Effect of Parathyroid Extract on Bone Phospholipid (35220)

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Cruess and Clark (1) studied the effect of hypervitaminosis D on the phospholipids of diaphyseal and metaphyseal long bone. All major phospholipid fractions were increased, and incorporation of ^{32}P into these fractions was enhanced. This study is concerned with the effect of parathyroid hormone administration on the concentration of total lipid, phospholipid, and ^{32}P incorporation into phospholipid in bone.

Materials and Methods. Male CFN rats (130–150 g, Carworth, Inc.) were maintained on a regular diet of Lab-Blox and water *ad libitum*. Parathyroidectomy was performed by the cautery procedure of Munson (2) 2–4 days prior to hormone treatment.[1] Control animals compared with parathyroid extract-treated animals were injected with a corresponding volume of solvent: 1.2% glycerol, 0.7% bovine serum albumin, and 0.2% phenol in physiological saline. Carrier-free $H_3{}^{32}PO_4$, glycerol-1,3-^{14}C(10.6 mCi/mmole), ethanolamine-1,2-^{14}C(3.7 mCi/mmole) and L-serine-^{14}C(U) (7.5 mCi/mmole) were purchased from New England Nuclear Corp. and Nuclear Chicago. Bones were prepared and incubated according to Cruess and Clark (3). Femora and tibias were removed and the marrow cavity was flushed out by washing with a stream of cold saline through a fine needle. Tissues were incubated in 4 ml of Krebs-Henseleit bicarbonate buffer containing 10 m*M* glucose. $H_3{}^{32}PO_4$, glycerol, and serine were present in appropriate incubation mixtures at a concentration of 1.0 μCi/μmole/ml. Ethanolamine was added at 0.5 μC/μmole/ml.

In experiments testing metabolic inhibitors, only a single tibia and femur were incubated in each flask; in all other experiments paired femora and tibias were used. All incubations were done at 37° for 4 hr. The gas phase was 95% oxygen–5% CO_2, except when the anaerobic effect of 95% N_2–5% CO_2 was evaluated. Bones for lipid extraction were pulverized in a mortar with Dry Ice and extracted with chloroform–methanol. Bones were extracted without decalcification since little additional lipid or lipid radioactivity could be recovered by removing calcium. The extract was filtered and then washed six times by the procedure of Folch *et al.* (4). The washed extracts were then completely evaporated in a stream of nitrogen. ^{32}P-labeled phospholipid extracts were dissolved in benzene–isoamyl alcohol (1:1) and ^{14}C-labeled lipid extracts were dissolved in chloroform. The lipid from liver slices was extracted by the method of Folch *et al.* (4) ^{14}C-labeled lipid extracts were separated into neutral lipid and phospholipid by adsorption on activated silicic acid according to the method of Thompson and DeLuca (5). Radioactivity was estimated in a Packard Tricarb liquid scintillation spectrometer. Suitable corrections for quenching and radioactive decay were applied to the data. Dry bone weight was determined by heating the fat-free bone at 110° overnight in an oven. Ash content was determined by heating in an electric furnace at 680° for 24 hr. Organic content was estimated as the difference between dry bone weight and ash. Total extracted lipid was determined gravimetrically and phospholipid was measured by the method of Marinetti (6). Rat liver slices were prepared with a Stadie-Riggs slicer; 200 mg of the slices were incubated for 1 hr at 37° in 95% O_2–5% CO_2.

Results. Table I shows the effects of para-

[1] Parathyroid extract was donated by Dr. E. L. Grinnan, Eli Lilly and Company. Purified parathyroid hormone was a gift of Dr. Paul Munson, University of North Carolina School of Medicine.

TABLE I. Effect of PTE on Bone Weight and Lipid.[a]

	Control (SEM) (9)	PTE (SEM) (10)	p
Bone ash (% dried defatted bone wt)	69.7 ± 0.1	69.5 ± 0.3	NS
Bone organic fraction (% dried defatted bone wt)	30.3 ± 0.8	30.5 ± 0.3	NS
Total extractable lipid (mg/g of dried defatted bone)	7.7 ± 1.3	7.5 ± 0.6	NS
Total extractable phospholipid (mg/g of dried defatted bone)	1.95 ± 0.11	2.59 ± 0.007	<0.001
$^{32}P_i$ Incorporation into phospholipid (cpm/10 mg of dried defatted bone—4 hr)	328.0 ±16.0	655.0 ±36.0	<0.001

[a] Number of animals is given in parentheses. Parathyroidectomized rats received 1.0 U/g of body weight sc daily for 3 days; and 0.5 U/g of body weight ip 4 hr prior to sacrifice. Control parathyroidectomized rats received comparable injections of solvent.

thyroid extract treatment on extractable lipid, extractable phospholipid, ^{32}P incorporation into phospholipid and the composition of bone. Parathyroidectomized rats treated with 1.0 U/g body weight sc daily for 3 days plus 0.5 U/g ip 4 hr prior to sacrifice showed striking differences in phospholipid metabolism when compared to untreated controls. No significant difference was found in the percentage of bone ash, organic fraction, or total extractable lipid. In contrast, the total extractable phospholipid and ^{32}P incorporation into phospholipid were significantly increased. A small number of experiments of similar type but with administration of purified hormone gave similar results.

Table II illustrates the effect of the time of administration of hormone on the enhanced incorporation of $^{32}P_i$ into phospholipid. Animals were treated with parathyroid extract, 1 U/g of body weight sc at specific intervals and also with 0.5 U/g ip, 4 hr prior to sacrifice. Hormone administration 16 hr before sacrifice consistently produced positive effects at a highly significant level. In some experiments terminated 4 hr after parathyroid extract administration, there was significant stimulation of phospholipid synthesis, but this result was not always obtained in the short interval following treatment. There is little tendency for cumulative effects with longer treatment periods. Treatment for 2 subsequent days with 1 U/g of body weight of highly purified parathyroid hormone gave entirely similar increases in phospholipid incorporation.

TABLE II. Effect of PTE on Incorporation of $^{32}P_i$ into Phospholipid.[a]

Treatment before sacrifice (hr)				Incorporation (cpm/10 mg of dried defatted bone; SEM)	p
76	52	28	16		
+	+	+		655 ± 36 (10)	<0.001
	+	+		999 ± 41 (4)	<0.001
		+		665 ± 28 (3)	<0.001
			+	440 ± 12 (4)	<0.001
Control				310 ± 14 (16)	

[a] Number of animals is given in parentheses. Parathyroidectomized rats received 1.0 U/g of body weight sc at indicated times. All hormone-treated rats also received 0.5 U/g of body weight, 4 hr prior to sacrifice.

Incorporation of other phospholipid precursors. The effects observed with inorganic phosphate as a precursor could be possibly explained by changes in pool-size or transport rates. Accordingly, other phospholipid precursors were evaluated and these results are illustrated in Table III. Ethanolamine, serine, and glycerol show significant increase in incorporation following hormone treatment.

Liver slices. The effect of PE on phospholipid metabolism could conceivably be a much more general response to the hormone, rather than a specific response of bone. Table IV shows that parathyroid extract administration to parathyroidectomized rats resulted in no change in incorporation of ^{32}P into phospholipid liver slices.

Effect of inhibitors on $^{32}P_i$ incorporation into bone. Table V shows the effect of a variety of inhibitors on incorporation of ^{32}P

TABLE III. The Effect of PTE on Incorporation of Serine-^{14}C, Ethanolamine-^{14}C, and Glycerol-^{14}C into Phospholipid.[a]

Precursors	Control (cpm; SEM)	PTE (cpm; SEM)	p
Serine-^{14}C	(4) 114 ± 13	(5) 247 ± 73	<0.001
Ethanolamine-^{14}C	(5) 211 ± 15	(5) 384 ± 18	<0.001
Glycerol-^{14}C	(5) 106 ± 35	(5) 166 ± 14	<0.001

[a] Number of animals is given in parentheses. Parathyroidectomized rats received 1.0 U/g of body weight sc daily for 2 days and 0.5 U/g of body weight ip, 4 hr prior to sacrifice. The incubation mixture contained either 0.5 m*M* DL-serine-^{14}C, 1.0 m*M* ethanolamine-^{14}C or, 0.5 m*M* glycerol-^{14}C as described in the text. The results are expressed as cpm/10 mg of dried defatted bone.

into the lipid fraction. Incorporation was almost completely inhibited by 1×10^{-3} *M* iodoacetate; 4×10^{-2} *M* KF; 1×10^{-3} *M* dinitrophenol; 1×10^{-3} dicyclohexylcarbodiimide; and 1×10^{-3} KCN. Incorporation was partially but significantly blocked by 2×10^{-3} NaN_3; 1×10^{-4} *M* Dicumarol; and 1×10^{-5} *M* rotenone. Incorporation was inhibited 84% by incubation under anaerobic conditions. When bone was incubated at 0° or when boiled bones were incubated, incorporation was 1 and 2%, respectively, of the controls. These results demonstrate that incorporated radioactivity actually represents lipid synthesis and is adequately freed from nonlipid precursor.

Discussion. Prior morphological observations associate bone phospholipid with physiological processes in calcification. The histochemical observations in this area have been summarized by Irving and Wuthier (7) and form an anatomical framework for the chemical effects observed in the current work. Thompson and DeLuca (5) demonstrated that administration of vitamin D produced increased incorporation of ^{32}P into the phospholipids of intestinal mucosa and kidney. These organs are involved in calcium and phosphate transport. This association between phospholipid and calcium was strengthened when Hosoya *et al.* (8) demonstrated a Ca^{2+}-stimulated exchange of serine into phosphatidyl serine in liver mitochondria. The association of calcium metabolism in bone with phospholipid metabolism is provided by the study of Cruess and Clark (1). These investigators showed that administration of vitamin D to rats produced a generalized increase in net bone phospholipid as well as enhanced incorporation of ^{32}P into all phospholipid fractions studied. These studies suggest a general role for phospholipid in calcium–phosphate metabolism. The present study demonstrates a net increase in bone phospholipid as well as evidence for enhanced phospholipid synthesis following administration of parathyroid hormone. The increased incorporation of serine, glycerol,

TABLE IV. The Effect of Parathyroid Extract on Incorporation of $^{32}P_i$ into Phospholipid of Liver Slices.[a]

	(cpm/10 mg of liver; SEM)	p
Control (3)	4297 ± 88	
PTE (3)	3978 ± 103	NS

[a] Number of animals is given in parentheses. Parathyroidectomized rats received 1.0 U/g of body weight sc daily for 2 days and 0.5 U/g body weight ip, 4 hr prior to sacrifice.

TABLE V. Effect of Metabolic Inhibitors on Incorporation of $^{32}P_i$ into Phospholipid of Diaphysis and Metaphysis.[a]

Inhibitor	Conc. (*M*)	Inhibition (%)
Potassium fluoride	4×10^{-2}	98
Dinitrophenol	1×10^{-3}	94
Dicyclohexylcarbodiimide	1×10^{-3}	91
Potassium cyanide	1×10^{-3}	88
Sodium azide	2×10^{-3}	71
Rotenone	1×10^{-5}	65
Dicumarol	1×10^{-4}	59
Iodoacetate	1×10^{-3}	100

[a] Rats weighing between 160 and 200 g were used. The samples were incubated for 30 min with inhibitor before addition of $^{32}P_i$. Incubation was carried out as described in the text.

ethanolamine and phosphate preclude the possibility that these phenomena represent an effect of hormone on precursor pool-size or transport. The incorporation of P_i depends on active cellular function since anaerobiosis, a variety of inhibitors, or boiling the bone effectively prevent incorporation.

The stimulation of phospholipid metabolism is not an early direct action of parathyroid hormone. Reproducible stimulation occurs more than 4 hr following administration of hormone; whereas the increase in bone adenyl cyclase activity following exposure to hormone occurs within a few minutes (9). Presumably, cyclic AMP activates a variety of enzymes concerned with ion-transport and lysosomal resorbtion. The latter enzymes studied by Vaes (10) bear no immediate relationship to phospholipid metabolism. The former processes are currently under consideration with regard to the transport model of Hokin and Hokin (11), involving a phosphatidic acid cycle. Vitamin D and parathyroid hormone cause bone resorbtion and increased phospholipid synthesis. Current studies attempt to find a central point of action for these substances.

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