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*DEDICATED TO PROFESSOR NIELS BOHR ON THE OCCASION OF HIS 70TH BIRTHDAY*

# CONSERVATION OF SKELETAL CALCIUM ATOMS THROUGH LIFE

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From the earliest beginning, Professor NIELS BOHR has shown great interest in the application of radioactive indicators to the study of the conservation of skeletal atoms through life. This fact has induced the writer to contribute to this volume with a communication of the results obtained in an investigation on the conservation of skeletal calcium atoms in the adult mouse and on the fate of maternal calcium atoms through generations.

The first application of an artificially radioactive isotope as a tracer in 1934 was that of $P^{32}$ in a study of the problem whether and to what extent the mineral constituents of the skeleton of the adult organism are replaced during lifetime[1,2,3,3a]. By using this radioactive indicator it was possible to demonstrate the dynamic nature of the building up of bone tissue. It was found that an initial rapid location of the circulating labelled phosphate in the mineral constituents of the skeleton is followed by a much slower second effect. The first effect was interpreted by us to be due to an interchange between the phosphate ions located in the surface layer of the bone apatite and in the plasma, the second one, however, to the fact that "the bone is destroyed at certain places and rebuilt under incorporation of labelled phosphate at others". Emphasis was given to the analogy between these phenomena and those observed when, in early experiments, naturally radioactive isotopes were applied as tracers. PANETH[4], when shaking solid lead sulfate with a solution containing labelled lead ions, observed an interchange of lead ions only between the uppermost molecular layer of the solid salt and the dissolved ions. In studies in which the interchange between the atoms of lead metal and the labelled lead ions of a solution, or vice versa, was investigated, the present author and others[5,6,7] found that many hundreds of atomic layers of the lead foil were converted into ions, and a corresponding number of ions into atoms,

making out the lead foil. Thus, a renewal of the constituents of a metal foil, involving dissolution and reprecipitation due to "local currents", was found to be a much deeper going process than that occurring between solid lead salts and the lead ions of the surrounding solution. In the early investigations mentioned above, it was pointed out that the rapid uptake of $P^{32}$ during the early phase of the experiment recalls the behaviour of a lead salt placed in the solution containing labelled lead ions, the recrystallization of the mineral constituents of the skeleton reminds of the behaviour of a lead foil immerged into a solution containing labelled lead ions, however, with the difference that, in the latter case, enzymic actions are involved. Or, as it was expressed later[8], "A restricted extent of renewal of the skeleton is due to the fact that, while the P atoms of the uppermost molecular layer of the bone apatite crystals can promptly interchange with the free P atoms of the plasma (actually not the P atoms, but the phosphate ions interchange), a renewal of the main part of the apatite P can take place only when the crystal is dissolved and when new crystals are formed from the plasma; from labelled plasma, labelled crystals are formed". Subsequent experiments confirmed the correctness of these early conclusions, showing that both a surface exchange between plasma phosphate and bone phosphate, and a recrystallization, thus a dissolution of some of the apatite crystals and the formation of new ones, take place in the skeleton. Different workers, however, arrived at divergent results about the share of both processes in the interaction of plasma and bone constituents.

The introduction of autoradiographic methods into the study of bone formation by LEBLOND and assoc.[9] was a very important advance, since it became possible to visualize the rapid formation and destruction of some parts of the calcified tissue. Numerous autoradiographic investigations such as those by LEBLOND and assoc. applying $P^{32}$, those by COMAR et al.[10] using $Ca^{45}$, $Sr^{89}$, and $P^{32}$, by SKIPPER et al.[11] with $C^{14}$, by KIDMAN et al.[12] with $Sr^{89}$, by ENGFELDT et al.[13] with $P^{32}$, by AMPRINO and ENGSTRÖM[14] with $Ca^{45}$, and by BAUER[15] with $Na^{22}$, clearly demonstrate that a great part of the bone salt crystals are more or less unchanged until they are reached by the process of resorption.

LEBLOND's autoradiographs clearly indicate that the cir-

culating phosphate enters the skeleton either by *exchange* or by *precipitation* in definite areas with the formation of new bone. While, in the autoradiographs, the exchangeable phosphate is depicted as diffuse reactions disappearing rapidly with time, the precipitated or stable phosphate appears as localized persistent reactions.

In contradistinction to all workers in this field, ENGFELDT, ENGSTRÖM, and ZETTERSTRÖM[13] arrived at the result that even the initial uptake of $P^{32}$ by the bone is due exclusively to some kind of recrystallization. This conclusion is based on their observation that the autoradiographic patterns of cross sections from long bones show an uneven distribution of radioactive phosphate. They found the fastest uptake of labelled phosphate to take place in Haversian systems with a low content of mineral salts. Since the major part of the tracer is found in limited areas, the initial rapid uptake of labelled phosphate –according to their view– cannot be due to an ion exchange on the crystal surface of the bone minerals, such an exchange being prevented by the organic constituents of the bone.

While there can hardly be any doubt that the main part of the renewal of the bone apatite of the skeleton is due to a recrystallization process, to a degradation and new formation of the mineral constituents of the skeleton, objections may be raised against the view that the initial uptake of $P^{32}$ is due exclusively to some kind of recrystallization.

Uneven distribution of radioactive phosphate as shown in autoradiographic patterns of cross sections from long bones cannot be interpreted as an absence of surface interchange. According to PANETH[4,16], the whole uppermost molecular layer of crystalline salt powders interchanges with the ions of a surrounding solution, while properly crystallized surfaces like those of natural crystals fail to do so. He states that his investigations suggest that the radioactive method of determining surfaces, based on the assumption that the whole uppermost layer molecular interchanges, should be employed in those cases only for which it is established that the fundamental supposition of kinetic exchange of the entire surface is valid. If we assume the bone apatite, or part of it, as occurring in vivo, to be a properly crystallized substance, we arrive at another explanation than

that of ENGFELDT, ENGSTRÖM, and ZETTERSTRÖM, according to which the organic constituents of fresh bone are responsible for preventing surface exchange. This alternative explanation is that the bone apatite, or large parts of it, behaves like a properly crystallized substance in Paneth's experiments and not like a crystal powder.

The exchange of ions on a crystal surface is thus far from being absent and, though restricted to a fraction of that surface, is responsible for an appreciable part of the early uptake of labelled ions by the mineral constituents of the skeleton. As shown by ARMSTRONG and assoc.[17], in the course of the first ten minutes, 2 % of the skeletal calcium of the dog are replaced by labelled calcium of the plasma; this is $^1/_{10}$ only of the amount which, according to FALKENHEIM's[18, 18a] calculations, would be necessary to replace the whole uppermost molecular layer of the bone apatite, or $^1/_6$ of the amount estimated by HENDRICKS and HILL[19]. A large part of these 2 % –or even 2 %– could be due to a surface interchange in spite of the uneven autoradiographic patterns of cross sections of long bones observed by ENGFELDT, ENGSTRÖM, and ZETTERSTRÖM. In view of the high specific activity of the plasma calcium in an early stage of the experiment, to a 2 % interchange corresponds a very much higher percentage decrease in the $Ca^{45}$ content of the plasma in the course of the first two minutes, more than 50 % of the injected radiocalcium leaving the circulation. From $Ca^{45}$ injected into the circulation of growing hogs, COMAR and assoc.[10] found only 2 % to be present after the lapse of an hour.

In the experiments of ENGFELDT and assoc., three hours was the shortest time after which the $P^{32}$ injected rats were sacrificed. The early phase of the experiment, in which a very rapid interchange of the mineral constituents of the bone takes place, is much shorter than three hours. When investigating the uptake of $Sr^{89}$ by the skeleton of outgrown rabbits during the first 30 seconds, 11.7 % were found to be taken up[20], while during the first six hours –thus a 720 times longer period– only about twice as large an uptake was observed. ARMSTRONG and assoc.[17] found during the first 20 minutes an interchanges of 4 % of the skeletal calcium with plasma calcium, this amount increasing less than three times during the following 160 minutes.

A change in the concentration of the bone apatite constituting ions, and still more a variation in the concentration of enzymes involved in recrystallization of the plasma and the lymph, is bound to influence the rate of recrystallization of the skeleton. Repeated administration of bone phosphatase extract by intravenous injection was found to lead to a decrease of the mineral constituents of the bone tissue[21], which are replenished after removal of the excessive phosphatase. HASTINGS[22], when replacing the plasma of a dog by plasma of low calcium content, found that the mobilization of bone calcium increased the calcium level of the plasma almost momentarily to a normal level. Parathyroid hormon is known to exert a direct action on bone[22a]. In this connection, also CARLSSON's[23] investigation should be mentioned; he found vitamin D deficient rats to be unable to utilize their bone stores for maintaining a normal serum calcium. However, in view of the very great difference in the distribution of the mineral constituents in the bone tissue and the corresponding tendency to remove these differences, the biological recrystallization of the skeleton, as rightly emphasized by ENGFELDT and ass.[13], is not due exclusively to these processes[24].

KING raised the idea that, though conventionally, the bony framework of the body is regarded as a means of making locomotion possible, it may be that this is no more than a secondary development, the primary function of bone in the body being to act as a reservoir for the maintenance of a constant blood calcium level.

At a very early date[25, 26, 3], it has already been observed that the diaphysial phosphate is replaced by administered labelled phosphate at an appreciably lower rate than epiphysial phosphates, and similar observations were made in the investigation of the incorporation of $Ca^{45}$ into the skeleton[27, 29]. Since the transition between diaphysial and epiphysial bone tissue is almost continuous, the specific activity of bone phosphorus or bone calcium varies considerably through the whole bone tissue. This great heterogeneity of the specific activity of the bone apatite phosphorus could be demonstrated by ZETTERSTRÖM and LJUNGGREN[30] by isolating bone fractions of different solubility and measuring their specific activity. The most soluble bone phosphorus was found to show the highest specific activity, thus the

most rapid rate of renewal. X-ray absorption and diffraction studies by AMPRINO and ENGSTRÖM[14] revealed also that the distribution of mineral components in the bone tissue is far from being uniform.

### Size of the Non-Renewable Part of the Skeleton.

The extent of renewal of apatite phosphate of the skeleton can be calculated from the mean value of the specific activity of the plasma phosphate during the experiment and the value of the specific activity of the apatite phosphate at the end of the experiment. During the early part of the experiment, the sensitivity of the radioactive indicator is comparatively low, thus a strong decline in the plasma activity corresponds to a comparatively low interchange figure. In the later part of the experiment, the same activity which indicated at the start the presence of 1 mg. of phosphorus in the plasma, for example, indicates 1/100 mg., thus the sensitivity of the radioactive indicator is strongly increased. Now, a further interchange will be indicated by a very small further loss of activity. Furthermore, following the interchange of plasma and bone phosphate for a longer time interval, increase and decrease in the specific activity of the plasma phosphate may alternate due to a variation in the phosphate intake or other reasons. Thus, it encounters great difficulties, by comparing the mean specific activity of the plasma phosphate during the experiment and the specific activity of the apatite phosphate at the end of the experiment, to find a reliable value for the extent of the renewable part of the mineral phosphate of the skeleton, and similar considerations apply to the determination of the renewable part of bone calcium in contrast to that of the bone sodium. Sodium, being mainly an extracellular element, is distributed between plasma and extracellular fluid within a few minutes, a distribution which results in a decrease in the specific activity of plasma sodium to about $^1/_6$ of its original value, followed by a very slow decrease with time only. Thus, as discussed on p. 16, the extent of the renewable part of the mineral bone sodium could be calculated from specific activity data. We can, however, determine the extent of renewal of bone phosphate from specific activity data when keeping the specific activity of

the plasma phosphate at a constant level during the experiment. This result was obtained by the author and his associates[31] by daily injecting the rabbit repeatedly with labelled phosphate. After the lapse of 50 days, the phosphorus of the femur epiphysis found to have a specific activity of 30 % of that of the plasma inorganic P, thus indicating that 30 %, and only 30 %, of the epiphysial bone apatite had been renewed, a much lower renewal figure (7 %) being obtained for the diaphysial phosphorus.

This method has the disadvantage of being cumbersome. Furthermore, the results may be influenced by the time that passes between the last injection of the rabbit and the killing of the animal. Therefore, when determining the renewable fraction of the skeleton calcium of the mouse, we have chosen another procedure. Mice were bred whose skeleton was labelled throughout with $Ca^{45}$ and the loss of the activity in the skeleton was followed with increasing age of the animals. Such mice can be obtained by administering to the mother food containing labelled calcium already weeks before gestation and continuing to feed the lactating mother and the growing offsprings with food containing labelled calcium. We assume every one of the offsprings to have the same $Ca^{45}$ content. If we stop administering labelled food after these offsprings are outgrown, they start to interchange their labelled bone calcium with the unlabelled calcium from the food with the result that the $Ca^{45}$ content of the skeleton decreases and, when the offspring is killed after two months, its $Ca^{45}$ content is lower than that of another offspring killed after one month. By killing members of a litter at different dates, we can follow the processes in the skeleton for years, viz. through the lifetime of the animal.

TABLE I.

Weight and activity of new-born mice.

| No. | Weight in gm. | Relative activity |
|---|---|---|
| 1 | 1.8 | 100 |
| 2 | 1.3 | 98.5 |
| 3 | 1.4 | 93.5 |
| 4 | 1.2 | 99.5 |

The $Ca^{45}$ content of every member of a litter is not strictly the same, and this applies also to the growth rate. The evidencee that a part of the curve depicted in Fig. 1 is discontinuous may presumably be due to a difference in the uptake of maternal $Ca^{45}$ by the offspring of the same litter. The variation in the radioactivity of different members of a litter, however, is restricted and does not suffice to frustrate the applicability of the method described (cf. Table I).

## Experimental.

In view of the difficulties in replacing all food calcium by labelled calcium, we added the labelled calcium as $CaCl_2$ (150 mg. per liter) to the drinking water, on the assumption that the quantity of water drunk by the mouse, kept at constant temperature, is about proportional to the intake of food which consisted of standard cakes. We started to administer two to ten weeks before parturition to 20—30 gm. mice the labelled $CaCl_2$ and continued administration of such drinking water till weaning. Then, the growing mice were given labelled drinking water until they were outgrown. From that date (when the mice were about 100 days old), administration of $Ca^{45}$ was discontinued. The offsprings were killed at different times, and the radioactivity of the ash of their skeletons was compared. 20 mg. of bone ash were placed under the Geiger counter, and the total activity of the skeleton was calculated from the measured activity and the total ash weight. In other experiments, the radioactivity of the total body ash samples was compared.

The ratio of the activity of 20 mg. of bone ash of outgrown and of newborn mice is not a correct measure of their relative $Ca^{45}$ content. The calcium content of the ash of the newborn being appreciably lower than that of the adult, the backscattering of the $\beta$-rays emitted by the $Ca^{45}$ of the first mentioned samples will be lower, furthermore the consistency of the samples and, thus, the distance of the sample from the counter window may slightly differ. By measuring once the activity of a 20 mg. sample of the bone ash of newborn mice, and then that of a small known aliquot of this sample brought up to 20 mg. through addition of inactive bone ash of an adult mouse, we arrive at the result

that the activity measured of the ash of the newborn mouse has to be multiplied by 1.05 in order to make it comparable with the activity of the bone ash of adult mice.

In other experiments, new-born mice were shifted from their active mothers to inactive mothers shortly after birth; determinations were made of the percentage of maternal labelled calcium taken up by the offspring after birth and the rate of loss of these calcium atoms during growth and later.

The $Ca^{45}$ activity of the mice remained below 0.05 $\mu$C per gm. and, in most cases, it was very appreciably less. SIMMONS and assoc.[32] observed the effect of radiation produced in mice during 108 weeks. When a dose of 0.034 $\mu$C was administered, they could not find anaemia; when the dose was raised to 0.068 $\mu$C, moderate changes in heterophylous values could be detected. In our experiments, no effect on growth or fertility due to the presence of $Ca^{45}$ could be observed. Our main litter size was 5.7. As shown by RUSSELL[33], the litter size of mice at term is reduced as a result of irradiation during preimplantation stages with 100 r or more, and when exposed shortly after implantation, by a minimum dose of 200 r.

The composition of standard cakes fed to our mice is seen from Table II.

TABLE II. Composition of cakes fed to our mice ("Gard-bred").*

| 100 gm. cakes contain | |
|---|---|
| Water | 7.6 gm. |
| Ash | 2.9 - |
| Proteins (5.7 × N) | 6.3 - |
| Carbohydrates | 67.6 - |
| Ca | 188 mg. |
| P | 424 - |
| Fe | 24 - |
| Combustion value (calculated according to Rubner) | 400 cal. |

* In Sweden, mice and rats are fed almost exclusively on these cakes, the exact composition of which was hitherto unknown. The author is much indebted to Professor E. BRUNIUS and Mrs. ESTHER SIHLBOM who most kindly made the analysis of these cakes at Statens Institut för Folkhälsan.

## Results.

The results of experiments in which mice born from mothers kept on a $Ca^{45}$ diet for weeks prior to and after parturition, and continuously kept on a $Ca^{45}$ diet till they reached an age of about 100 days, thus were outgrown, are shown in Table III.

TABLE III.

Loss of $Ca^{45}$ by the uniformly labelled skeleton of mice with time, indicated by measurements of the radioactivity of the skeleton of different members of a litter killed at various times. The mice were born from active mothers and were administered $Ca^{45}$ until the first member of the litter was killed.

| No. of litter | Age in days | $Ca^{45}$ content |
|---|---|---|
| I | 111 | 100 |
| | 329 | 66.7 |
| | 519 | 57.0 |
| II | 108 | 100 |
| | 327 | 90.7 |
| | 517 | 78.8 |
| III | 108 | 100 |
| | 326 | 88 |
| | 501 | 69.4 |
| IV | 115 | 100 |
| | 220 | 79.9 |
| | 393 | 64.4 |
| V | 106 | |
| | 106 | 100 |
| | 231 | 66 |
| | 325 | 63.8 |
| VI | 56 | 100 |
| | 129 | 81.4 |
| | 266 | 69.7 |
| VII* | 99 | 100 |
| | 214 | 77.7 |
| | 308 | 55.5 |
| | 392 | 55.9 |
| | 503 | 50.1 |

* Cheese and egg shells were added ad libitum to the standard bred diet.

The mean conservation of $Ca^{45}$ by the uniformly labelled skeleton of the mice in the course of 390 days, representing a mean value of the duration of the experiments, works out to be $64.7 \pm 7.34$ per cent, the standard error of the mean being 2.78. If we disregard the last experiment in which the mice were

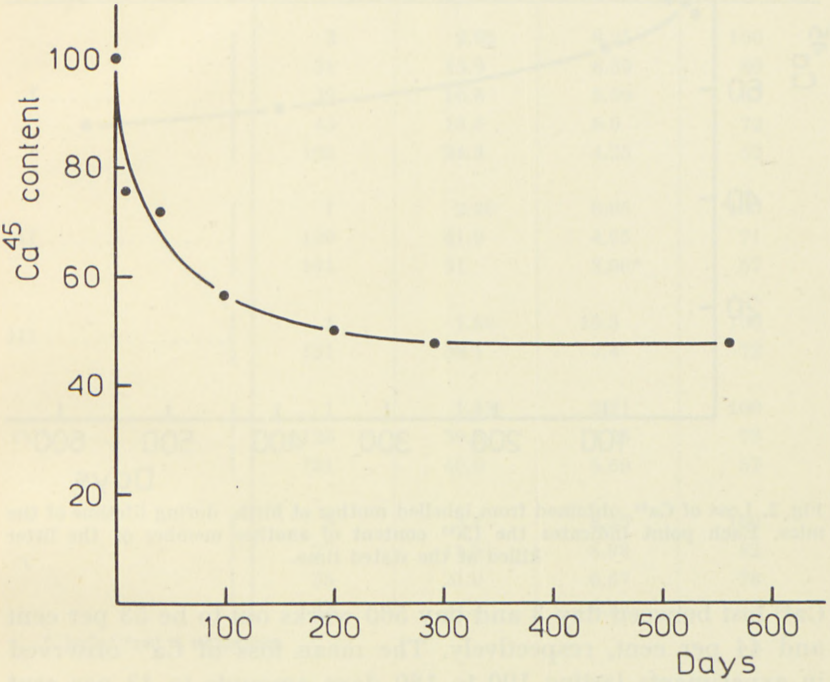


Fig. 1. Loss of $Ca^{45}$, obtained from labelled mother at birth, during lifetime of the mice. Each point indicates the $Ca^{45}$ content of another member of the litter killed at the stated time.

kept on a high calcium diet, the mean value is $67.2 \pm 7.86$ per cent, the standard error of the mean being 3.23. Thus, $^2/_3$ of the calcium atoms present in the skeleton of the outgrown mice are present after the lapse of more than a year and can thus be considered to be unreplaceable during life.

Figs. 1 and 2 and Table IV show the results of some of our experiments in which the litter, born from active mothers, was kept from birth on a $Ca^{45}$-free diet. These experiments include the results obtained between the third and the 560th day after birth, thus almost the lifetime of the mouse. The percentage of

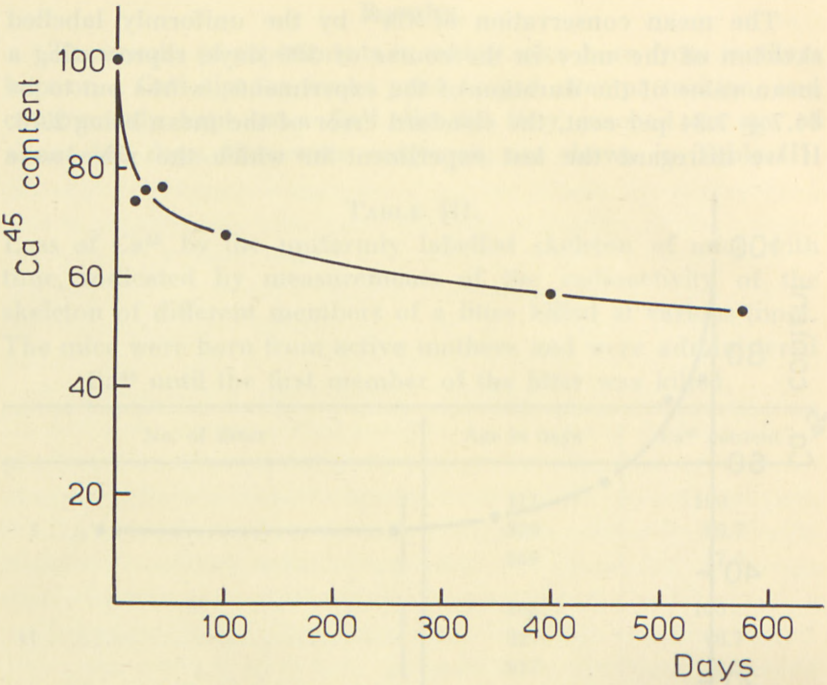


Fig. 2. Loss of $Ca^{45}$, obtained from labelled mother at birth, during lifetime of the mice. Each point indicates the $Ca^{45}$ content of another member of the litter killed at the stated time.

$Ca^{45}$ lost between day 3 and day 560 works out to be 53 per cent and 44 per cent, respectively. The mean loss of $Ca^{45}$ observed in experiments lasting 100 to 180 days amounts to 43 per cent (Table IV). The loss of $Ca^{45}$ during the first three days of life is less than 10 per cent; thus half of the maternal calcium atoms are preserved during life.

The calcium content of our newly born mice, weighing 1.23—1.37 gms. varied between 0.28 and 0.35 per cent of the body weight, not much differing from the calcium content of the new-born rat (4.7 gm.) for which data varying between 0.27 and 0.35 per cent are reported[42]. The calcium content of 1 gm. fresh weight of newly born mouse amounts to 0.3 times that of 1 gm. of the adult animal, which is 1.05 per cent. If all the maternal calcium atoms had the same chances to supply calcium to the offspring, and all were labelled, we would find 1 gm. of newly born mouse to be 0.3 times as active as 1 gm. of the mother.

TABLE IV. Retention of maternal calcium atoms by the offsprings.

| No. of litter | Age in days | Weight in gms. | Per cent of mothers' activity present in the offspring | Number of maternal atoms present |
|---|---|---|---|---|
| I | 3 | 2.95 | 8.25 | 100 |
| | 31 | 13.9 | 6.59 | 80 |
| | 39 | 16.8 | 5.50 | 67 |
| | 43 | 18.6 | 6.0 | 72 |
| | 103 | 24.3 | 4.25 | 52 |
| II | 1 | 2.20 | 6.95 | 100 |
| | 129 | 41.9 | 4.95 | 71 |
| | 181 | 31 | 3.96* | 57 |
| III | 1 | 1.80 | 10.3 | 100 |
| | 131 | 38.1 | 7.4 | 72 |
| IV | 1 | 1.45 | 9.91 | 100 |
| | 128 | 39.6 | 7.25 | 73 |
| | 181 | 40.0 | 5.69 | 57 |
| V | 3 | 3.1 | 8.55 | 100 |
| | 27 | 11.5 | 6.98 | 82 |
| | 35 | 21.0 | 6.67 | 78 |
| | 42 | 24.2 | 6.27 | 73 |

* Incl. $C^{45}$ of 3 offsprings.

We find the $Ca^{45}$ content of 1 gm. of new-born mouse to amount to 1.7 times that of 1 gm. of the adult mouse. The $Ca^{45}$ taken in by the mother has thus only an opportunity of interchanging in the average with about $^1/_5$ to $^1/_6$ of the body calcium before being utilized in the building up of the embryo.

## Discussion.

*a) Conservation of the calcium atoms of the outgrown skeleton through life.*

The fact that a very appreciable part of the skeletal calcium is preserved in the outgrown animal throughout its lifetime results

from experiments carried out by SINGER, ARMSTRONG, and PREMER[34], by CARLSON[28,35], and by BAUER[15]. Similar results werse obtained in investigations on the renewal of the mineral constituents of the skeleton, performed by the present author and his assoc. who used $P^{32}$ as an indicator[31].

From specific activity data of the plasma and the skeleton of the outgrown rat, the percentage of renewable skeletal sodium was calculated by BAUER[36] to amount to 30—40 per cent of the sodium present (disregarding the extracellular sodium); a similar figure – 45 per cent – is reported by EDELMAN[38] and by BADEN and MOORE[39]. Since sodium is mainly an extracellular element, the specific activity of plasma sodium decreases only slowly with time, not so the specific activity of calcium. The calculation of the percentage of renewable skeletal calcium from specific activity data is therefore encumbered with great difficulties (cf. p. 8). From data collected during five days, BAUER[15] estimates, however, that less skeletal calcium than skeletal excess sodium is exchangeable in the rat, thus less than 30—40 per cent. As it was shown above (p. 13), the mobilization of some further skeleton calcium is still going on in the mouse after the lapse of more than 100 days and the non-exchangeable part of the skeleton amounts to 67 per cent.

It is interesting to note that, when injecting $Ca^{45}$ at the start of the experiment interperitoneally to outgrown rats whose skeletal calcium content was increased appreciably during the experiment, SINGER and ARMSTRONG[40] found a $Ca^{45}$ retention of 42—45 per cent in the skeleton after the lapse of 52 days and the release of only small amounts of radiocalcium after that date. BUCHANAN[41], who exposed mice to air containing $C^{14}O_2$, found that 30 per cent of the bone carbonate are replaced within 12 days, while 45 per cent only are renewed in the course of three months.

b) *Conservation of maternal calcium atoms by the offspring through life.*

Our results demonstrate the very pronounced ability of the skeleton to conserve maternal atoms.

In the first mentioned experiments, one third of the $Ca^{45}$ content of the outgrown mouse was found to be replaceable by

inactive food calcium. In the latter experiments with growing mice, released $Ca^{45}$ had a further outlet, viz. utilization in the formation of additional skeleton, which takes place in the growing organism.

Investigations were carried out earlier on the loss of $P^{32}$ through the lifetime of mice born from active mothers[37]. Some results of these investigations are shown in Table V.

TABLE V.

Loss of $P^{32}$ through the lifetime of mice born from active mothers. Mother injected with $P^{32}$ on February 9. Gestation: February 18. Replacement of the active by an inactive mother: February 22.

| No. of offspring | Killed: date | Relative activity | Weight in gm. |
|---|---|---|---|
| 1..... | 22/2 | 100 | 3 |
| 2..... | 3/3 | 82 | 7 |
| 3..... | 16/3 | 73 | 15 |
| 4..... | 30/3 | 48 | 18 |
| 5..... | 13/4 | 41 | 25 |
| 6..... | 13/5 | 40 | 35 |

Loss of $P^{32}$ in the course of 81 days: 60 per cent.

The fact that a very appreciable percentage of the maternal phosphate is preserved –though less than of the maternal calcium– is presumably due to the lower share of the bone phosphorus in the total body phosphorus than the part of bone calcium in body calcium. 17 per cent of the phosphorus content of the mouse are present in the soft tissues, but only 1 per cent of its calcium content is located there. The phosphorus and calcium atoms present in various components of the soft tissues –with the exception of desoxyribo nucleic acid phosphorus of some tissues– are poorly conserved and, consequently, maternal calcium may be expected to be better conserved than maternal phosphorus.

From the fact that during the first 40 days of life –thus during a phase of intense skeleton formation– only less than a third of the maternal calcium atoms of the mouse is lost, we can conclude that the largest part of the calcium atoms leaving the circulation is utilized to skeleton formation and remains largely conserved in the skeleton.

Le Blond and assoc.[9] injected labelled phosphate into newborn rats and followed the $P^{32}$ uptake by the humerus and the lower jaw. Denoting the total $P^{32}$ taken up by the humerus in the course of the first hour by 100, the uptake after eight hours was found to be 150, after one day 117, and after three days 116. In spite of the rapid growth of the humerus, the $P^{32}$ present after the lapse of a day is thus conserved through the following days; similar results were obtained in investigations on the $P^{32}$ uptake by the lower jaw.

The incorporation of calcium atoms in the rapidly growing bone tissue can also be studied by following its uptake into the incisor of outgrown animals. Carlson[23, 28, 35] performed extensive and highly instructive studies on the calcium metabolism of outgrown rats, among others with the result that the calcium atoms incorporated with the rapidly growing incisors are conserved to a very large extent in contrast to those incorporated with the outgrown skeleton.

It is rather difficult to determine the calcium intake and excretion by the suckling mouse. Our adult mice (36—37 gm.), however, were found daily to consume $4 \pm 0.6$ gm. of standard bread containing $8.3 \pm 1.2$ mg calcium; further 0.2 mg. calcium was contained in the 4 ml. of daily consumed water. The calcium recovered daily in the feces amounted to 8 mg. A very appreciable part of the feces calcium may be assumed to be of endogenous origin, thus having passed the circulation before excretion. The share of endogenous phosphorus in the feces phosphorus was calculated from the specific activity of feces P and urine (plasma) P[44, 45]; these calculations lead to the result that 74 per cent of the phosphorus of the human food and 72 per cent of the rat food are absorbed into the circulation. About the same percentage of the food P can be expected to be taken up by the mouse. As to the utilization of calcium, data are available only for the uptake by humans[43]. Here, the mean percentage uptake was found to be 56. From the above data it follows that, out of the daily uptake of 8 mg. calcium by our mice, at least 4 mg. have passed the circulation, representing a minimum amount of 2 gm. in the course of 500 days. From our results it thus follows that these 2 gm. were prevented from interchanging with $^2/_3$ of the 370 mg. calcium present in the skeleton of a mouse weighing 36 gm. The pro-

tected part of the skeleton calcium did not come into contact with the plasma or lymph and, correspondingly, an exchange between the unlabelled food calcium and labelled skeleton calcium could not take place; the same is true for the new-formation of the protected apatite crystals of this part of the skeleton under participation of food calcium. A possible rearrangement within the protected area would not manifest itself in our experiment.

The inaccessibility of parts of the skeleton minerals manifests itself also by the observation that radium, which like calcium is a strongly bone-seeking element, can find a life-long abode in the skeleton. The fact that a large fraction of radium administered to human subjects remains for decades in the skeleton is due presumably to the incorporation of the radium into parts of the skeleton which are covered by apatite layers and thus become inaccessible and, even if released, are incorporated again with the apatite structure. Aub and associates[46] report a case in which no decrease in the radium content of a woman was found to take place between 1934 and 1945. This woman had been administered radium in 1924.

## Conservation of Ancestoral Atoms.

The radiocalcium atoms going over from the first generation of mice into the second (cf. p. 14) do not indicate the total amount of maternal calcium atoms passing from the mother to the offspring, since the mother is not uniformly labelled. Chemical data indicate a passage of about 1.3 per cent. Since the calcium of the second generation is uniformly labelled, the passage of the ancestoral calcium atoms from the second into the third generation is properly indicated by the radioactive tracer. About one third of the $Ca^{45}$ content of the second generation is lost prior to gestation, while about 0.5 per cent or less of the remainder passes into the third generation. From the calcium atoms present at birth in each generation, thus $^1/_{300}$ part or less goes over to the following generation. As our mice contained $6.10^{21}$ calcium atoms, the eleventh generation did no longer contain a single ancestoral calcium atom.

It is of interest to compare the life cycle of the ancestoral

calcium atoms of the mouse with that of easily accessible water molecules. Applying deutoriated or tritiated water as an indicator first the half life of water molecules present in the rat was found to vary between 3.6 and 2.5 days[35, 36], that of the mouse is expected to be somewhat shorter. Thus, in the course of 165 days, all $10^{24}$ water molecules present at the start of the experiment in the mouse are replaced. About 4 per cent of the maternal water molecules go over to the offsprings and, from these, the second and third generations of offsprings will take up a share which depends on the age of gestation; the fourth generation, however, will hardly contain any more ancestoral water molecule.

When the rate of disappearance of labelled water was followed in the rat during a long period, which was made possible by using tritiated water as an indicator, it was observed[36] that, after the lapse of 30 days, the labelled water disappeared at an appreciably slower rate than with a half life of 2.5 days. The controlling factor of the disappearance of labelled water from the organism is now the release of firmly bound tissue tritium which again becomes a constituent of the water molecules. Due to this fact, it lasts 60 days until the number of labelled water molecules of this type, present in the mouse, decreases to a $10^{-2}$th of its initial value.

If we disregard those water molecules whose hydrogen atoms were temporarily incorporated in tissue constituents and released appreciably later to become constituents of water molecules again, then all ancestoral water molecules are lost by the mouse during two generations.

While the loss of ancestoral calcium is determined mainly by the loss at birth, many ancestoral water molecules are lost during the lifetime of a generation, none of them reaching the third generation of offsprings.

## Summary.

Since it was desirable to obtain uniform labelling of all calcium present in the skeleton of the mouse, $^{45}CaCl_2$ was added to all water administered to mice for weeks before and after gestation. Such water was also given to new-born mice after weaning until adult age was reached. The members of the litter, having almost the same radiocalcium content, were then sacrificed at different dates within 560 days.

From the labelled calcium atoms present in the skeleton of the outgrown mice, $67.2 \pm 7.9$ per cent were found still to be present in the skeleton of sister mice sacrificed after the lapse of 390 days.

When administration of $Ca^{45}$ was interrupted after the birth of the litter, and its members reared by inactive mothers were sacrificed at different dates within 560 days, a mouse killed shortly after birth contained 8 per cent of the maternal $Ca^{45}$ atoms, another mouse killed after 510 days contained 4 per cent. Half of the calcium atoms present at birth is thus conserved during the lifetime of the mouse.

From the figures obtained for the passage of labelled calcium from one generation to the next, it follows that the eleventh generation does not contain a single calcium atom present in the first generation of its ancestors.


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