bone salt derived by this tracer technique thus is valid for the initial one-two days after the administration of the radiocalcium even if calculated from activity values obtained up to five days after the administration of the radiocalcium.

The validity of this statement is supported by an experiment in which P^{32} was administered to mature rats, 14 days after they had been subjected to femur fractures (*Bauer,* 1954 e). The deposition rate of phosphorus in the fracture callus (determined in the same way as above) was found to be 0.035 mg per hour. With a calcium/phosphorus ratio of 2: 1 in the bone salt, this figure corresponds to a deposition rate of calcium in the 14-day-old fracture callus of 0.07 mg per hour. This higher figure is of the expected magnitude if the values of the activity uptake drawn in graph 8 are proportional to the deposition rate of bone salt in the fracture callus.

It is thus possible to obtain a picture not only of the relative rate but also of the absolute rate of deposition of bone salt in a fracture callus.

It should be mentioned that with a knowledge of only the rate of deposition of lime salt in just one skeletal part, the tracer technique makes it possible to obtain a figure for the rate of the deposition of Ca in any other skeletal part without a separate determination of the specific activity of the blood:

It has been shown above how the deposition rate of lime salt in the incisor teeth can be calculated without the aid of tracer data. The rate of Ca deposition in any other part is then the ratio of the activity of this part to that of the incisors in the same animal times



GRAPH 7

Ratios of the Activities[1]*, Ash Weights, and Specific Activities*[2] *of the Metaphyseal Ends*[3] *of the Fractured*[4] *Femur to Those of the Metaphyseal Ends of the Uninjured Femur at Varying Intervals of Time After the Administration of Radiocalcium to Mature Rats.*

Explanation of symbols:
□ Activity ratio.
△ Ash weight ratio.
○ Specific activity ratio.

1 The activity values have been expressed in per cent of dose when used for this calculation. These values appear in table 1.

2 Specific activity means activity in per cent of dose per mg ash. The ash values appear in table 2.

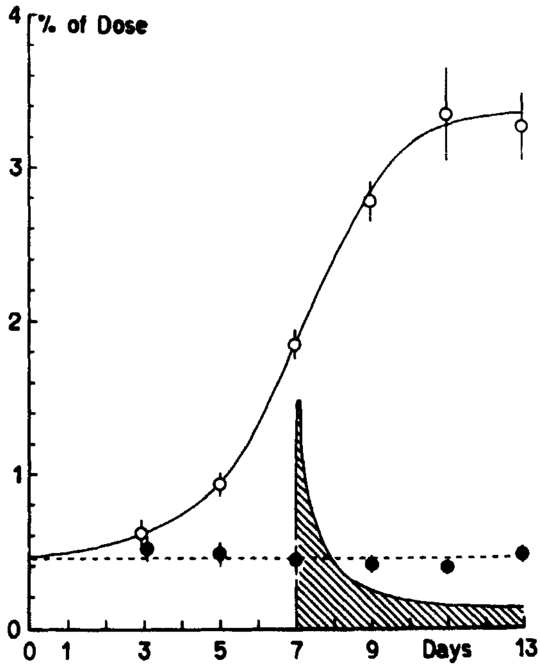
3 Consult plate 1 for an explanation of how the bones were divided in ends and shafts.

4 One femur in each animal was fractured manually one week before the administration of the radiocalcium.

Note: A logarithmic time scale has been used in this graph.

the deposition rate of Ca in the incisors in question. This formula may be written $U_s = \frac{A_s \times U_i}{A_i}$ where U_s and A_s mean the deposition rate resp. the absolute activity of the skeletal part investigated, and U_i and A_i mean the deposition rate resp. the absolute activity of the incisors of the same animal.

The only important precaution when this "simplified U-formula" is used, is to chose an interval of time long enough to exclude the influence of the factor of "exchange" and short enough to preclude the



GRAPH 8

Activity Values[1] *of the Fractured*[2] *and Intact Femur Shafts Recorded Twenty-Four Hours After the Administration of* Ca^{45} *to Mature Rats.*

Explanation of symbols:
○ The fractured femur shaft.
● The intact femur shaft.

1 The activity values have been expressed in per cent of dose. The mean values are indicated in the graph ± the standard error. Five animals were killed in each group.

2 One femur in each animal was fractured two to twelve days before the subcutaneous injection of Ca^{45}. The animals were killed twenty-four hours after the isotope injections. The time scale of the graph indicates days after fracture at death of the different groups of rats.

Note: In this graph has been superimposed the curve drawn for the specific activity of the blood Ca appearing in graph 2.

importance of resorption of the newly built-in bone salt. The seven-day-old fracture callus in the mature rats of this investigation meets these requirements between two and five days after the administration of the radiocalcium. The deposition rate of the shafts can be calculated from this formula during all intervals ranging from two to thirty-two days after the administration of the radiocalcium. The only reasonably sure interval for the ends seems to be about eighteen hours after the administration of the radiocalcium.

For purpose of comparison the rate of deposition of Ca in the fracture callus has been calculated with the help of this "simplified U-formula". It appears (see graph 6) that there is an agreement between the two sets of data, viz. that the rate of new bone salt formation in the callus is about 0.04 mg per hour.

It is seen in table 2 that the ash weight of the fractured femur shaft is higher than that of the intact shaft. In table 5 it is seen that this effect gradually develops after the fracture. The difference in ash weight between the fractured and the intact shaft five days after the fracture is 5.2 mg. Eleven days after the fracture this difference is 15.2 mg. During the intervening six days the rate of the increase in this difference has been 1.7 mg ash per day. With a Ca content of the ash of 38 % this means an increase at the rate of 0.026 mg Ca per hour. This increase in ash weight is the net result of bone salt deposition and bone salt resorption. The resorption of the preexisting bone may be assumed to be enhanced in a fractured bone (cf. below). The newly formed bone salt is reached by a resorption process (cf. above). The figure for the increase in ash weight of 0.026 mg Ca per hour will thus be lower than the true rate of bone salt deposition.

By comparison with the figure for the deposition rate arrived at by means of the above tracer technique (0.04 mg Ca per hour) it is seen that these values are of the same order.

From table 1 it appears that the activity values of the metaphyseal ends of the fractured femur drop at a faster rate than do those of the metaphyseal ends of the uninjured femur. *Bohr* and *Sørensen* (l. c.) stated that the metaphyseal ends of the fractured femur exhibited a higher uptake of radioactivity than did the metaphyseal ends of the uninjured femur. The authors attributed this enhanced uptake to an increased "exchange" compared to the normal side. *Bohr* and *Sørensen* expressed the activity uptake in terms of *specific activity* (activity per unit weight of ash).

From graph 7 it appears, however, that not only the activity, but also the ash weights of the fractured femur metaphyseal ends drop to values far below those of the normal femur ends.

The data of the present investigation thus show that there is an enhanced loss of bone tissue from the metaphyseal ends of the fractured bone. During early intervals of time after the injection of the radiocalcium, most of the extra bone tissue removed is nonlabelled and therefore the *specific activity* values are higher in the ends of the fractured bone than in those of the intact bone. Later during the investigation, even the labelled bone salt is reached by the enhanced removal process and thus the *specific activity* tends to be lower in the fractured bone ends than in the intact bone ends.

A similar distinct effect on the rate of bone salt formation and resorption is observed also in the tibia ends and shaft on the fracture side. The nature of this phenomenon will be discussed more in detail in a forthcoming paper.

SUMMARY

Mature rats were killed one hour to thirty-two days after a subcutaneous injection of Ca^{45}. One week prior to the isotope administration, one femur had been fractured in each animal. Serum samples and various portions of the long bones and the incisor teeth were analyzed for their activity and calcium (ash) content.

It proved possible to correlate the amount of activity recovered from the bones to the amount of bone salt formed in these bones in the course of bone growth and bone repair.

The advantages of the tracer technique used in the present investigation seem to be:

1) It is possible to differentiate between formation and resorption of bone salt.

2) The rate of formation of bone salt (bone salt deposition) can be determined in a simpler, yet more exact way than has hitherto been possible.

From the data of this paper it is seen that

a) the rate of bone salt formation in the seven-day-old callus in the femur of a mature rat is the equivalent of 0.04 mg of Ca per hour;

b) the newly formed bone salt of the fracture callus stays at least four-five days until it is reached by a resorption process;

c) the bone salt of the metaphyseal ends of the fractured femur is resorped at a faster rate than is the bone salt of the corresponding ends of the intact femur;

d) even in the tibia of the fractured side, a distinct effect on the normal rate of bone salt formation and resorption is observed.

RESUME

Des rats adultes ont été tués à un intervalle variant entre une heure et 32 jours après une injection sous-cutanée de Ca^{45}. Une semaine avant l'administration de l'isotope, on a provoqué la fracture d'un fémur chez chaque animal. Des échantillons de sérum et de différentes portions de l'os long, ainsi que des incisives ont été examinés afin de déterminer leur activité et leur teneur en calcium (cendres). On a

constaté qu'il était possible d'établir une corrélation entre l'activité recouvrée dans les différentes parties des os et la quantité de sel osseux qui s'était formée dans ces os au cours de la croissance de l'os et de sa guérison.

Les avantages de la technique utilisée dans les présentes recherches semblent être les suivants:

1) il est possible d'établir la différenciation entre la formation et la résorption du sel osseux

2) le taux de formation du sel osseux (dépôt de sel osseux) peut être fixé d'une manière plus simple et plus exacte que jusqu'ici. D'après les indications recueillies, il semble en effet:

a) que le taux de la formation de sel osseux dans le 7ème jour du cal du fémur d'un rat adulte est équivalent à 0,04 mg de Ca par heure;

b) que le sel osseux nouvellement formé du cal de la fracture se maintient au moins 4 à 5 jours avant d'être atteint par le processus de résorption;

c) que le sel osseux de l'extrémité métaphyséale du fémur fracturé est résorbé plus rapidement que celui de l'extrémité correspondante du fémur intact;

d) que même dans le tibia du côté fracturé on peut observer un effet distinct sur le taux normal de la formation de sel osseux et de résorption.

ZUSAMMENFASSUNG

Voll ausgewachsene Ratten wurden eine Stunde bis zweiunddreissig Tage nach einer subkutanen Injection von Ca^{45} getötet. Eine Woche vor der Einverleibung des Isotops war ein Femur in jedem der Tiere gebrochen worden. Serumproben und verschiedene Teile von langen Röhrenknochen, sowie von Zähnen wurden auf Radioaktivitet und Calcium (Asche) untersucht. Es war möglich den Grad der Aktivitet, der in den verschiedenen Knochenteilen bestimmt wurde und die Menge der Knochensalze, die in diesen Knochen im Verlaufe des Wachstums und der Knochenheilung gespeichert wurden, in ein gewisses Verhältnis zu bringen.

Die Vorteile der „Tracer" Technik, die in den vorliegenden Untersuchungen verwendet wurde, scheinen zu sein:

1) Es ist möglich zwischen Speicherung und Aufsaugung von Knochen-salzen zu unterscheiden.

2) Der Grad der Speicherung von Knochensalzen kann in einer einfacheren, jedoch genaueren Weise bestimmt werden als das bis jetzt möglich war. Aus den in dieser Arbeit gemachten Angaben ergibt sich:

a) Die Geschwindigkeit der Knochensalzspeicherung in dem sieben Tage alten Kallus des Femurs einer ausgewachsenen Ratte entspricht 0,04 mg Ca in der Stunde.

b) Das neulich gespeicherte Knochensalz des Bruchkallus wird zumindest vier bis fünf Tage festgehalten ehe es vom Aufsaugungsprozess erreicht wird.

c) Die Knochensalze der Metaphysen des gebrochenen Femurs werden mit grösserer Geschwindigkeit resorbiert als die der entsprechenden Partien des intakten Femurs.

d) Selbst in der Tibia der gebrochenen Seite kann ein ausgesprochener Effekt auf die Speicherungs- und Aufsaugungsgeschwindigkeit von Knochensalzen beobachtet werden.

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. The Journal of Bone and Joint Surgery, *36-A* (April) 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. The Journal of Bone and Joint Surgery, *36-A* (April) 1954 b.

– Metabolism of Bone Sodium in Rats Investigated with Na^{22}. Acta Physiologica Scandinavica, in press, 1954 c.

– Bone Repair in Rats Studied by Means of Radio-Autography. To be published, 1954 d.

– To be published 1954 e.

– and *Carlsson, A.:* To be published, 1954.

Bohr, H. and *Sørensen, A. H.:* Study of Fracture Healing by Means of Radio-Active Tracers. The Journal of Bone and Joint Surgery. *32-A*: 567–574, 1950.

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

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

Clark, E. P. and *Collip, J. B.:* A Study of the Tisdall Method for the Determination of Blood Serum Calcium with a Suggested Modification. The Journal of Biological Chemistry. *63*: 461–464, 1925.

Leblond, C. P., Wilkinson, G. W., Bélanger, L. F. and *Robichon, J.:* Radio-Autographic Visualization of Bone Formation in the Rat. The American Journal of Anatomy. *86*: 289–341, 1950.

McLean, F. C. and *Urist, M. R.:* Newer Knowledge of Post-Fetal Osteogenesis. Bulletin of the Hospital for Joint Diseases. *12*: 431–442, 1951.

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

Schour, I. and *Massler, M.:* The Teeth, page 103, in *Griffith, J. Q.* and *Farris, E. J.:* The Rat in Laboratory Investigation, Philadelphia, 1942.

Urist, M. R. and *McLean, F. C.:* Calcification and Ossification. I. Calcification in the Callus in Healing Fractures in Normal Rats. The Journal of Bone and Joint Surgery. *23*: 1–16, 1941.