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Observations on the Relationship of Parathyroid Hormone and Calcitonin to Plasma and Liver Phosphate[1] (39495)

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Parathyroid hormone and calcitonin, along with vitamin D, are considered to be calcium-regulating hormones. However, all three influence phosphate homeostasis, although by different mechanisms. Parathyroid hormone increases renal excretion of phosphate (1), vitamin D increases intestinal absorption of this ion (2), and calcitonin increases its transport out of plasma (3). None of the three is thought to specifically control the plasma phosphate concentrations, since no "feedback" system related to this ion has been identified. Other hormones also affect plasma phosphate. Two of the most important are insulin and glucagon (4). Following the administration of either of these, plasma phosphate concentrations fall. Also, the administration of glucose produces a temporary fall in plasma phosphate (5).

While it has been known for many years that plasma phosphate levels in the laboratory rat rise during the daylight hours, only recently have the daily fluctuations of this ion been quantitated (6). One of the interesting results of this study was the finding that in the normal rat, plasma calcium and phosphate fluctuated in the same direction during the daily cycle, except that the changes in phosphate were considerably greater.

After it was observed that calcitonin decreased plasma phosphate by moving this ion out of the ECF (3), attempts have been made to determine into which tissue this phosphate was moved. While some phosphate may be lost in urine following calcitonin injection, this loss is not considered to be sufficient to account for decreases in plasma phosphate (7). Recently, it has been reported that liver inorganic phosphate is reduced by parathyroid hormone and increased by calcitonin (8, 9).

This study was carried out to examine in greater detail the relationship of parathyroid hormone and calcitonin to the daily fluctuation in plasma phosphate and to investigate the possible involvement of the liver in these events.

Materials and methods. Holtzman rats weighing between 150–260 g were used for these experiments. The phosphate-deficient diet was obtained from Nutritional Biochemical Co. It contained less than 0.15% P. Most animals were maintained on standard Purina Laboratory Chow under 12-hr light and 12-hr dark conditions in which food was available during the dark period.

All surgery was performed under light (ether) anesthesia. Parathyroid glands, when autotransplanted, were individually placed laterally in the hyoid muscle. After surgery, rats were allowed a minimum of 3 days for recovery, extended to 2 weeks following gland transplantation. The day before rats were used experimentally, a blood sample was obtained and plasma calcium was analyzed to determine adequacy of surgical procedures. Thyroidectomized (TX) rats were maintained on T_4 (50 μg/kg three times weekly).

The calcitonin used was purified salmon calcitonin (SCT) furnished by Armour Pharmaceutical Co. and assayed at 400 MRC U/mg. Parathyroid hormone was a TCA powder obtained from Inolex Pharmaceutical Division and assayed at 179 U/mg.

Sacrifice was by decapitation to permit rapid blood drainage. The central (ventral) lobe of the liver was rapidly removed, frozen in liquid nitrogen, and transferred in dry ice to a freezer maintained at −40°C.

For phosphate analysis, a procedure modified from Wahler and Wollenberger (10) was utilized. The lobe of liver was weighed frozen, homogenized in 0.5 *N* perchloric acid (PCA), centrifuged at 0°C, and the supernatant was kept on ice for the remain-

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der of the procedure. An aliquot was immediately shaken with 0.015 *M* sodium molybdate in isopropyl acetate. After separation, an excess of stannus chloride was added to the upper phase which was analyzed for inorganic phosphate. A separate aliquot of the PCA supernatant was hydrolyzed at 70°C for at least 2 days in sealed ampules and analyzed for inorganic phosphate colorometrically by the method of Chen *et al.* (11). The resultant data were recorded as milligrams of inorganic phosphate and milligrams of organically bound acid soluble phosphate per gram wet weight of liver. The latter value (organically bound) was obtained by subtracting the value for inorganic phosphate from the total acid soluble phosphate value.

Results. 1. Daily changes in plasma phosphate concentrations. Plasma phosphate concentrations rise during the daily fasting portion of the feeding cycle in the rat and fall abruptly during the feeding portion (6). These daily changes were reported to be less marked in thyroidectomized rats bearing functional parathyroid glands (TX). In the present study, data were obtained for plasma phosphate levels in thyroparathyroidectomized (TPTX) rats and further observations were made in TX rats. These data are summarized in Fig. 1.

In the absence of the parathyroids, plasma phosphate concentrations are elevated. However, the daily cyclic nature of these concentrations is retained. Plasma phosphate levels, as in normal rats, reach their maximum near the end of the fasting portion of the daily cycle and fall rapidly during feeding (Fig. 1A).

Since one of the purposes of this study was to relate calcitonin function to phosphate homeostasis, changes in plasma phosphate concentrations in TX rats were reexamined. The study focused on that period of the day just prior to, and for a few hours after the rats were provided food. In normal rats conditioned to a regular feeding schedule, plasma phosphate concentrations start their fall several hours prior to feeding and continue to fall during the first few hours of feeding, even if, on any one day, food is withheld for a few hours (12). The same pattern can be seen in the data obtained from TX rats (Fig. 1B). While the changes are less marked than in normal rats, plasma phosphate values fall during the fed portion of the daily cycle. It also occurred in rats fasted for several additional hours.

It can be concluded from these results that, while both hormones, affect plasma phosphate concentrations, the daily cyclic nature of these concentrations is due at least in part to other causes.

2. The effect of extended fasting on plasma PO_4 changes. In these experiments three groups were studied: normal rats, rats maintained for 2–3 weeks on a phosphate deficient diet, and rats TPTXed for 10 days. In each group, food was removed from half the animals late in the evening. Plasma phosphate concentrations in plasma from blood samples taken in the evening were compared to those taken the next morning. It should be borne in mind that after an extended period of time on a phosphate deficient diet, the parathyroid glands atrophy and are no longer functional (13). The data are summarized in Table I. In each of the three groups, comparison is made between fasted and fed conditions. In normal rats, the early morning plasma phosphate values were the same whether or not the rats had been fed or fasted during the night hours. In both the TPTX group and that on a low PO_4 diet the fasted values in the morning were higher than the corresponding fed values. These results emphasize the reciprocal relationship of plasma phosphate changes to digestive processes.

3. The effect of feeding versus fasting on liver phosphate concentrations. For these experiments, the acid-soluble fraction of liver phosphate was divided by the procedures described above into inorganic phosphate and that organically bound. Plasma phosphate values at time of sacrifice are given in micromoles for ease of comparison to liver concentrations. The data are summarized in Table II. In each experiment, the rats were weighed the afternoon prior to separation into "fed" and "fasted" groups and were divided such that the average body weight of those to be "fasted" or "fed" was not significantly different. Three groups were studied: normal rats, TPTX-rats, and rats on the low PO_4 diet.

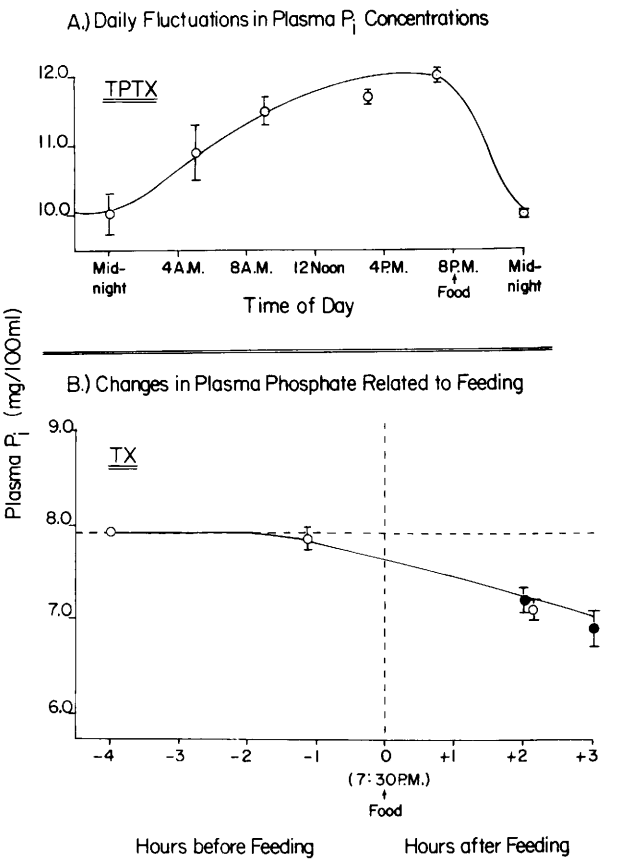

FIG. 1. Plasma phosphate changes in thyroidectomized (TX) and in thyroparathyroidectomized (TPTX) rats: (1) All rats were maintained on a 12-hr light, 12-hr dark schedule. Food was provided at the time indicated for the following 12 hr. Availability of food was not necessarily correlated with either the light or dark period. (2) All values represent mean of five to 15 different plasma samples ± SE. (3) In 1 B, ○ = nonfed rats; ● = fed rats. ○ after feeding represents a single experiment when availability of food was delayed 2 hr. (4) All plasma samples were obtained from rats maintained on a food and light schedule for two or more weeks.

The wet weight of the lobe of liver analyzed was approximately 30% greater in "fed" than in the "fasted" condition in all groups. Since this appeared to be too great a difference to be accounted for solely by the uptake or loss of liver glycogen, a separate study was done to determine how much of this additional weight was due to fluid incorporation into liver. These data are given in Table IIA. The percentage of water remained relatively constant, indicating both water and organic addition/or loss in the two groups.

Because of this difference in liver weight, the inorganic phosphate values for "fasted" rats were greater than for "fed" when expressed as P_i per gram wet weight. However, when expressed as P_i per lobe of liver, all values were surprisingly constant regardless of diet or group. Only in the normal rats was there any significant increase in the inorganic phosphate concentration per lobe of liver due to feeding.

For the acid-soluble organically bound phosphate pool, values are given only as total bound phosphate/lobe of liver. The phosphate concentration was much higher in the "fed" condition in all groups.

The particular points of interest concerning these data to our present study are: (1) The inorganic phosphate/lobe of liver was constant regardless of the plasma PO_4 concentration at the time of sacrifice and regardless of whether the rats had been fed or

TABLE I. EFFECT OF OVERNIGHT FASTING ON PLASMA PHOSPHATE CONCENTRATIONS

Time sample obtained	Plasma phosphate (mg P/100 ml)	
	10 PM[a]	9 AM
Controls		
(a) Fed	> 9.05 ± 0.16 (17)[b]	8.52 ± 0.21 (8)
(b) Fed		8.06 ± 0.17 (8)
Low PO_4 diet		
(a) Fed	> 3.88 ± 0.28 (6)	3.50 ± 0.11 (17)
(b) Fed		6.90 ± 0.14 (11)[Δ]
TPTX		
(a) Fed	> 14.59 ± 0.30 (20)	12.1 ± 0.25 (20)
(b) Fed		14.45 ± 0.19 (22)[Δ]

[a] Blood sample taken prior to removal of food from cages.
[b] Values = mean ± SE with number of animals utilized in parentheses.
[Δ] Significantly different from "fed" rats of same group with $P < 0.01$.

TABLE II. LIVER PHOSPHATE CONCENTRATIONS: FED VERSUS FASTED ANIMALS

	Body wt (g)[a]	Liver wt (mg)	Plasma P_i (μM/ml)	Liver P_i		
				μM/g wet wt	μM/lobe	μM/Lobe of liver ASB[b]
1. Normals						
Fed (8)[c]	165 ± 3.7	988 ± 52	2.7 ± 0.07	6.54 ± 0.23	6.49 ± 0.33	21.81 ± 1.32
Fasted (8)	167 ± 4.3	699 ± 34[Δ]	2.6 ± 0.05	7.59 ± 0.27[Δ]	5.31 ± 0.33[Δ]	15.64 ± 0.89[Δ]
2. TPTX						
Fed (9)	152 ± 4	880 ± 33	4.0 ± 0.1	5.93 ± 0.13	5.22 ± 0.23	21.46 ± 0.77
Fasted (9)	149 ± 3	603 ± 20[Δ]	4.6 ± 0.1[Δ]	9.28 ± 0.45[Δ]	5.48 ± 0.22	15.76 ± 0.56[Δ]
3. Low PO_4 diet						
Fed (11)	177 ± 4	1072 ± 52	1.16 ± 0.04	5.22 ± 0.30	5.86 ± 0.47	28.59 ± 1.97
Fasted (11)	174 ± 4	782 ± 24[Δ]	2.23 ± 0.05[Δ]	8.20 ± 0.24[Δ]	6.38 ± 0.21	19.89 ± 0.76[Δ]

[a] Animal weight obtained late afternoon prior to separation into "fed" and "fasted" groups. All other values obtained at 9 AM the following day.
[b] Acid-soluble bound phosphate.
[c] Numbers in parentheses equal number of animals. Values given ± SE.
[Δ] Statistically different from "fed" rats of same group with $P < 0.01$.

TABLE IIA. RAT LIVER WEIGHTS (WET WT VS DRY WT)

	Body weight (g)		Liver weights (AM)		
	PM	AM	Wet wt (mg)	Dry wt (mg)	Water (%)
Fed (3)[a]	289 ± 4.7	286 ± 4.2	1276 ± 29	385 ± 8	70.9 ± 1.2
Fasted (3)	290 ± 4.2	274 ± 4.9	966 ± 30[Δ]	288 ± 14[Δ]	69.8 ± 0.1

[a] Numbers in parentheses equal number of animals.
[Δ] Statistically different from "fed" rats run concurrently, with $P < 0.01$.

fasted; (2) neither TPTX nor a low phosphate diet appeared to exert any significant influence on the increase in the concentrations of phosphate in the acid-soluble organically bound pool which occurred as the result of feeding, or the decrease due to overnight fasting; and (3) the inorganic phosphate concentration of liver when compared on the basis of micromolar per gram wet weight was always greater than that found in plasma.

4. Liver phosphate concentrations in "fed" rats as influenced by the absence of the thyroids. Since a previous study (12) had determined that the actual entrance of food into the digestive tract was a major cause for the secretion of calcitonin, these experiments were done to determine whether the presence of calcitonin increased the uptake of phosphate by liver during a single feeding period. The data are summarized in Table III. Three groups of rats were compared: normal versus TPTX; control rats (bearing parathyroid transplants and with active thyroid glands) versus TX rats with parathyroid transplants; and TPTX versus PTX rats. The only significantly different values were in plasma phosphate levels at time of sacrifice, as was expected from the treatment. Neither the absence of the thyroids or para-

thyroids appeared to influence either the liver inorganic phosphate or the uptake of phosphate by the liver as the result of overnight feeding.

5. The influence of exogenous hormones on liver phosphate. Since it has recently been reported (8, 9) that both PTH and calcitonin influence liver inorganic phosphate, a series of experiments was run in which these two hormones were injected singly or in combination to TPTX rats fed overnight. In the first experiment, four hourly injections of the hormones were made before the rats were sacrificed and liver samples were analyzed. In the second experiment, the time was extended to 8 hr. The data are summarized in Table IV.

It can be noted that plasma phosphate fell as expected following hormone treatment and was maintained at the lower levels throughout the experiment (see column 4 of Table IVA and B). The inorganic phosphate component of liver (per lobe) was also lower in all three experimental groups at the 4-hr post-hormone time period (Table IVA). However, by 8 hr, despite continued hormone treatment, liver inorganic phosphate values all had returned to their normal range (Table IVB). While PTH appeared to cause a slight drop in the acid soluble organically bound component, this difference was not statistically significant.

Discussion. These and earlier studies have demonstrated that plasma phosphate concentrations drop as the result of anticipation of feeding and actual entrance of food into the digestive tract. Conversely, plasma phosphate concentrations rise during extended fasting. This rise is prevented by the presence of an actively secreting parathy-

TABLE III. LIVER PHOSPHATE CONCENTRATIONS IN THE ABSENCE OF THE THYROID

	Body weight[a] (g)	Liver weight (mg)	Plasma P_i (μM/ml)	Liver P_i (μM/lobe)	Liver ASB[b] (mm/lobe)
Normal (5)[c]	167 ± 11.5	879 ± 112.42	2.51 ± 0.06	6.98 ± 0.74	21.46 ± 2.74
TPTX (6)	161 ± 6.9	967 ± 87.67	3.95 ± 0.09[Δ]	7.56 ± 0.45	22.63 ± 1.89
PTT (15)	264 ± 3.56	1437 ± 57.27	3.29 ± 0.07[Δ]	7.09 ± 0.39	29.44 ± 0.88
TX (14)	264 ± 4.04	1335 ± 39.38	3.29 ± 0.06[Δ]	6.10 ± 0.25	28.05 ± 0.85
PTX (12)	227 ± 6.2	1148 ± 48.7	4.37 ± 0.11[Δ]	6.06 ± 0.29	27.00 ± 0.94
TPTX (10)	212 ± 7.0	1035 ± 45.9	4.31 ± 0.08[Δ]	5.37 ± 0.22	24.49 ± 1.17

[a] Animal weight obtained late afternoon prior to the availability of food. All other values obtained at 9 AM the following day.
[b] Acid-soluble bound phosphate.
[c] Numbers in parentheses equal number of animals. Values given ± SE. TPTX = thyroparathyroidectomized. PTT = thyroidectomized with parathyroid transplants. TX = thyroidectomized. PTX = parathyroidectomized.
[Δ] Statistically different from normal rats, with $P < 0.01$.

TABLE IV. LIVER PHOSPHATE CONCENTRATIONS FOLLOWING HORMONE ADMINISTRATION

	Body wt[a] (g)	Liver wt (mg)	Plasma P_i (μM/ml)	Liver P_i (μM/lobe)	Liver ASB (μM/lobe)
A. Injected hourly for 4 hr					
Veh. (12)[b]	172 ± 3.7	870 ± 29.5	4.13 ± 0.16	5.54 ± 0.26	19.9 ± 0.96
SCT (13)[c]	175 ± 4.5	896 ± 27.6	3.26 ± 0.09[Δ]	4.94 ± 0.19[▲]	20.9 ± 1.22
PTH (6)[d]	179 ± 5.4	886 ± 29.4	2.49 ± 0.04[Δ]	4.55 ± 0.43[▲]	18.6 ± 1.55
Comb. (7)[e]	167 ± 6.0	827 ± 43.0	2.13 ± 0.10[Δ]	4.50 ± 0.16[Δ]	20.9 ± 1.23
B. Injected hourly for 8 hr					
Veh. (12)	179 ± 5.7	848 ± 50.04	3.57 ± 0.14	5.08 ± 0.40	20.42 ± 1.60
PTH (6)	180 ± 3.0	843 ± 17.80	2.14 ± 0.18[Δ]	5.62 ± 0.32	19.79 ± 1.43
Comb. (7)	180 ± 8.7	859 ± 51.30	1.25 ± 0.13[Δ]	5.05 ± 0.19	21.17 ± 1.90

[a] Animal weight taken late afternoon, all other values the following day. All values given ± SE.
[b] Veh. = vehicle injected controls.
[c] SCT dose = 0.2 mU/g/hr.
[d] PTH dose = 0.1 U/g/hr.
[e] Comb. = both hormones injected together.
[Δ] Statistically significant from vehicle injected controls run concurrently, with $P < 0.01$.
[▲] Statistically significant from vehicle injected controls run concurrently, with $P < 0.05$.

roid gland and conversely is most dramatically illustrated in rats maintained several weeks on a phosphate deficient diet and then fasted overnight. In the fasted condition, the plasma phosphate concentration of the phosphate deficient rats almost doubles, rising into the low normal range for this species. This suggests that the phosphate changes may be secondary to other metabolic events.

The difference between the size of the acid-soluble organically bound phosphate pool in the livers of fed and fasted rats also supports the conclusion that phosphate changes may largely be the result of other metabolic events. These liver values are influenced markedly by digestive activity but not by a phosphate deficient diet or by plasma phosphate concentrations varying from 1 to 4 μM/ml.

The inorganic phosphate concentrations of liver remain relatively constant and unaffected by feeding or fasting. These levels were higher than that in plasma, even in thyroparathyroidectomized rats, and were not statistically reduced after 3 weeks on a phosphate deficient diet. Abrupt changes in plasma phosphate by hormone injection tended to cause a temporary drop in liver inorganic phosphate, but these returned to normal even under continued hormone administration, which produced even lower plasma phosphate levels. It appears, therefore, that the liver inorganic phosphate pool is under some type of homeostatic control.

The primary purpose of this study was to relate parathyroid hormone and calcitonin action to these phosphate changes. It is concluded that neither hormone directly affects liver phosphate. No evidence was obtained to support our hypothesis that the hypophosphatemic action of calcitonin could be attributed at least in part to its ability to move phosphate from plasma to liver. However, movement into other soft tissue or bone has not been ruled out. The discrepancy between these results with CT and those reported earlier (8, 9) may be accounted for by the previous use of only fasted TPTX rats. CT injection in such rats may lead to severe tetany. Any rats in tetany were discarded from our experiments, since such rats tended to have variable and usually elevated phosphate values. Neither PTH or CT appears to be a primary causative agent in the plasma phosphate drop occurring at the onset of feeding, since it occurred in the absence of both hormones. The role of insulin or other intestinal-related hormones remains yet to be determined, being beyond the scope of this project.

While these data do not suggest any role for PTH on plasma phosphate other than its action on renal phosphate excretion, they do raise the question as to why plasma phosphate values continue to rise throughout the fasting segment of the daily feeding cycle. If feeding is delayed, extending the fasting period, plasma phosphate values are maintained low as long as PTH is available. It is obvious that while parathyroid hormone sets the plasma phosphate level by its renal action, it is unable to prevent the daily rise during the fasting portion. Yet PTH secretion must occur during the fasting portion of the daily cycle or the plasma calcium concentrations would not be at their highest level (6) at this time.

Summary. The relationships of parathyroid hormone and calcitonin to plasma and liver phosphate concentrations were studied in rats. While parathyroid hormone affected plasma phosphate levels, it did not control the daily fluctuations in rats adapted to standard feeding and light conditions. The endogenous presence of this hormone did prevent the additional rise in plasma phosphate concentrations which occurs in its absence following an overnight fast.

Inorganic phosphate concentrations of liver were always higher than plasma phosphate and were surprisingly constant despite almost fourfold variation in plasma concentrations. They remained relatively constant even 3 weeks after the rats were placed on a low phosphate diet and after extended thyroparathyroidectomy. Only minor differences were seen in liver inorganic phosphate levels in fed and fasted rats. It is concluded that this phosphate pool is under some type of homeostatic control.

The organically bound phosphate in the acid-soluble pool of liver increases rapidly after feeding, decreasing just as rapidly on fasting. These changes were not influenced

by the phosphate level of plasma nor the presence or absence of either endogenous parathyroid hormone or calcitonin and occurred while the rats were on a low phosphate diet. It is concluded that neither parathyroid hormone nor calcitonin directly affects these two acid soluble phosphate pools in liver.

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