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TRANSDUODENAL CALCIUM-45 TRANSFER IN THE PRESENCE OF LEAD NITRATE

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Transduodenal transfer of calcium-45 was studied in eight-week-old female albino rats by the *in vitro method* of »everted gut sac« in the presence of lead nitrate in the incubation media. Lead concentration ranged from 0 (control) to 1000 μmol/L. Although it showed a tendency to decrease, calcium transfer was not significantly influenced by 20 and 40 μmol Pb/L, whereas it was significantly inhibited by concentrations exceeding 100 μmol Pb/L. Inhibition was inversely proportional to lead concentration: from 60 per cent of the control at 100 μmol Pb/L to 10 per cent at 1000 μmol Pb/L.

The importance of calcium is well recognized in physiology while lead is assuming great importance as a toxic agent not only for occupationally exposed people but also for the general population (1).

There are plenty of data about calcium-lead interrelationship, mostly concerning the influence of calcium on lead toxicity (2—4). Our earlier studies show that lead administered orally significantly decreased the transduodenal calcium-45 transfer when it was measured by the *in vitro* method of »everted gut sac« (5). On the other hand the same lead doses either did not alter, or even increased radiocalcium absorption from the whole intestinal tract in *in vivo* experiments (6,7). In both cases lead was administered orally whereas calcium-45 transport was determined in the first case *in vitro,* and in the second case as a measure of the cumulative calcium transfer *in vivo.*

The aim of the present experiments was thus to study calcium-45 transfer through the rat's duodenal wall by bringing lead in direct contact with the mucosa of a duodenal segment.

MATERIALS AND METHODS

Seventy eight-week-old female albino rats, 150—170 g body weight were used in the experiments. They were fed a standard laboratory

diet (1.1% Ca, 0.65% P) and received drinking water ad libitum. The animals were deprived of food eighteen hours before they were killed by decapitation and exsanguination.

The duodenum was dissected and the everted duodenal sac prepared according to the method of *Wilson* and *Wiseman* (8). A 3.5-cm-long duodenal segment was washed, everted and tied at one end. It was then injected with 0.6 ml of the basic solution and tied at the other end. The composition of the medium on either side of the intestinal wall was as follows: 135 mM NaCl, 11 mM KCl, 0.05 mM $CaCl_2$ and 100

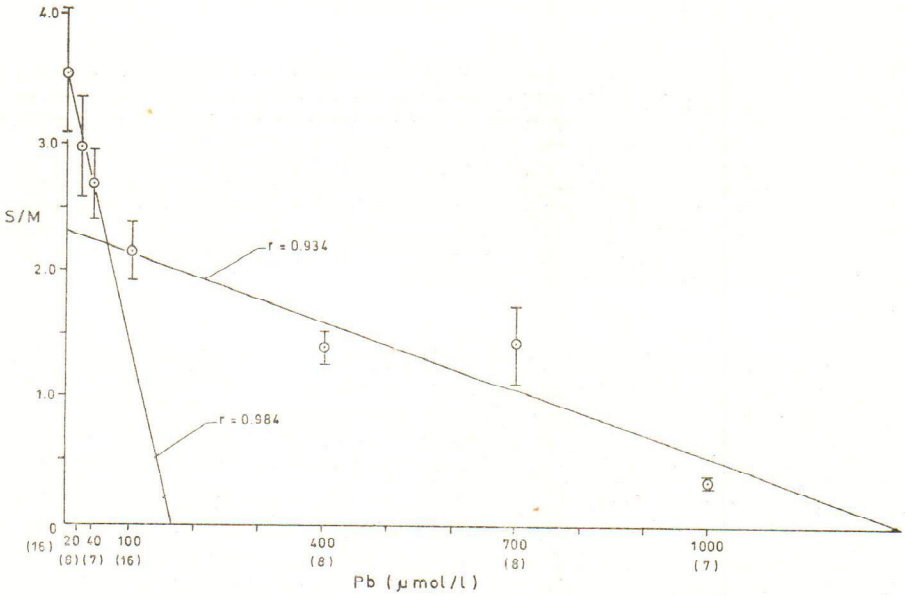

Fig. 1. *Transduodenal transport of calcium-45 in the presence of lead nitrate. The results are the means ($\bar{x} \pm SE$) of the S/M ratio, i. e. the ^{45}Ca-activity ratio of the serosal to the mucosal solution at the end of the incubation period. The number of animals in each group is indicated in the parenthesis below the abscissa.*

ml sodium phosphate buffer pH 7.4 (prepared by titration of 0.2 M Na_2HPO_4). The samples were divided into seven groups according to the amount of lead nitrate added to the outside incubating solution: 0 (control) -20-40-100-400-700 and 1 000 μmol Pb/L ($2x10^{-5}$ — 10^{-3} M). Calcium-45 was added as chloride to the outside medium in an essen-

tially carrier free from supplied by the Radiochemical Centre, Amersham, England. The activity was adjusted to about 220 KBq (6 μCi) of ^{45}Ca per 100 ml of the buffer solution. The tied segments were then incubated in 2.5 ml of the medium equilibrated with oxygen for 45 minutes. After incubation at 37 °C in 25 ml flasks with a metabolic shaking incubator the sacs were drained.

The activity of calcium-45 was determined in plated dried samples of 100 λ mucosal and serosal solutions in an end-window Geiger-Müller counter.

Student's t test was used to calculate the statistical significance of the difference between the groups.

RESULTS AND DISCUSSION

There are data suggesting that lead affects the active calcium transport through the intestinal wall (9, 10). However, our earlier results (5, 11) indicate that lead does not affect the active component of transduodenal calcium transport under our experimental conditions. In the present experiments we therefore determined only the total calcium transfer.

The results expressed as the S/M ratio ($\pm$ SE), i. e. the ^{45}Ca-activity ratio of the serosal to the mucosal solution at the end of the incubation period are shown in Fig. 1. in relation to lead concentrations in the incubating medium. The first three points (no lead, or a low lead concentration) do not differ significantly among themselves. However, the whole set of the results in Fig. 1 is suggestive of two straight-line dependences as limiting behaviours. Any higher order curve matching of these data would not be more significant nor would it alter the following conclusions.

The latter observation may be understood in terms of two binding sites for lead in the calcium transporting channels of the duodenal wall, one with a much higher affinity for lead than the other. The first sites would be saturated with lead with solutions of up to about 100 μmol Pb/L whereas the other do not show saturation at even ten times larger lead concentrations.

As calcium transport was involved in these measurements, one is led to conclude that the same binding sites could be occupied by both ions, so that lead blocks competitively the calcium transfer, which is, hence, diminished (5). This is also in agreement with the findings that lead binds to the intestinal membrane in greater amounts and much more strongly than calcium (12, 13).

Comparison of our present and earlier (5) findings shows a great deal of similarity despite a difference in animals' age (5 vs. 8 weeks here) and in the way the duodenal mucosa was in contact with lead. Another difference between these two sets of experiments is the presence of the lead anion in the medium, i. e. lead acetate in the earlier

vs. lead nitrate in the present experiment. As the results of both are quite similar, one should conclude that it is indeed the lead (cation) which is important.

In the earlier experiment (5) calcium transfer was constant (at S/M = 2.2) up to about 100 μmol Pb/L, to attain abruptly the lower plateau (at S/M = 1.7) for lead concentrations greater than about 900 μmol Pb/1. Unfortunately, no data exist for the lead concentration range in between (100—900 μmol Pb/L), so that we may conclude that the calcium transduodenal transport is similarly affected by a higher lead concentration (1000 μmol Pb/L) irrespective of whether the duodenal wall was brought into direct contact with lead ions, as is the case here, or whether lead was administered orally (5). In relative terms, this effect was much clearer in the present experiment, where the S/M ratio diminished to about one tenth, whereas in the former (5) it diminished to about three quarters. This difference may be understood as follows. In the »pure« *in vitro* experiments (here) the small part of the duodenum is practically equilibrated with the incubation medium containing lead, so that many more available binding sites could be occupied by lead than by the orally administered lead solution passing through the whole of the duodenum — at an unknown rate.

The present study definitely confirms that the *in vitro transfer* of calcium is indeed diminished by the presence of lead ions, and this should be distinguished from the results of calcium *absorption in vivo* as discussed in ref. 6 and 7. High lead doses did not inhibit calcium absorption in the experiments of *Mykkänen* and *Wasserman* (13) either. These authors claim that there is no direct interaction between these cations at the limiting step in the absorptive process. Therefore further studies are needed to understand definitively the relation between calcium and lead absorption in the intestinal tract.

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Sažetak

TRANSDUODENALNI TRANSPORT KALCIJA-45 U PRISUTNOSTI OLOVNOG NITRATA

Ispitivan je utjecaj olovnog nitrata na transport kalcija-45 kroz duodenalnu stijenku osmotjednih ženki bijelog štakora *in vitro* metodom »izvrnute crijevne vreće«. Olovni nitrat je dodan otopini za inkubiranje uzoraka u koncentracijama od 0 (kontrola) do 1 000 μmola Pb/L. Već 20 i 40 μmola Pb/L snizuje transport radiokalcija, ali ne značajno. Kod doza iznad 100 μmola/L inhibitorni učinak olova je značajan i obratno proporcionalan njegovoj koncentraciji. Transport radiokalcija iznosi 60% od kontrolne vrijednosti kod 100, a svega 10% od kontrole kod 1 000 μmola u litri otopine za inkubiranje.