Effect of Phosphate on Serum Strontium (35499)

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Inorganic phosphate is of value in the therapy of hypercalcemia, specially in hypercalcemic crisis (1). Rationale of this therapy is based on the fact that phosphate reduces serum calcium level (1–3), although the exact mechanism of action was not definitely known. This had stimulated a variety of studies on possible mechanism of action. Studies have been done with another alkaline earth metal, namely, strontium, whose biologic distribution and metabolic pathways are similar to those of calcium (4). Therefore, the effect of phosphate on strontium should reflect its effect on calcium. Decreased bone resorption (2, 5) and increased bone formation (6) have been previously postulated as theories for the mechanism of action of phosphate on serum calcium. Extraskeletal deposition of calcium following the administration of phosphate was suggested by Hebert *et al.* (3) as another theoretical mechanism, but the exact anatomical site was obscure. The present study was undertaken to observe the altered tissue distribution of strontium and hence calcium after administration of phosphate. The study was performed on rats using radioactive strontium (^{85}Sr) and subsequent radioactivity counting of various tissues and organs. Whole body scans with a Nuclear-Chicago gamma camera were also made to correlate with the findings of tissue analysis.

Methods. Sprague-Dawley rats (340–360 g) were used in our experiments. Strontium-85 chloride in aqueous solution was obtained from Abbott Laboratories (48.8 μCi/ml; 3.169 μg/ml). Phosphate solution was prepared by dissolving monobasic and dibasic salts of sodium in distilled water to give a final dilution of 43.8 mg/ml of NaH_2PO_4 and 199.6 mg/ml of Na_2HPO_4 (pH 7.4). One-half ml of phosphate solution[1] was suitable for a single intravenous injection per rat.

Each rat received 20 μCi of strontium-85 in aqueous solution intravenously. Ten min later, intravenous injection of phosphate was started and completed in 1-min time. This was expressed in all of our data as zero time. The control group received the same radioactive strontium but no phosphate. Instead they received 0.5 ml of normal saline thus keeping the volume of injection constant. Ten min postinjection was considered zero time for this group. Following 1 ml of blood collection from the aorta, five animals at a time from each group were sacrificed at each time interval. Liver, spleen, thigh muscle, bone (one femur), and kidney were removed, washed, and weighed. Radioactivity was measured in a well-type gamma scintillation counter keeping a comparable geometry each time. Whole-body scans with a gama camera were performed on two rats from each group at time intervals of 10 min, 30 min, 1 hr, and 5 hr.

Results. Figure 1 graphically presents strontium activity in whole liver, one femur, and 1 ml of blood, respectively, in two groups of animals at different time intervals after administration. Relative radioactivity was plotted on the ordinate and time after administration was plotted on the abscissa. The curves were drawn through the mean value obtained from a group of 5 rats for each point. The standard deviation was shown by

[1] This dose of (0.75 m*M*) phosphate was chosen by adjustment, on a body weight basis, from that used by Eisenberg (10). We noted that if we gave larger amounts to our animals it was commonly lethal.

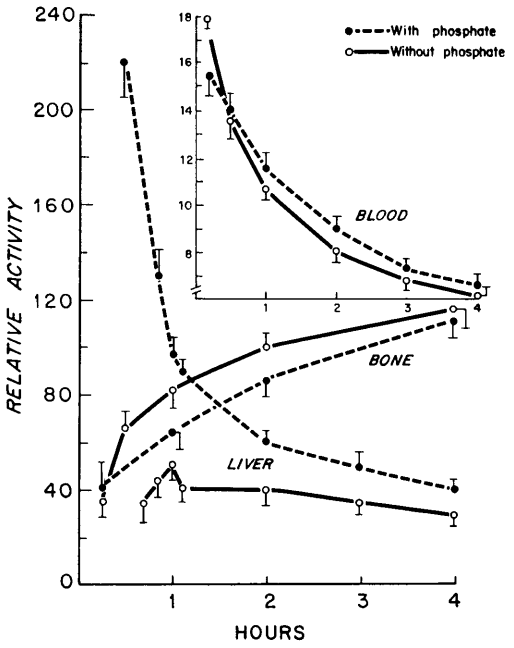

FIG. 1. Relative ^{85}Sr activity in liver (whole organ), bone (one femur), and blood (1 ml) in the control group (○—); and in the phosphate group (●- -). Five rats were used for each point. (inset) Abscissa is also in hours. Activity in the liver in phosphate group at 30 min corresponds to 33% of the injected dose.

line drawn only on one side of the curves from each point. Activity in the liver was expressed for the whole organ, whereas activity in bone is expressed for one femur and that in blood for 1 ml of blood. In the present context, observed activity in spleen, muscle, and kidney were not very significant above those due to blood activity in those organs; hence, they are not presented here.

Figure 1 shows that, in the phosphate group, the liver showed maximum activity in 30 min following which the activity decreased gradually returning to almost blood background in 4-5 hr. Decrease in liver activity was accompanied by relative rise in blood activity indicating release of ^{85}Sr from the reticulo-endothelial cells. However, this could not stay longer in blood, because it was utilized by the bone (as shown in Fig. 1). In bone, the strontium level was at least 20% lower when phosphate was given, than that of the control group. With time, the bone strontium content of the former was approximating the latter.

Figure 2 shows the results of sequential whole-body scan in one rat from each group. Maximum concentration of radioactivity in the organs, revealed by scan, had good correlation with tissue activities measured by well counter.

In control rat (Fig. 2a) bones were concentrating radiostrontium with time, with a consequent drop of blood activity as seen in the surrounding tissues. The activity in the pelvic region was mainly due to accumulation of urine in the bladder, which was shown by drawing the urine in a syringe and keeping it beside the animal while scanning (2-hr scan of Fig. 2a). Rat receiving phosphate (Fig. 2b), showed a good delineation of the liver. As time progressed liver activity was diminishing and dropped to blood background by 5 hr. At the same time bone activity was increasing and 5-hr scans were essentially same in both rats. Increased activity in the pelvic region in this rat (2b) was obviously due to the bladder also.

Discussion. Although inorganic phosphate has been recognized as a potential therapeutic agent in hypercalcemic crisis, the mechanism of action was not clear. The mechanism of action of phosphate on serum calcium or strontium was thought to be either decreased bone resorption or increased bone formation. The former theory has been suggested by Goldsmith and Ingbar (2) and Albright *et al.* (5). Evidence against this theory was obtained from the study of Eisenberg (7), who demonstrated that oral administration of phosphate could maintain normal serum calcium level for 4 months but failed to halt the progression of primary hyperparathyroid bone disease in one patient. Other evidences are from the study of Pechet *et al.* (8) and Rasmussen and Tenenhouse (9), where phosphate infusion failed to inhibit bone resorption induced by parathyroid hormone in rats.

The theory of increased bone formation has been suggested by Milhaud *et al.* (6); but no evidence supporting this conclusion was obtained. Eisenberg (10) suggested that phosphate lowers serum calcium by removing

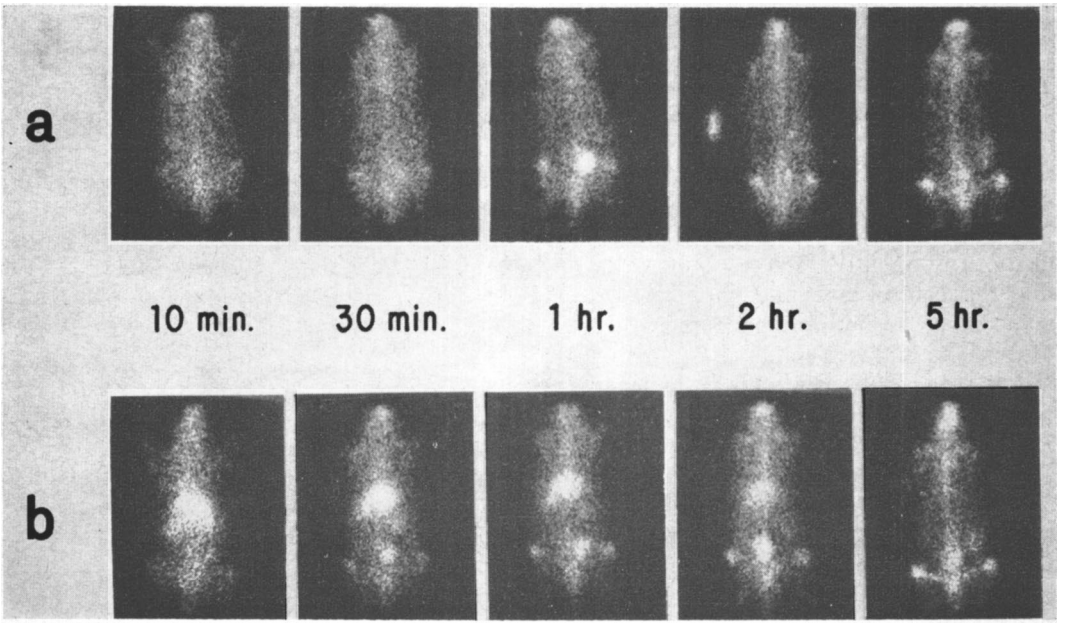

FIG. 2. Sequential whole body scans of one rat from each group (tails were eliminated from the field): (a) strontium only; (b) strontium in combination with phosphate.

it from the circulation; but the site of sequestration of calcium was not identified. A metabolic pool has been postulated; but the size of the pool and its exchange characteristics are not known. Hebert *et al.* (3) showed indirect evidence of precipitation of calcium phosphate. The metastatic soft tissue calcification observed by Goldsmith and Ingbar (2) and Carey *et al.* (11) support this conjecture. However, the prolonged use involved large amounts of phosphate in these cases. The data obtained from our investigation suggest that excess phosphate forms presumably a colloid with calcium or strontium and the resulting strontium phosphate or calcium phosphate is ingested by the reticuloendothelial cells, retained there for a short time, and then released back into the circulation.

Blood activity curves (Fig. 1 inset) were essentially the same in both groups except the initial activity in blood (15-min sample) in the phosphate group was approximately 12% less than that of control group. This was due to quicker removal of strontium phosphate colloid by the reticuloendothelial system in comparison to less quick heterionic exchange of free strontium into bones. Presumably colloid was being formed *in vivo* after high phosphate came in contact with tracer strontium. Later, the blood activity in the former group became slightly higher than the other due to release of ^{85}Sr from the reticuloendothelial system. Blood activity in the control group was decreasing with time due to ionic strontium excretion in the urine and deposition into the bone.

Removal of ^{85}Sr from blood after phosphate infusion is not as complete as that of colloidal gold or technetium–sulfur colloid. It appears that beyond the point of solubility, the insoluble portion of strontium would form a colloid with phosphate. The soluble portion would be freely circulating in blood and thus become available for heterionic exchange with calcium ions in the bone. Decreased uptake of ^{85}Sr by reticuloendothelial cells is due to less formation of its colloid.

Liver had more uptake than the spleen and the bone because of greater blood flow to that organ. This is consistent with any other colloid distribution in these organs. Initial ^{85}Sr activity in rat femur was possibly from two sources. One is normal deposition of strontium, *i.e.*, accretion (coming from ionic plasma strontium) and the other due to strontium

colloid uptake by bone reticuloendothelial (RE) cells.

Summary. The effect of phosphate on serum strontium and hence serum calcium is described. A possible mechanism of action is proposed. Organ distribution data in rats at different time intervals after iv injection of strontium and strontium with excess phosphate are presented. Studies show that, unlike the control animals, phosphate-treated animals had an initial large trapping of radiostrontium in liver, followed by its release into the circulation. Initial bone radioactivity was less in the phosphate-treated group than in the other. Within a 4-hr period, bone activity of the former gradually approached that of the latter. We propose that initial high activity in the liver was due to formation of strontium phosphate colloid and its removal by liver RE cells. This also explains the low initial activity in bone, as well as the lowering of serum strontium (or calcium), following phosphate therapy. The similarity of bone ^{85}Sr content in the two groups after 4 hr demonstrates the transitoriness of the altered body distribution of strontium and/or calcium which follows phosphate load.

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