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THE EFFECT OF PHOSPHATES ON STRONTIUM AND CALCIUM METABOLISM IN CONTROL, PARATHYROIDECTOMIZED AND PARATHORMONE-TREATED RATS

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The influence of the phosphate content of the diet on strontium and calcium metabolism was studied in control, parathyroidectomized and parathormone treated rats after oral and intraperitoneal application of radioactive isotopes.

It was shown that skeletal retention of radioactive calcium ($^{45,47}Ca$) and strontium (^{85}Sr) and the $^{85}Sr/^{45,47}Ca$ ratio depend upon the phosphate content in the diet as well as upon the route of radioactive isotope application. The effect of phosphates on calcium and strontium metabolism, however, does not depend upon the function of the parathyroid gland.

In parathyroidectomized, parathormone-treated and control rats both the urinary elimination and gastrointestinal absorption of radiocalcium and radiostrontium are influenced by changes in dietary phosphates in the same way.

An increase in the phosphate content in the diet has been shown to cause decreased absorption of strontium and calcium from the gastrointestinal tract and a decreased urinary elimination of calcium and strontium (1, 2).

The mechanism of this action of phosphates on strontium and calcium metabolism is not yet certain. It is known that high phosphate diets or phosphate infusions cause a rise in the concentration of inorganic phosphates in the serum (3, 4, 2) and a fall in plasma total and ultrafiltrable calcium (3, 5). This is followed by a simultaneous fall in urinary calcium elimination (2, 3, 5, 6).

The mechanism of the reciprocal depression of serum calcium by phosphates is uncertain. The results published by *Herbert et al.* (5) support the hypothesis that phosphates lower the serum calcium concentration

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as a result of $CaHPO_4$ precipitation. *Smith* and *Nordin* (3) assume that high phosphate diets produce a state of secondary hyperparathyroidism by depression of the calcium concentration in the extracellular fluid.

The purpose of the present experiments was to investigate whether the effect of dietary phosphorus level on the metabolism of calcium and strontium, observed in our previous experiments (1, 2) is dependent on the function of the parathyroid gland.

METHODS

Female albino rats, 8–9 weeks old, and weighing 130–160 g were used. The metabolism of calcium and strontium was followed by oral or intraperitoneal application of radioactive isotopes of these cations. The experiment lasted 16 days. The rats were divided into 3 groups:

Group of parathyroidectomized animals (the parathyroidectomy was perfomed under ether narcosis by removing the upper part of both thyroid gland lobes – approximately 30% of each lobe). Radioisotopes were applied eight days after parathyroidectomy; group of parathormone-treated animals. (Each rat received altogether 525 USP parathyroid units in 15 i. m. injections of parathormone (P-20) »Lilly« containing 100 USP parathyroid or 20 Collip units in 1 ml solution); a control group.

During the experiment all animals were fed diets which differed from the standard rat diet only in the phosphorus content. With regard to the dietary phosphorus content each group was further divided into two subgroups receiving 0.5% and 1.2% phosphorus respectively (the calcium content of both diets being 1.2%).

All animals were put on the experimental diets for 16 days. To some animals a dose of 0.01 μCi ^{45}Ca and 0.2 μCi ^{85}Sr was administered intraperitoneally in 1 ml of solution 8 days after the beginning of the experiment. On the 16th day after the beginning of the experiment all animals were sacrificed. (The radioisotopes were supplied by the Radiochemical Centre, Amersham, England).

Skeletal retention of radioisotopes was estimated in the femora of the experimental animals. Femora were dissected from the soft tissues, thermally ashed at 800° C, dissolved in concentrated hydrochloric acid and made up to volume.

^{85}Sr and ^{47}Ca were assayed directly in acid digests on a well type scintillation counter in conjunction with a single channel analyzer.

^{45}Ca was precipitated as oxalate from the acid digests and the dried precipitate was assayed on an end window Geiger counter (7).

Serum calcium was determined by the method described by *Clark* and *Collip* (8). The calcium content of the diet was determined by the standard oxalate-permanganate procedure (7).

Phosphorus content of the diet was estimated by the method of *Lucena-Conde* and *Prat* (9).

The statistical treatment of the results was performed by the method of analysis of variance (10).

RESULTS

1. *The effect of dietary levels of phosphates on the serum calcium in control, parathyroidectomized and parathormone-treated rats*

In Fig. 1 the serum calcium content is shown for control, parathyroidectomized and parathormone-treated animals as a function of the phosphorus level in the diet. Each point represents the arithmetic mean of 12–18 animals and the standard error of the mean.

The highest serum calcium content is found in the parathormone treated group of animals (curve A).

In all animals receiving diet with the higher phosphorus level (curves A, B, C) the serum calcium is decreased.

In parathyroidectomized animals the serum calcium is most dependent upon phosphorus content of the diet.

It seems that the parathyroidectomized animals on a high phosphorus diet had more difficulties in keeping the serum calcium constant than the parathyroidectomized animals on a low phosphorus diet. This is in agreement with observations on patients with hypoparathyroidism who do not tolerate a high phosphorus diet. (3).

2. *The effect of the phosphorus content in the diet on the skeletal retention of orally administered radioisotopes in control, parathyroidectomized and parathormone-treated rats*

The results of ^{85}Sr and ^{47}Ca retention (expressed as the percentage of the dose administered) in rats' femora after oral administration of radioisotopes are presented in Table 1. The retention of both strontium and calcium was lower in animals fed a high phosphorus diet. The effect was most evident in the parathyroidectomized animals.

No significant difference between the parathyroidectomized or parathormone-treated and the control group of animals was observed.

The effect of phosphates proved to be much stronger on radiostrontium than on radiocalcium retention. Increased discrimination against strontium as indicated by a decreased $^{85}Sr/^{47}Ca$ ratio in the femur was recorded in all the three groups of animals when fed higher phosphate diets. The results presented in Table 2 indicate that the discrimination

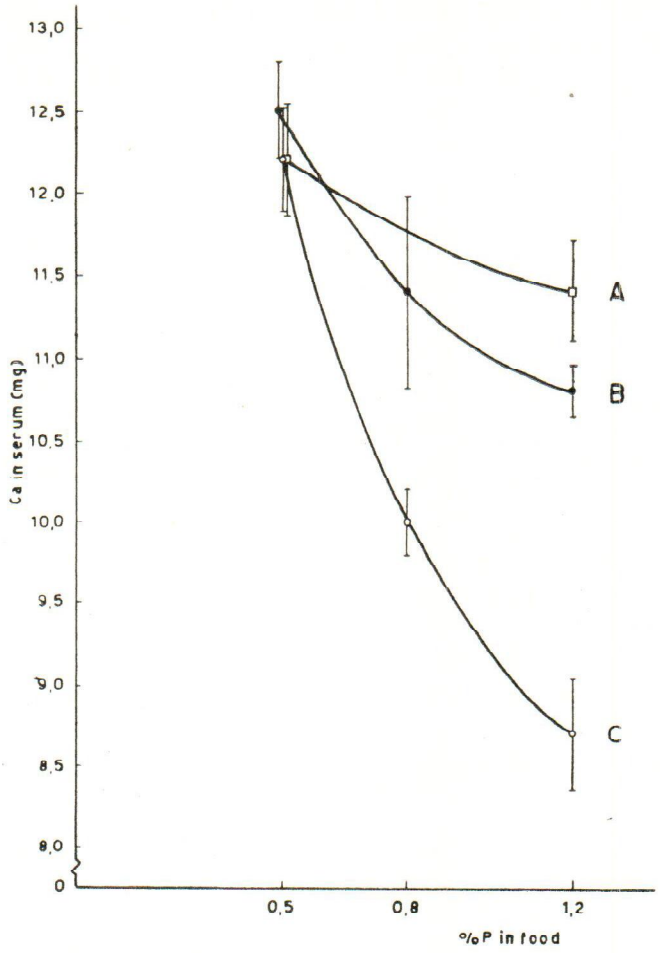

Fig. 1. *Serum calcium content shown as a function of phosphorus in the diet*
curve A – parathormone-treated groups
curve B – control group
curve C – parathyroidectomized group

against strontium depends upon the phosphate content in the diet ($P<0.001$) and not upon the function of the parathyroid gland ($P<0.05$).

Table 1

Retention of radioactive calcium and strontium in femora of control, parathyroidectomized and parathormone-treated rats receiving different amounts of phosphates in the diet (oral application of radioactive isotopes)

Group	No. of rats	Diet 0.5% P		No. of rats	Diet 1.2% P	
		Femora % dose			Femora % dose	
		^{85}Sr	^{47}Ca		^{85}Sr	^{47}Ca
E	9	0.38 ± 0.01	1.10 ± 0.03	8	0.19 ± 0.02	0.82 ± 0.05
K	7	0.37 ± 0.03	1.10 ± 0.08	7	0.25 ± 0.03	1.01 ± 0.04
H	6	0.55 ± 0.10	1.58 ± 0.07	6	0.33 ±0.05	1.34 ± 0.08

E = parathyroidectomized animals
K = control animals
H = animals who received daily i. m. injections of 35 I. U. parathormone (P-20) »Lilly« for 15 days
Rats on diet 16 days. Radioactive isotopes applied in drinking water from the 8th to 14th day. All animals sacrificed on the 16th day.

Table 2

$^{85}Sr/^{47}Ca$ ratio in the femora of control, parathyroidectomized and parathormone-treated rats fed diets with different phosphate content (oral application of radioactive isotopes)

Group	No. of rats	Diet	
		0.5% P	1.2% P
E	9	0.346 ± 0.012	0.234 ± 0.012
K	7	0.336 ± 0.020	0.250 ± 0.038
H	6	0.346 ± 0.014	0.242 ± 0.033

E = parathyroidectomized animals
K = control animals
H = animals who received daily i. m. injections of 35 I. U. parathormone (P-20) »Lilly« for 15 days
Rats on diet 16 days. Radioactive isotopes applied in drinking water from the 8th to 14th day. All animals sacrificed on the 16th day.

3. *The effect of the phosphate content in the diet on the retention of intraperitoneally administered radioisotopes in control, parathyroidectomized and parathormone-treated rats*

The results of ^{85}Sr and ^{47}Ca retention in the rats' femora after intraperitoneal application of the radioactive isotopes are presented in Table 3. Animals on a higher phosphate diet showed increased skeletal retention of intraperitoneally administered radioisotopes. It is again obvious that the skeletal radiostrontium retention is much more dependent on the phosphorus content in the diet than the retention of radiocalcium. This is in agreement with the results of *Kostial et al.* (1, 2).

No effect of the function of the parathyroid gland on the skeletal retention of either radiostrontium or radiocalcium could be observed.

Table 3

Retention of radioactive calcium and strontium in femora of control, parathyroidectomized and parathormone-treated rats fed diet with a different phosphate content (intraperitoneal application of radioisotopes)

Group	No. of rats	Diet with 0.5% P		No. of rats	Diet with 1.2% P	
		Femora % dose			Femora % dose	
		^{85}Sr	^{45}Ca		^{85}Sr	^{45}Ca
E	10	1.71 ± 0.06	2.3 ± 0.15	5	2.69 ± 0.19	2.8 ± 0.10
K	6	1.92 ± 0.07	2.9 ± 0.11	6	3.00 ± 0.06	3.5 ± 0.13
H	6	2.20 ± 0.07	3.1 ± 0.07	6	2.71 ± 0.09	3.3 ± 0.10

E = parathyroidectomized animals

K = control animals

H = animals who received daily i. m. injections of 35 I. U. parathormone (P-20) »Lilly« for 15 days

Rats on diets 16 days. Radioactive isotopes applied as a single injection on the 8th day. All animals sacrificed on the 16th day.

There was no significant difference in this respect between the control parathyroidectomized and parathormone treated group of animals.

The $^{85}Sr/^{47}Ca$ ratio is shown on Table 4. It is higher for all groups of animals which were on the higher phosphate diet. The statistical treatment of these results also indicates that the ^{85}Sr to ^{45}Ca ratio in the bone depends on the phosphate content in the diet ($P < 0.001$) and not upon the function of the parathyroid gland (< 0.01).

Table 4

$^{85}Sr/^{45}Ca$ ratio in the femora of control, parathyroidectomized and parathormone treated rats fed diet with different phosphate content (intraperitoneal application of radioactive isotopes)

Group	No. of rats	Diet	
		0.5% P	1.2% P
E	10	0.742 ± 0.025	0.960 ± 0.033
K	6	0.658 ± 0.014	0.847 ± 0.025
H	6	0.707 ± 0.019	0.827 ± 0.024

E = parathyroidectomized animals
K = control animals
H = animals who received daily i. m. injections of 35 I. U. parathormone (P-20) »Lilly« for 15 days

Rats on diet 16 days. Radioactive isotopes applied as a single injection on the 8th day. All animals sacrificed on the 16th day.

DISCUSSION

Under the conditions of our present experiments a decrease or increase in the amount of parathyroid hormone did not produce a statistically significant effect on either absorption or elimination of calcium and strontium from the body. *Neuman* and *Neuman* (11) previously concluded that parathormone has a negligible effect on absorption of calcium from the intestine. *Wasserman* and *Comar* (12) who investigated the effect of parathyroidectomy on the intestinal absorption of calcium, strontium and phosphate *in vivo* and *in vitro* also found that parathyroidectomy did not alter the movement of ^{45}Ca and ^{85}Sr across the intestine. It is, however, known that the effect of parathormone is extremely sensitive to particular conditions under which the experiments are carried out.

Phosphates caused the same effect on calcium and strontium metabolism as observed in our previous experiments (1, 2). Our results seem to justify the assumption made in our previous papers that phosphate ions act at two sites in the body; in the gastrointestinal tract – reducing the radioisotope absorption, and in the kidney – diminishing the strontium and calcium excretion in urine. The results of our experiments do not show any difference in the phosphate action on calcium and strontium metabolism in parathyroidectomized, parathormone-treated or control animals. This finding is in agreement with data reported by

Herbert et al. (5) who found that phosphate infusion causes the same changes in calcium metabolism in all subjects – regardless of the presence or absence of those endocrine organs normally involved in the regulation of calcium homeostasis.

We can therefore conclude that the mechanism by which phosphate ions act on strontium and calcium absorption and elimination from the body does not depend upon the function of the parathyroid gland. It is much more likely that the complexing of calcium and strontium by phosphates and other physico-chemical factors regulating the mineral equilibrium in the body are responsible for this action of phosphates.

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Sadržaj

UTJECAJ PARATHORMONA I FOSFATA NA METABOLIZAM KALCIJA I STRONCIJA U ŠTAKORA

Rad je izvršen sa svrhom da ustanovimo do koje mjere utjecaj fosfata na metabolizam kalcija i stroncija ovisi o funkciji paratireoidne žlijezde.

Metabolizam kalcija i stroncija pratili smo primjenom njihovih radioaktivnih izotopa (stroncij-85 i kalcij-45, 47) intraperitonealnim odnosno oralnim putem. U pokusu su bile tri grupe životinja: kontrolne, paratireoidektomirane i životinje koje su primale intramuskularno dodatak paratireoidnih hormona. Svaka grupa životinja bila je razdijeljena u dvije podgrupe, od kojih je jedna primala hranu s nižim (0.5%), a druga s višim sadržajem fosfata (1.2% P).

Iz rezultata skeletne retencije radioaktivnih izotopa nakon oralne i intraperitonealne aplikacije vidimo da je djelovanje fosfata bilo nezavisno od funkcije paratireoidne žlijezde. U životinja na dijeti s višim sadržajem fosfata bila je snižena apsorpcija radioaktivnog stroncija i kalcija iz probavnog trakta i snižena eliminacija tih radioaktivnih izotopa u urinu.

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