THE INFLUENCE OF PYROPHOSPHATE ANALOGUES (DIPHOSPHONATES) ON THE PRECIPITATION AND DISSOLUTION OF CALCIUM PHOSPHATE IN VITRO AND IN VIVO

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In recent years we have shown that pyrophosphate inhibits both the precipitation (Fleisch and Neuman, 1961; Fleisch et al., 1966a) and dissolution (Fleisch et al., 1966b) of calcium phosphate in vitro. Pyrophosphate also prevents some types of experimentally induced soft tissue calcification (Schibler et al., 1968) in vivo, when it is administered subcutaneously but not when given orally. Pyrophosphate does not however inhibit bone resorption in vivo. The failure of pyrophosphate to affect the calcification of certain tissues e.g. kidney, and its failure to act when administered orally was ascribed to the fact that pyrophosphate is rapidly broken down at certain sites within the body, where pyrophosphatase levels are presumed to be high. Indeed kinetic studies with P-32-pyrophosphate showed the turnover of pyrophosphate in plasma to be very high. The failure of pyrophosphate to influence bone resorption in vivo was similarly attributed to its rapid local breakdown in bone.

Francis has shown that the polyphosphonates, which have a P-C-P bond rather than a P-O-P bond, inhibit the crystal growth of hydroxylapatite through chemisorption and that they are stable to hydrolysis; he suggested that these compounds might be of use in disease states involving aberrant calcium and phosphate metabolism (Francis).

To test this hypothesis we have now performed a series of experiments with simple compounds of the diphosphonate series. The P-C-P bond proved to be resistant to pyrophosphatase. The action of two such diphosphonates, disodium ethane-1-hydroxy-1, 1-diphosphonate (EHDP) and disodium methanediphosphonate (MDP), has been compared with that of a monophosphonate, pentane-1-phosphonate (PMP), which contains only a single C-P bond. Like pyrophosphate (Schibler et al., 1968), the diphosphonates EHDP and MDP inhibit both the precipitation and dissolution of calcium phosphate in vitro and inhibit aortic calcification induced by Vitamin D_3 in rats, when they are given subcutaneously. In contrast to pyrophosphate, the latter action is also present when the compounds are given orally and the compounds are effective against kidney calcification. A further difference from pyrophosphate is that the diphosphonates prevent bone resorption. Thus they were found to block the resorption induced by parathyroid extrace in mouse calvaria in tissue culture at concentrations as low as 0.1 ug P/ml. They also partially or completely prevent the hypercalcimia induced by parathyroid extract in living in thyroparathyroidectomized rats. The monophosphonate, PMP, is almost without activity in these various systems, suggesting that the P-C-P bond is essential for activity.

Since the diphosphonates are close structural analogues of pyrophosphate these results strengthen our earlier hypothesis that pyrophosphate could inhibit bone resorption in vivo and that its apparent failure to do so when given exogenously may be due to its local enzymic destruction.

The effect of these new compounds on the calcification of soft tissues and their "thyrocalcitonin-like" effect on bone resorption may have interesting possibilities in the treatment of diseases that involve disturbances in the deposition and resorption of calcium phosphate.

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We are grateful to Dr. M.D. Francis and The Procter and Gamble Company for providing the compounds and for their support and encouragement.

Table 1 Influence of two diphosphonates, EHDP and MDP, and a monophosphonate, PMP, on: a) the amount of calcium in the aortas of rats that received 75,000 I.U. Vitamin D_3 per kg body weight for five days (for experimental details see ref (4); the aortic calcium content of 26 normal rats not receiving Vitamin D_3 was 5.4 ± 2.6 /ugCa/mg dry weight); and b) the rise in plasma calcium induced by 50 I.U. parathyroid extract (PTE) per 100 g. body weight in thyroparathyroidectomised rats. Numbers of animals in parentheses.

	Route of administration of the phosphonate (10 mgP/kg body weight/day)	Aortic calcification		Bone resorption in thyroparathyroidectomised rats
		/ugCa/mg.dry weight ± SE	log /ugCa/g.dry weight ± SE	Rise± SE in plasma calcium (mg/100 ml)induced by PTE
Control	---	68.3± 7.6 (51)	4.64± 0.07 (51)	1.26 ± 0.27 (17)
EHDP	s.c.	2.2± 0.4 (9)*	3.33± 0.06 (9)*	0.49 ± 0.21 (10)
	p.o	2.7± 0.6 (6)*	3.38± 0.09 (6)*	----
MDP	s.c.	2.5± 1.1 (13)*	3.18± 0.11 (13)*	- 0.22 ± 0.19 (14)*
	p.o	3.8± 1.5 (17)*	3.28± 0.11 (17)*	----
PMP	s.c.	79.1±25.3 (9)	4.59± 0.22 (9)	1.72 ± 0.40 (11)
	p.o	64.0±16.5 (8)	4.66± 0.15 (8)	----

* denotes difference from control statistically significant at less than 5% level