2. Phospholipase A has been shown to occur in fresh or aged frozen human pancreas. The tissue requires special treatment to render the enzyme fully water-soluble.

3. A relatively simple and rapid method for the purification of phospholipase A from human pancreas is described.

4. Some of the properties of the pancreatic enzyme have been investigated, including heat-stability, pH optimum and the effect of certain inhibitors.

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The Absorption of Calcium, Strontium, Barium and Radium from the Gastrointestinal Tract of the Rat

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The comparative metabolism of calcium and strontium has been widely studied and it is now established that calcium is utilized in preference to strontium in many species of animals, including man (Comar, Whitney & Lengemann, 1955; Comar, Wasserman & Nold, 1956; Comar, Russell & Wasserman, 1957).

However, little attention has been paid to the metabolism of the two heavier members of the alkaline-earth series, namely barium and radium; and there are few reports in the literature on the comparative metabolism of the four elements.

In the present paper comparative studies of the effect of age, and other factors, on the absorption of calcium, strontium, barium and radium from the gastrointestinal tract of the rat are described.

In order to reduce biological variation, and to allow ready inter-comparison of results, absorption has been measured by means of double radioactive-tracer techniques with the following pairs of isotopes: ^{45}Ca and ^{85}Sr; ^{85}Sr and ^{140}Ba; ^{85}Sr and ^{226}Ra.

EXPERIMENTAL

Animals. The animals used were female rats of a highly inbred, brown-hooded August strain, aged between 14 days and 70 weeks. Animals less than 28 days old were suckled by the mother up to the start of each experiment; all other animals were maintained on M.R.C. diet no. 41B, both food and water being allowed *ad libitum*.

This diet contains about 0·8% by wt. of calcium.

Radioactive materials. Solutions of $^{45}CaCl_2$, $^{140}BaCl_2$ and $^{226}RaCl_2$ in dilute HCl were obtained from The Radiochemical Centre, Amersham, Bucks. The specific activity of the ^{45}Ca was from 5 to 10 mc/mg. of Ca. The ^{140}Ba was carrier-free; ^{85}Sr was prepared as a carrier-free solution of $^{85}SrCl_2$ from RbCl bombarded with deuterons in a cyclotron (Overstreet, Jacobson, Scott & Hamilton (1951)).

The activity administered, as a single dose, to each animal was approx. 1 μc of ^{45}Ca or ^{85}Sr, 10 μc of ^{140}Ba and approx. 0·01 μg. of ^{226}Ra.

Measurement of absorption. Appropriate amounts of the pair of isotopes being studied, contained in 0·2–0·5 ml. of 0·01 N-HCl, or in some experiments in cow's milk, were administered by intragastric intubation. The activity was always administered between 9.00 a.m. and 9.45 a.m., and the animals were killed approx. 7 hr. later. The entire gastrointestinal tract was carefully dissected out, and the radioactivity in the gastrointestinal tract and the remainder of the carcass determined in the manner described below.

In the three younger age groups the urinary excretion of the administered material was negligible. The older animals, however, excrete a few per cent of the administered activity in the urine during the experimental period; these animals were therefore kept in individual metabolism cages during the experiment and the urine was collected on filter paper.

'Absorption' has been calculated as the sum of the percentages of the administered material remaining in the carcass plus that in the urine at the end of the 7 hr. period, less that in the gastrointestinal tract.

In some experiments the animals were deprived of food, but not water, for 18 hr. before the administration. In all other cases the animals were allowed free access to food and water up to the commencement of each experiment.

Preparation of samples and measurement of radioactivity. All samples were ashed overnight in a muffle furnace at 550°; the gut and dried urine samples were ashed in silica crucibles and the whole carcasses in large porcelain dishes. With the exception of the samples containing ^{85}Sr and ^{226}Ra, the resulting ash was dissolved in 3 N-HCl and diluted to a suitable volume. The ash from the samples containing ^{85}Sr and ^{226}Ra was weighed and ground to a fine powder. The radioactivity in the various samples was then determined in one of the following ways.

(*a*) ^{45}Ca and ^{85}Sr: for the assay of ^{45}Ca the total calcium was precipitated as calcium oxalate from samples of the ash solutions containing about 5 mg. of calcium. The precipitated calcium oxalate was mounted on a tared polythene planchet, dried in air, weighed and counted in an end-window Geiger–Müller counting assembly. The resulting counting rates were corrected for background counts, self-absorption and for the resolving time of the counter. ^{85}Sr was determined by counting a suitable fraction, usually 5 ml., of the ash solutions in a well-type sodium iodide crystal-scintillation counter. Since ^{45}Ca emits only β particles of 0·25 Mev this isotope does not interfere with the assay of ^{85}Sr. Under the conditions used here the 0·51 Mev γ rays from the ^{85}Sr made only a negligible contribution to the observed counting rate of the ^{45}Ca samples.

(*b*) ^{85}Sr and ^{140}Ba: the decay of ^{140}Ba gives rise to a radioactive daughter product ^{140}La, and it is necessary to allow the ^{140}Ba–^{140}La mixture to build up to equilibrium before counting the samples. A period of 12 days, the half-life of ^{140}Ba, was found to be sufficient to allow equilibrium to be re-established and all samples were stored for this period before counting. The ^{140}Ba–^{140}La equilibrium mixture emits a number of β particles of varying energy and γ rays of energies from 0·03 to 2·57 Mev. The samples containing ^{85}Sr and the ^{140}Ba–^{140}La mixture were assayed in a well-type scintillation counter which was coupled to a single-channel pulse-height analyser. The counting rate was measured in the region of the 0·51 Mev γ peak of ^{85}Sr and again in the region of the 1·60 Mev γ peak of ^{140}Ba–^{140}La. The counting rate in the 1·60 Mev region is a direct measure of the activity due to ^{140}Ba–^{140}La. The activity due to ^{85}Sr was obtained by subtracting the counting rate due to ^{140}Ba–^{140}La in the 0·5 Mev region from the total counting rate in that region. The ratio of the counting rate due to ^{140}Ba–^{140}La in the 0·5 and 1·60 Mev region was determined by counting a pure sample of the ^{140}Ba–^{140}La equilibrium mixture with each batch of samples. The contribution to the counting rate in the 0·5 Mev region due to ^{140}Ba–^{140}La for any sample was calculated from the counting rate of that sample in the 1·60 Mev region.

(*c*) ^{85}Sr and ^{226}Ra: For the measurement of ^{85}Sr weighed portions of the ash were dissolved in 5 ml. of 3 N-HCl and counted in the scintillation counter. The counting rate was determined in the region of the 0·5 Mev peak only. Since the quantity of ^{226}Ra used was only 1 % of the activity of ^{85}Sr the contribution from the ^{226}Ra γ rays to the counting rate in the 0·5 Mev region could be ignored. The ^{226}Ra content of the finely ground ash was determined by α-scintillation counting with the method of sample preparation and counting described by Turner, Mayneord & Radley (1958).

In all cases standards consisting of suitable dilutions of the solutions administered to the animals were prepared and assayed with each batch of samples.

All results are expressed as a percentage of the administered activity.

RESULTS

The retention in the body, less that in the gastrointestinal tract, at 7 hr. after oral administration does not give a true measure of the amount of the material which has been absorbed, since no correction is made for any of the absorbed material which has been returned to the gastrointestinal tract by excretion. For the purpose of these comparative studies, however, it has not been considered necessary to correct for excretion into the gastrointestinal tract, since other experiments in which these four elements were administered intravenously have shown that the faecal excretion does not vary greatly from element to element. Hence the ratio of the measured retention to the true absorption will be approximately the same in each case.

The observation period of 7 hr. was considered to be sufficiently long to allow virtually complete absorption to take place since Cramer (1959) and Cramer & Copp (1959) have shown that absorption of ^{89}Sr is virtually complete 6 hr. after oral administration and that the maximum rate of absorption occurs 1–2 hr. after ingestion. This is supported by one or two experiments in which blood samples have been collected from our animals during the 7 hr. period; the highest amounts of ^{45}Ca and ^{85}Sr were found between 0·5 and 1 hr. after oral administration of these isotopes.

The effects of the age of the animal, deprivation of food and administration of the isotopes in cow's milk, instead of in dilute HCl, on the absorption of ^{45}Ca, ^{85}Sr, ^{140}Ba and ^{226}Ra from the gastrointestinal tract of the rat are shown in Table 1.

In Table 2 the absorption of the four elements is shown as a fraction of the amount of ^{45}Ca absorbed by the fed animals in each age group.

Table 1. *Effect of age, deprivation of food and administration of the metal in cow's milk on the absorption of calcium, strontium, barium and radium from the gastrointestinal tract of the rat*

Results are expressed as percentages of the administered dose ± S.E.M. with numbers of animals used given in parentheses.

Age of animal	Fed rats				Starved rats				Milk administration to fed rats			
	Ca	Sr	Ba	Ra	Ca	Sr	Ba	Ra	Ca	Sr	Ba	Ra
14–18 days	97·5 ±0·8 (9)	95·2 ±0·4 (31)	84·6 ±2·4 (10)	78·6 ±3·1 (12)	—	—	—	—	—	—	—	—
22 days	—	74·4 ±2·4 (5)	63·0 ±4·1 (5)	—	—	—	—	—	—	—	—	—
6–8 weeks	63·0 ±2·6 (15)	24·6 ±1·0 (45)	6·8 ±0·3 (5)	11·3 ±1·5 (20)	53·7 ±2·4 (5)	24·2 ±2·4 (19)	20·0 ±2·7 (5)	17·5 ±2·6 (9)	71·5 ±4·6 (10)	31·9 ±1·0 (25)	7·2 ±1·4 (5)	12·7 ±4·1 (10)
60–70 weeks	31·6 ±2·0 (10)	11·1 ±0·8 (24)	7·5 ±1·9 (10)	3·2 ±0·7 (4)	24·8 ±1·9 (10)	16·7 ±1·8 (18)	19·9 ±6·8 (5)	8·4 ±1·3 (3)	30·2 ±3·4 (5)	9·9 ±0·9 (5)	—	—

Table 2. *Absorption of strontium, barium and radium relative to that of calcium*

Results are expressed as fractions of amount of ^{45}Ca absorbed by the fed animals in each age group.

Age group	Fed rats				Starved rats				Milk administration			
	Ca	Sr	Ba	Ra	Ca	Sr	Ba	Ra	Ca	Sr	Ba	Ra
14–18 days	1·00	0·97	0·87	0·80	—	—	—	—	—	—	—	—
6–8 weeks	1·00	0·39	0·11	0·18	0·85	0·38	0·32	0·28	1·13	0·51	0·11	0·20
60–70 weeks	1·00	0·35	0·24	0·10	0·79	0·53	0·63	0·27	0·96	0·31	—	—

DISCUSSION

The results given in Table 1 show that the absorption of these four alkaline-earth metals decreases as the age of the animal increases, but not in the same way for all elements. In the animals between 14 and 18 days of age, whose diet consists entirely of maternal milk, there is almost complete absorption of calcium and strontium and about 80% of the barium and radium are absorbed; at this age there is little or no discrimination between calcium and strontium in absorption from the gastrointestinal tract. By 6–8 weeks of age the absorption of all four elements has decreased but the decrease is most marked with barium and radium, where absorption has fallen to little more than one-tenth of the amount absorbed by the 14–18-day-old animals. In animals at 60–70 weeks of age the absorption of calcium, strontium and radium has been reduced to between one-half and one-third of the amount absorbed by the 6–8-week-old animals. Barium absorption, however, has not significantly altered between these two ages. This last observation implies that, relative to calcium, barium absorption is greater in the older animal than in the animal 6 weeks old.

The percentage of radioactive material remaining in the carcass 7 hr. after administration gives no indication of the true amount of calcium that has been absorbed unless the specific activity of the calcium in the gastrointestinal tract is known. Thus the decreasing percentage of ^{45}Ca found in the carcass with increasing age could represent an increased or a decreased or an unchanged total calcium absorption according to how the dietary intake of calcium varies with increasing age. The measured daily food intake of the rats used in these experiments varies only from 15 g. at 6–8 weeks of age to 17 g. at 60–70 weeks of age; thus the total daily calcium intake remains fairly constant at about 130 mg., hence the specific activity of the calcium in the gut also remains fairly constant.

The decreasing uptake of ^{45}Ca with age represents therefore a decreasing absorption of calcium. If the absorption of strontium, barium and radium occurs by a similar mechanism to that of calcium, the absorption of these elements might also be expected to be affected by the total calcium intake. Thus the increased absorption of ^{140}Ba and ^{226}Ra, and, in the older animals, of ^{85}Sr, by starved animals may result in part from the decreased concentration of calcium in the gut. No figures are available for the calcium intake of the young rat during suckling, but the results for the starved animals at 6–8 weeks of age suggest that the high uptake of all four isotopes at 14 days of age cannot be explained solely on the assumption that the specific activity of the calcium in the gut is greater than that in the older animals.

It is not possible to determine the extent to which the high absorption of all four elements in the very young animal results from some effect of the diet of maternal milk, from the high physiological requirement for calcium at this time or from the fact that the mechanisms which may control the absorption of metals from the gastrointestinal tract have not yet developed.

Hansard & Crowder (1957) have also reported almost complete absorption of calcium by rats between 1·5 and 4 weeks of age. Lengemann, Comar & Wasserman (1957) have shown that in rats, cattle and man the absorption of calcium and strontium is enhanced when the animals are fed on a predominantly milk diet. These workers found that the effect disappeared when the animal was transferred to a non-milk diet; in the 22-day-old rats, which are beginning to supplement the maternal milk with the normal diet, the absorption of both strontium and barium is less than that in the 14-day-old animals.

Absorption of other metals in high amounts by 14-day-old rats has also been observed; Ballou (1960) has shown that more than 80% of a test dose of ^{65}Zn was absorbed at this age and D. M. Taylor (unpublished work) has shown that 14-day-old August rats absorb more than 80% of a tracer dose of ^{59}Fe or ^{58}Co but only 8% of a similar dose of ^{64}Cu. Of these last-named elements only cobalt absorption is known to be enhanced by milk (Taylor, 1962).

The fact that the presence of food, or substances derived from the food, in the gastrointestinal tract markedly affects the absorption of these elements is shown by the results given in Table 1 for the absorption of ^{45}Ca, ^{85}Sr, ^{140}Ba and ^{226}Ra by rats that had been starved for 18 hr. before the administration of the isotopes. Statistical analysis of the data given in Table 1 shows that the absorption of calcium is probably reduced in starved animals at both 6–8 and 60–70 weeks of age ($P < 0{\cdot}05$ in each case). Conversely there are significant increases in the absorption of barium ($P < 0{\cdot}01$) and radium ($P < 0{\cdot}05$) in the starved animal at 6–8 weeks of age. In the older animals the absorption of strontium ($P < 0{\cdot}01$), barium ($P < 0{\cdot}05$) and radium ($P < 0{\cdot}02$) is increased. In the starved animal at 6–8 weeks the percentage of the test doses of strontium, barium and radium that are absorbed are similar. This is also true for strontium and barium in the older animals.

It is well known that substances such as phytic acid and oxalic acid that may be present in food can markedly reduce the amount of calcium and other metals absorbed from the gastrointestinal tract by the formation of insoluble calcium compounds. Similarly the presence of SO_4^{2-} ions in the diet might be expected to reduce the absorption of barium and radium by the formation of their insoluble sulphates. The normal diet of these animals contains about 1·5 mg. of SO_4^{2-} ion/g. of food and the daily intake has been calculated to be about 25 mg. of SO_4^{2-} ion. However, in a series of experiments in which animals of the two older age groups were given ^{45}Ca, ^{85}Sr, ^{140}Ba or ^{226}Ra orally, followed immediately by oral administration of 300 μg. of SO_4^{2-} ion, the percentage absorption in both fed and starved animals did not differ significantly from the results given in Table 1.

The data recorded in the last section of Table 1 confirm that for fed animals, when the isotopes are administered in cow's milk instead of in 0·01 N-hydrochloric acid, there is a significantly increased absorption of calcium ($P < 0{\cdot}05$) and strontium ($P < 0{\cdot}001$) in 6–8-week-old animals, the increase in strontium absorption being greater than that of calcium. This effect was not observed in animals 60–70 weeks old, nor was there any effect on the absorption of barium and radium by either age group. Comar *et al.* (1956) have shown that the enhancement of calcium and strontium absorption results from the presence of substances such as lysine, arginine and lactose in the milk. However, in the experiments described by Lengemann *et al.* (1957) the animals were transferred to milk diets some time before the administration of the isotopes whereas our data have been obtained by administration of the isotopes in a small amount of cow's milk to animals maintained on a standard non-milk diet.

By comparison of the absorption of these four elements and their response to the various factors studied it becomes apparent that strontium corresponds fairly closely to calcium in its response to the different conditions, whereas barium and radium show wide differences from calcium but correspond closely to each other. For example, the decrease in the absorption of barium and radium between 14 days and 6 weeks is very much greater than that of calcium and strontium; similarly deprivation of food produces a much greater relative increase in barium and radium absorption than occurs with calcium and strontium, calcium absorption being in fact slightly depressed by starvation.

The absorption of calcium, except by the very young rats, was always two to three times as great as that of strontium. Schachter & Rosen (1959) and Wasserman (1960) have shown by experiments *in vitro* that ^{45}Ca is transported from the mucosal to serosal surfaces of duodenal sacs against a concentration gradient. This process, which is dependent on oxidative phosphorylation, is relatively specific for calcium. However, this 'active transport' is apparently influenced by the calcium content of the diet and it would appear likely that in animals maintained on a diet with fairly high calcium, such as the rats used in the present studies, this process might be only partly responsible for the preferential utilization of calcium over strontium.

SUMMARY

1. The absorption of calcium, strontium, barium and radium from the gastrointestinal tract of the rat has been studied by means of double radioactive-tracer techniques.

2. At 14–18 days of age there is almost complete absorption of calcium and strontium and about 80 % of barium and radium is absorbed. For animals 6–8-weeks old absorption of calcium has decreased to about 60 %, strontium to 25 %, barium to 7 % and radium to 11 %. In animals at 60–70 weeks of age the absorption of all the elements except barium is decreased to between one-third and one-half of these levels.

3. Deprivation of food before administration markedly increases the absorption of barium and radium and slightly reduces calcium absorption.

4. Administration of the elements in cow's milk significantly enhances the absorption of calcium and, more particularly, strontium in 6–8-week-old rats. The absorption of barium and radium is not affected and the effect is not observed in old animals.

5. The results for the absorption of barium and radium show a closer similarity to each other than to the results for strontium and calcium.

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Protein Synthesis in Mitochondria

2*. RATE OF INCORPORATION *IN VITRO* OF RADIOACTIVE AMINO ACIDS INTO SOLUBLE PROTEINS IN THE MITOCHONDRIAL FRACTION, INCLUDING CATALASE, MALIC DEHYDROGENASE AND CYTOCHROME *c*

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It has been demonstrated previously that, under suitable conditions, isolated rat-liver mitochondria can incorporate radioactive amino acids into their proteins (Reis, Coote & Work, 1959*a*). In a more detailed study of the system (Roodyn, Reis & Work, 1961*a*) it was shown that the incorporation was not due to bacterial or microsomal contamination, was fully energy-dependent, and was very sensitive to incubation in the absence of an oxidizable substrate. The incorporation was not due to adsorption on the mitochondrial protein, or to the

* Part 1: Roodyn, Reis & Work (1961*a*).

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