catalysed by acid and may be due to an initial phosphorylation of histidine followed by migration of the phosphoryl group to the hydroxyl group of serine.

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A Comparative Study on the Metabolism of ^{140}Ba and ^{45}Ca in Rats

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The tracer technique has made possible measurements of various processes involved in growth and remodelling of the skeleton, and has thus contributed to a better understanding of bone physiology (Bauer, Carlsson & Lindquist, 1955a). We have recently described a method with ^{32}P as tracer for calculating the accretion rate of bone salt in humans (Bauer, Carlsson & Lindquist, 1955b). Results have been obtained by this method in human cases of rickets and osteoporosis which indicate significant changes from the normal rate of accretion of bone salt (Bauer, Carlsson & Lindquist, 1956). It seems probable that isotope methods can be used in humans, not only for basic research on skeletal metabolic disorders but also as diagnostic tests. Part of our research has been to find useful isotopes for this purpose.

Theoretically, skeletal accretion and resorption processes can be studied by means of isotopes of almost any element which forms an appreciable part of the skeletal tissue. For obvious reasons,

however, isotopes labelling bone salt are better suited for this purpose than are those which label the organic tissue. Not all the phosphorus outside the bone salt is freely exchangeable, and therefore ^{32}P is not suitable for calculations of the accretion rate in the whole skeleton. All extra-skeletal calcium is freely exchangeable, but the commonly available ^{45}Ca has too long a half-life (152 days) to permit a widespread use of this isotope in human experiments. Carrier-free ^{90}Sr is metabolized by the rat skeleton exactly as is ^{45}Ca (Bauer, Carlsson & Lindquist, 1955c) but again the half-life (2.5 years) is too long. Other isotopes of strontium, however, may prove useful in humans [e.g. ^{85}Sr (Spencer, 1955), half-life 65 days]. The similarity in the skeletal metabolism of ^{90}Sr and ^{45}Ca has prompted us to investigate isotopes of other elements with even shorter half-lives. The data of Hamilton (1947) suggest that the metabolic behaviour of barium and strontium are similar and ^{140}Ba has a half-life of only 12.8 days.

The present experiment demonstrates that in young rats ^{140}Ba is metabolized essentially in the same way as calcium. This isotope may therefore prove a useful tool in studies of skeletal metabolism.

MATERIALS AND METHODS

Two-month-old rats of both sexes, which had been fed on the stock diet (Bauer *et al.* 1955*c*), were used.

At about 9 a.m. the animals were injected intraperitoneally with 1 ml. of an isotonic saline solution containing about $15\mu\text{C}$ of high-specific activity ^{45}Ca and $1.5\mu\text{C}$ of carrier-free ^{140}Ba . This was found to be the most suitable ratio of activities in the solution administered for differentiating between the two isotopes by means of absorption, since the radiation emitted from ^{140}Ba (together with its daughter isotope ^{140}La) is stronger than that from ^{45}Ca . The isotopes were obtained from the Atomic Energy Establishment, Harwell, England.

At the time of injection, the urinary bladder was emptied. The animals were divided into eleven groups of four animals. Care was taken to make the groups as similar as possible with respect to litter, sex, and body weight. The different groups of animals were killed at varying intervals ranging from 15 min. to 48 hr. after injection. Immediately after injection, each of the animals intended to survive for 4 hr. or more was placed in an individual cage provided with an arrangement for separation of urine and faeces. Before these animals were killed, their bladders were emptied. After death the bladder of each animal was examined and in the few rats in which urine was still present it was added to that previously excreted. Urine and faeces were not collected from the animals allowed to survive less than 4 hr. Food was available from 8 hr. after injection. However, none of the animals of the 12 hr. group took any food, because the food intake of rats is mostly nocturnal. The animals allowed to survive more than 12 hr. appeared to consume normal amounts of food.

The animals were killed by bleeding under ether anaesthesia. Serum calcium was determined on serum samples (1–3 ml.) by the method of Clark & Collip (1925). In order to avoid precipitation of BaSO_4 (see below), the oxalate precipitate was dissolved in 0.3N-HCl (instead of H_2SO_4) containing a small amount of MnCl_2 as a catalyst (Kolthoff & Sandell, 1945). For each animal the supernatant serum and the washings were pooled, 2 ml. of N-BaCl₂ together with excess of K_2CrO_4 were added, and the samples were centrifuged. The supernatants were found to be radioactive. The precipitation was therefore repeated by adding 1 ml. of N-BaCl₂ to each sample. The supernatants were then free from activity. The precipitates were dissolved in the titrated-oxalate solution, 1 ml. of 10N-HCl was added, and the samples were diluted with distilled water to 5 ml.

In each animal the gastro-intestinal tract together with the faecal collection, the ends and shafts of both right and left tibiae and fibulae (subsequently referred to as tibiae), and the four incisor teeth after removing the pulp were ashed in an electrically heated furnace. The urine was wet-ashed with HClO_4 and HNO_3 .

In order to re-establish equilibrium between ^{140}Ba and its daughter isotope ^{140}La (half-life 40 hr.), the samples were not counted until at least 12 days after the animals had been killed. Counting was made on liquid samples after suitable dilution with HNO_3 (d 1.10). Each sample was counted with

and without an aluminium absorber, which absorbed all the ^{45}Ca radiation and 61% of the ^{140}Ba , ^{140}La radiation. The activity emanating from ^{45}Ca and ^{140}Ba , ^{140}La respectively was calculated and compared with the activity of standard solutions of either of the two isotopes. The values obtained were expressed as a percentage of the dose administered.

For further details on the collection, pretreatment and counting of the samples, see Carlsson (1951).

In one of the 16 hr. rats the isotope injection was made in the intestinal tract, and one of the 48 hr. rats died from a lesion produced by the injection needle. Results from these animals have been omitted.

Calculation of results

Calcium can be taken up by the skeleton and be removed again by two different mechanisms: 1, 'physical exchange reactions' between the bone salt and the surrounding fluid; 2, accretion (formation) and resorption (dissolution) of bone salt. By definition the exchange reactions are reversible processes and do not result in any net change in bone-salt mass. Accretion and resorption, on the other hand, can be regarded as two irreversible processes working more or less independently of each other at different sites. As a rule there is a distinct interval of time between accretion and resorption which is equal to the life span of the tissue and varies with anatomical localization in the skeleton. Thus in the rat skeleton this interval is short in the ends and long in the shafts of the long bones and in the incisor teeth.

To make a valid comparison of the skeletal metabolism of ^{45}Ca and ^{140}Ba the above-mentioned processes must be analysed separately. The following method of calculation will therefore be applied to the observed values in this investigation. The principles involved are described in detail in Bauer *et al.* (1955*a*).

The amount of an isotope such as ^{45}Ca present in a calcified tissue can be written as follows:

$$^{45}\text{Ca}_{\text{Obs.}} = ^{45}\text{Ca}_E + ^{45}\text{Ca}_A - ^{45}\text{Ca}_R, \quad (1)$$

($^{45}\text{Ca}_{\text{Obs.}}$, Total amount of ^{45}Ca present in a calcified tissue; $^{45}\text{Ca}_E$, amount of ^{45}Ca present in the exchangeable fraction of the bone salt; $^{45}\text{Ca}_A$, amount of ^{45}Ca incorporated into the non-exchangeable fraction of the bone salt through accretion; $^{45}\text{Ca}_R$, amount of ^{45}Ca removed through resorption).

Assuming that the specific activity, i.e. $^{45}\text{Ca}/(^{40}\text{Ca} + ^{45}\text{Ca})$, of the exchangeable Ca fraction rapidly attains the same value as the specific activity of the plasma Ca and that these two specific activities can then be taken as equal, we get

$$^{45}\text{Ca}_E = E \times S, \quad (2)$$

(E , Amount of Ca ($^{40}\text{Ca} + ^{45}\text{Ca}$) in the exchangeable fraction of the bone salt; S , specific activity of the serum (plasma) Ca).

Since it must be assumed that ^{45}Ca and ^{40}Ca are deposited in the bone salt in the same proportions as those simultaneously occurring in the blood, we get

$$^{45}\text{Ca}_A = A \times T \times S_M, \quad (3)$$

(A , Rate of Ca accretion; T , interval of time between administration of ^{45}Ca and observation; S_M , average specific activity of serum Ca during this time interval).

Combination of equations (1), (2) and (3) gives

$$^{45}\text{Ca}_{\text{Obs.}} = E \times S + A \times T \times S_M - ^{45}\text{Ca}_R. \quad (4)$$

At sufficiently short intervals ⁴⁵Ca_R can be disregarded. From observations made at two different intervals, *T*₁ and *T*₂, we then get

$$^{45}\text{Ca}_{1-\text{Obs.}} = E \times S_1 + A \times T_1 \times S_{1-M}, \tag{4a}$$

$$^{45}\text{Ca}_{2-\text{Obs.}} = E \times S_2 + A \times T_2 \times S_{2-M}. \tag{4b}$$

From these two equations we obtain

$$\frac{^{45}\text{Ca}_{1-\text{Obs.}} - A \times T_1 \times S_{1-M}}{^{45}\text{Ca}_{2-\text{Obs.}} - A \times T_2 \times S_{2-M}} = \frac{S_1}{S_2}. \tag{5}$$

After *A* has been calculated from equation (5), *E* can be obtained by introducing the calculated *A* value in equation (4a).

Equation (5) can be used for calculating the accretion rate not only in different calcified tissues but also in the whole skeleton. In this calculation the whole body is treated in the same manner as a calcified tissue. ⁴⁵Ca_{Obs.} is then equal to 100 minus the percentage of dose lost via the kidneys and the gastro-intestinal tract, and *E* is the amount of Ca present in the exchangeable fraction of the bone salt plus the amount of Ca of the fluid phase of the body.

Table 1. Mean specific activities (*S_M*) of serum ⁴⁵Ca and ¹⁴⁰Ba during various intervals after simultaneous intraperitoneal injection of the two isotopes to young rats

Specific activity expressed as percentage of dose/mg. of Ca. Legend to Fig. 1 explains how the *T* × *S_M* values of this table have been calculated. *S_M* has been obtained by dividing these values by the corresponding *T*.

Interval <i>T</i> (hr.)	⁴⁵ Ca		¹⁴⁰ Ba	
	<i>T</i> × <i>S_M</i>	<i>S_M</i>	<i>T</i> × <i>S_M</i>	<i>S_M</i>
0-2	14.7	7.35	10.2	5.10
0-4	19.0	4.75	11.5	2.88
0-6	21.7	3.62	12.2	2.03
0-8	23.8	2.97	12.8	1.60
0-12	27.0	2.25	13.4	1.12
0-16	29.5	1.84	13.8	0.86
0-24	33.4	1.39	14.3	0.60
0-48	40.5	0.84	14.9	0.31

Fig. 1. ⁴⁵Ca and ¹⁴⁰Ba activity of blood serum. The values are expressed in percentage of dose/mg. of Ca (group values in Table 2). ●—●, ⁴⁵Ca; ○—○, ¹⁴⁰Ba. Inset: *T* × *S_M* = product of time (hr.) and mean specific activity of blood serum (% of dose/mg. of Ca) = area covered by ⁴⁵Ca and ¹⁴⁰Ba blood-serum activity curves during intervals 0—*T* hours. (Measurements of these areas have been made with a planimeter on large scale paper, and checked by cutting out and weighing the areas enclosed by the curves.) Numerical values of *T* × *S_M* are given in Table 1.

The ^{140}Ba results were treated in exactly the same way as the ^{45}Ca results. For both isotopes the blood activities were thus expressed as percentage of dose/mg. of Ca. This procedure permits the various metabolic processes to be studied with respect to similarities or differences between the two elements. It should perhaps be noted that the activity/mg. of Ca was, on an average, practically equal to the activity/10 ml. of serum, the average concentration of Ca in the entire material being 9.9 mg./100 ml. The standard error of the single observation was 0.60 mg./100 ml.

Examples of calculations. The accretion rate (A) of the tibia shafts was calculated from the ^{45}Ca results by introducing in equation (5) the 8 and 16 hr. values of Table 3

($^{45}\text{Ca}_{\text{Obs.}}$ of the tibia shafts) and of Fig. 1 (S from the blood-activity curve drawn through the values of Table 2; $T \times S_M$ from inset of Fig. 1, numerical values given in Table 1):

$$\frac{1.08 - A \times 23.8}{1.17 - A \times 29.5} = \frac{0.93}{0.57}; A = 0.034 \text{ mg. of Ca/hr.}$$

The 8–24 and 8–48 hr. values give A as 0.035 and 0.031 respectively. Practically the same value is thus obtained at different intervals. The mean of these three values (0.033) introduced in the 8 hr. values of equation (4a) gives $E = 0.3$ mg. of Ca. If earlier values than those at 8 hr. are used for the calculation of E , the criterion $^{45}\text{Ca}_E = E \times S$ is not met, and results which are too low will be obtained. At a

Fig. 2

Fig. 3

Fig. 2. Excretion and overall metabolism of ^{45}Ca . A: $\circ$, ^{45}Ca in gastro-intestinal tract plus faeces; $\bullet$, ^{45}Ca in urine. The curves have been drawn to fit the individual values recorded. (Group values are given in Table 2.) B: for explanation of symbols, see Calculation of results. $^{45}\text{Ca}_{\text{Obs.}}$: $\circ$, retention of ^{45}Ca in the whole body. These values have been obtained by subtracting from 100% the values of the urine and faecal curves (A) at each interval. $^{45}\text{Ca}_A$ and $^{45}\text{Ca}_E$ have been constructed from the A and E values of Table 4. $^{45}\text{Ca}_A + ^{45}\text{Ca}_E$ is the sum of $^{45}\text{Ca}_A$ and $^{45}\text{Ca}_E$ in this graph. $^{45}\text{Ca}_R$ has been obtained by subtracting $^{45}\text{Ca}_{\text{Obs.}}$ from $^{45}\text{Ca}_A + ^{45}\text{Ca}_E$. The marked scatter in the ^{45}Ca excretion values is of but limited importance for the validity of the retention values. The 24 hr. excretion value was 7%, corresponding to a retention value of 93%. A 50% deviation in the former value would give rise to a 4% deviation in the latter value.

Fig. 3. Excretion and overall metabolism of ^{140}Ba . See legend of Fig. 2. Both graphs have been drawn in the same way as those of Fig. 2, except that ^{140}Ba instead of ^{45}Ca values have been used.

Table 2. Mean values with their S.E.M. for ^{45}Ca and ^{140}Ba activities of the blood serum and of urinary and faecal excretions of young rats after simultaneous intraperitoneal injection of the two isotopes

Interval after injection (hr.)	No. of rats	Serum (% of dose/mg. of Ca)		Urine (% dose excreted from start of expt.)		Faeces* (% dose excreted from start of expt.)	
		^{45}Ca	^{140}Ba	^{45}Ca	^{140}Ba	^{45}Ca	^{140}Ba
$\frac{1}{2}$	4	11.4 (0.955)	14.4 (0.869)	†	†	†	†
$\frac{1}{2}$	4	12.6 (0.755)	7.09 (0.326)	†	†	†	†
1	4	5.45 (0.155)	2.52 (0.054)	†	†	†	†
2	4	2.91 (0.348)	1.01 (0.109)	†	†	†	†
4	4	1.54 (0.125)	0.431 (0.028)	0.50 (0.23)	5.8 (0.39)	6.2 (0.30)	18.4 (1.93)
6	4	1.24 (0.123)	0.430 (0.132)	0.66 (0.35)	7.4 (0.74)	6.6 (1.10)	19.3 (1.76)
8	4	0.93 (0.039)	0.216 (0.021)	0.90 (0.35)	6.4 (0.33)	6.1 (0.93)	19.4 (1.84)
12	4	0.72 (0.041)	0.118 (0.028)	0.57 (0.21)	5.5 (1.1)	5.6 (0.98)	19.9 (0.48)
16	3	0.57 (0.063)	0.051 (0.016)	0.91 (0.48)	5.6 (2.0)	6.3 (1.00)	21.1 (1.19)
24	4	0.41 (0.014)	0.060 (0.019)	0.95 (0.19)	7.5 (1.9)	6.0 (1.35)	21.1 (0.34)
48	3	0.23 (0.084)	0.143 (0.040)	0.57 (0.11)	6.6 (2.3)	7.1 (1.01)	22.7 (1.98)

* Gastro-intestinal tract plus faeces collection.

† Not collected.

Table 3. Mean values and S.E.M. for ^{45}Ca and ^{140}Ba activities of the incisor teeth and of the ends and shafts of the tibiae following simultaneous intraperitoneal injection of the two isotopes to young rats

Interval after injection (hr.)	No. of rats	Incisor teeth* (% of dose)		Tibia ends† (% of dose)		Tibia shafts‡ (% of dose)	
		^{45}Ca	^{140}Ba	^{45}Ca	^{140}Ba	^{45}Ca	^{140}Ba
$\frac{1}{2}$	4	0.47 (0.021)	0.49 (0.026)	2.34 (0.133)	2.35 (0.118)	0.47 (0.019)	0.45 (0.016)
$\frac{1}{2}$	4	0.80 (0.088)	0.82 (0.074)	3.79 (0.071)	3.68 (0.119)	0.82 (0.037)	0.73 (0.037)
1	4	1.09 (0.032)	1.04 (0.035)	4.88 (0.132)	4.57 (0.039)	0.99 (0.031)	0.90 (0.026)
2	4	1.45 (0.077)	1.05 (0.043)	5.52 (0.184)	4.84 (0.119)	1.04 (0.045)	0.90 (0.047)
4	4	1.51 (0.167)	1.03 (0.106)	5.23 (0.332)	4.73 (0.171)	1.08 (0.064)	0.87 (0.044)
6	4	1.81 (0.054)	1.16 (0.034)	5.61 (0.193)	4.80 (0.158)	1.08 (0.053)	0.88 (0.031)
8	4	1.85 (0.133)	1.14 (0.068)	5.49 (0.188)	4.83 (0.296)	1.08 (0.039)	0.86 (0.019)
12	4	2.07 (0.079)	1.33 (0.080)	5.44 (0.238)	4.76 (0.088)	1.19 (0.032)	0.96 (0.040)
16	3	2.21 (0.075)	1.23 (0.085)	5.45 (0.192)	4.82 (0.194)	1.17 (0.017)	0.84 (0.059)
24	4	2.34 (0.019)	1.26 (0.045)	5.47 (0.114)	4.30 (0.158)	1.28 (0.074)	0.93 (0.071)
48	3	2.47 (0.347)	1.36 (0.077)	5.26 (0.245)	3.85 (0.208)	1.35 (0.039)	0.93 (0.053)

* The four incisor teeth in each animal (pulp removed).

† Ends of right and left tibiae and fibulae in each animal.

‡ Shafts of right and left tibiae and fibulae in each animal.

Table 4. Skeletal metabolism of ^{45}Ca and ^{140}Ba in young rats

	Ca content (mg.)	Accretion rate (A)*			Exchangeable Ca (E)†		
		(mg. of Ca/hr.) calculated from		Ratio $^{140}\text{Ba}/^{45}\text{Ca}$	(mg.) calculated from		Ratio $^{140}\text{Ba}/^{45}\text{Ca}$
		^{45}Ca	^{140}Ba		^{45}Ca	^{140}Ba	
Whole skeleton	1000‡	2.4	5.2	2.2	39§	41§	1.1
Tibia ends	35	0.14	0.34	2.4	2.3	2.1	0.9
Tibia shafts	27	0.034	0.064	1.9	0.3	0.3	1.0
Incisors	44	0.067	0.092	1.4	(0.3)	(0.2)	—

* Accretion rate (A) calculated according to equation (5) (see under Calculation of results, p. 536).

† Exchangeable Ca (E) calculated by entering the A values of this table in the 8 hr. values of equation (4a).

‡ Roughly estimated from body weight (120 g.).

§ This figure includes the Ca of the soft tissues of the body.

longer interval, $E \times S$ is too small to allow any precise calculation of E . The A and E values used for construction of the various graphs and in Table 4 have been calculated from values obtained from curves drawn through the respective observed values of Tables 2 and 3. The values of the tibia shafts and the incisor teeth have been calculated from the 8-48 hr. values. The 8-48 hr. values of A for the tibia ends and total skeleton show a drop compared with values

calculated from earlier intervals, e.g. 8-16 hr. This drop may be due to loss of activity from resorption ($^{45}\text{Ca}_R \neq 0$). Therefore the end and total skeleton values of A have been calculated from the 8-16 hr. values only.

The results for Ba have been treated in a similar manner. Owing to the more rapid drop in Ba than in Ca activity in the blood it was possible to calculate E and A from the 4 hr. results.

Fig. 4

Fig. 5

Fig. 4. Role of accretion and exchange reactions for the uptake of ^{45}Ca in various calcified tissues. ●, Single observations ($^{45}\text{Ca}_{\text{Obs.}}$). (Group values given in Table 3.) For explanation of symbols, see under Calculation of results. For construction of curves, see legend of Fig. 2.

Fig. 5. Role of accretion and exchange reactions in the uptake of ^{140}Ba in various calcified tissues. ●, Single observations ($^{140}\text{Ba}_{\text{Obs.}}$). (Group values given in Table 3.) For explanation of symbols, see under Calculation of results. The curves have been drawn in the same way as those of Fig. 2, except that ^{140}Ba instead of ^{45}Ca values have been used.

RESULTS

Presentation of results. The activity values observed are presented in Tables 2 and 3, and in Figs. 1–5. The *A* and *E* values (calculated as described above) are given in Table 4. These values have been used for construction of the theoretical curves for the changes of $^{45}\text{Ca}_A$ and $^{140}\text{Ba}_A$, $^{45}\text{Ca}_E$ and $^{140}\text{Ba}_E$, $^{45}\text{Ca}_A + ^{45}\text{Ca}_E$ and $^{140}\text{Ba}_A + ^{140}\text{Ba}_E$ in time, drawn in Figs. 2–5.

Blood activities. From the 0.5 hr. interval onwards, the Ba activities of the serum were lower than the Ca activity. Consequently, $T \times S_M$ was constantly lower for Ba than for Ca (Fig. 1 and Table 1).

The ^{45}Ca activity values declined throughout the 48 hr. period studied, and the ^{140}Ba values possibly increased somewhat after the 16 hr. interval (Table 2), which might be explained by a liberation of ^{140}Ba from the bones owing to bone resorption.

Excretion. About 7% of the injected ^{140}Ba was recovered from the urine during the first 24 hr. after injection, compared with 0.9% of the injected ^{45}Ca (Figs. 2, 3). A similar difference in faecal excretion was observed, the 24 hr. values being about 20 and 6%, for Ba and Ca respectively.

As the activity/mg. of Ca in the serum was lower for ^{140}Ba than for ^{45}Ca , it is evident that the clearance of ^{140}Ba was higher than that of ^{45}Ca both in the kidneys and, to a lesser degree, in the digestive tract, both of which are thus able to distinguish between the two elements.

Retention. As a consequence of the higher excretion of ^{140}Ba , the retention of this isotope in the whole body (Figs. 2, 3) and in the calcified tissues (Figs. 4, 5, Table 3) was lower than that of ^{45}Ca .

Accretion rate. The value for the accretion rate (*A*) in the tibia ends was 2.4 times higher when calculated from the ^{140}Ba results, compared with the ^{45}Ca results (Table 4). For the tibia shafts this factor was 1.9, and for the incisors it was 1.4. It would thus seem that ^{140}Ba was incorporated relatively faster in the less compact areas of the skeleton. In the whole skeleton this factor was 2.2, i.e. in this respect the whole tibiae were representative of the whole skeleton.

Exchange reactions. Practically the same values for the size of the exchangeable fraction (*E*) of Ca were obtained from the ^{140}Ba and ^{45}Ca data in the whole body as well as in the calcified tissues. In the incisors the exchangeable fraction is too small to be estimated with any great accuracy.

Bone resorption. After computing *A* and *E* the curves for $^{45}\text{Ca}_A$, $^{45}\text{Ca}_E$, and $^{45}\text{Ca}_R$ were drawn (Figs. 2, 4). The corresponding curves for ^{140}Ba are given in Figs. 3 and 5. ^{140}Ba is transferred more rapidly than ^{45}Ca from the exchangeable to the non-

exchangeable fraction of the bone salt. The values for $^{45}\text{Ca}_R$ ($^{45}\text{Ca}_A + ^{45}\text{Ca}_E - ^{45}\text{Ca}_{\text{obs.}}$) and for $^{140}\text{Ba}_R$ are small even at the end of this short-term study and may hardly be significant in the present material. In the tibiae shafts and in the incisors it is undetectable. At longer intervals the influence of bone resorption is easily detectable (Bauer *et al.* 1955*a*).

DISCUSSION

The present investigation demonstrates that ^{140}Ba is irreversibly deposited in the skeleton. The clearance of ^{140}Ba from the plasma by skeletal accretion is about twice that of ^{45}Ca , but removal by reversible exchange reactions is about the same for the two isotopes.

It therefore seems worth trying ^{140}Ba as a tool for studying skeletal metabolism in humans. It is true that ^{140}Ba and ^{45}Ca are not dealt with by the skeleton in an identical manner. However, the behaviour of the two isotopes is qualitatively similar, and there is little reason to doubt that skeletal disturbances would result in altered ^{140}Ba , as well as ^{45}Ca , metabolism. The suitable half-life and low cost of ^{140}Ba may well prove to outweigh its disadvantages.

SUMMARY

1. In a comparative metabolic study of ^{140}Ba and ^{45}Ca , the two isotopes were given simultaneously to young rats by intraperitoneal injection.
2. The plasma activity (as a percentage of dose given) was lower for ^{140}Ba than for ^{45}Ca .
3. For ^{140}Ba the urinary and faecal excretions in 24 hr. were about 7 and 20% respectively, and for ^{45}Ca were 0.9 and 6% respectively.
4. The clearance of ^{140}Ba from the plasma through skeletal accretion was about twice that of ^{45}Ca .
5. The clearance from the plasma through reversible exchange reactions was the same for the two isotopes.
6. Since there was no fundamental difference in the metabolism of the two isotopes, and the half-life of ^{140}Ba is only 12.8 days, this isotope should be suitable for use as an indicator of skeletal metabolism in humans.

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Colorimetric Estimation of Ketopentoses and Ketohehexoses

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The introduction of the cysteine-carbazole reaction by Dische & Borenfreund (1951) has greatly simplified the estimation of ketopentoses in the presence of aldopentoses. In this reaction ketopentoses give a purplish-red colour (λ_{max} 540 m μ .) with little interference from aldopentoses; but a disadvantage of the method is that ketohehexoses (λ_{max} 560 m μ .) interfere with the estimation of ketopentoses.

Cohen (1953) and Dische (1951), using respectively the techniques of Roe (1934) and of Roe, Epstein & Goldstein (1949), have reported that ketopentoses give a green colour in the resorcinol reaction. The absorption spectrum of the green colour developed with both these methods appears to be unsuitable for the estimation of ketopentose in the presence of ketohehexose. Using the resorcinol method of Cole (see Bacon & Bell, 1948), McCorkindale & Edson (1954) also noticed that ketopentoses gave a green colour (λ_{max} 640 m μ .), whereas ketohehexoses gave a red colour (λ_{max} 480 and 415 m μ .).

Since these differences seemed to afford a basis for the estimation of ketopentoses and ketohehexoses in the same solution, the conditions of the resorcinol reaction have been investigated and a satisfactory quantitative method has been developed.

MATERIALS AND METHODS

D-Xylulose was prepared according to Levene & Tipson (1936). The monoacetone xylulose obtained (m.p. 68°) was shown to be free from other sugar derivatives by paper chromatography with ethyl acetate-acetic acid-formic acid-water (18:3:1:4, by vol.) as the mobile phase (Hough & Jones, 1953) and *m*-phenylenediamine dihydrochloride (Chargaff, Levine & Green, 1948) as spray reagent. Hydrolysis of the monoacetone derivative yielded xylulose as a colourless syrup, $[\alpha]_D^{25} + 34.8 \pm 0.6^\circ$ in water (*c*, 2.42). No aldose could be detected in the free sugar by paper chromatography with ethyl acetate-pyridine-water (Isherwood & Jermyn, 1951) as the mobile phase and aniline hydrogen phthalate (Partridge, 1949) as the spray. All solutions of

xylulose were standardized with periodate (Salo, 1953). After storage for about 2 months at 4° a solution of xylulose gave approximately 5% less colour than a portion of the same solution stored at -35°.

D-Tagatose (a gift from Mr D. R. D. Shaw) was prepared according to Reichstein & Bosshard (1934) and had m.p. 130° $[\alpha]_D^{25} - 2.9 \pm 0.3^\circ$ in water (*c*, 5.03). Paper chromatography showed the absence of aldose.

D-Fructose, L-sorbose and dihydroxyacetone were obtained from Pfanstiehl Chemical Co., U.S.A. and mannoheptulose from General Biochemicals, Inc., U.S.A. Perseulose hemihydrate was a gift from Dr N. K. Richtmyer. A solution of L-ribulose was prepared by oxidation of ribitol with an enzyme preparation made from *Acetobacter suboxydans* (Arcus & Edson, 1954).

Ketose reagent. This was a modification of Cole's (Bacon & Bell, 1948) reagent, and consisted of two solutions: A, resorcinol, 0.05% (w/v) in absolute ethanol and B, HCl (sp.gr. 1.18) containing $\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$, 0.216 g./l. This reagent was used in all experiments unless stated otherwise.

General procedure for ketose estimation. A solution (2 ml.) containing 5–50 μg . of ketose/ml. is placed in a 12 mm. $\times$ 125 mm. test tube. To this are added 3 ml. of solution A, followed by 3 ml. of solution B. The contents of the tubes are mixed thoroughly and the tubes, covered with glass marbles, are immersed in a water bath at $80^\circ \pm 3^\circ$ for 40 min. (60 min. for sorbose). A reagent blank containing 2 ml. of water instead of ketose solution is included with each batch of tubes. Unknown solutions are compared with standards, extinctions being measured at specific wavelengths in a Beckman quartz spectrophotometer Model D.U., Corex cells with a 1 cm. light-path being used.

When ketohehexose alone is being estimated, tubes are placed in the bath simultaneously and cooled together in ice water at the end of the heating period. The extinction at 480 m μ . is stable for more than 5 hr. If ketopentose is present, tubes are placed in the bath in groups of three at 10 min. intervals, removed after 40 min. and cooled in ice water for 2 min. Extinctions at 640 m μ . are measured 5 min. after removal of the tubes from the bath by reading against the reagent blank heated with the first three tubes. This procedure avoids error due to the instability of the ketopentose colour.