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Research Article

A circadian variation in serum calcium, albumin and PTH concentration in normal subjects has been demonstrated. The levels of the three blood constituents were remarkably constant during the day, but striking night and early morning changes occurred. Serum calcium levels were highest at 8:00 p.m. and reached a nadir between 2:00 and 4:00 a.m. Serum albumin levels were parallel to those of serum calcium. PTH levels began to rise after 8:00 p.m., reached the highest levels between 2:00 and 4:00 a.m., and fell to baseline values by 8:00 a.m. The nocturnal fall in serum calcium levels appears to be secondary to dilution of serum proteins by increasing blood volume. The nocturnal rise in PTH levels appears to be independent of serum calcium levels within the normal range but it can be abolished by induced hypercalcemia.

Serum phosphate levels were lowest between 8:00 a.m. and 10:00 a.m. and highest between 2:00 a.m. and 4:00 a.m. The data presented suggest that circadian changes in serum phosphate levels are not mediated *in toto* by parathyroid hormone but they are exaggerated when the secretion of this hormone is inhibited. They are independent of growth hormone levels and activity but they are greatly modified during a prolonged fast.

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Circadian Rhythm in Serum Parathyroid Hormone Concentration in Human Subjects: Correlation with Serum Calcium, Phosphate, Albumin, and Growth Hormone Levels

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ABSTRACT A circadian variation in serum calcium, albumin and PTH concentration in normal subjects has been demonstrated. The levels of the three blood constituents were remarkably constant during the day, but striking night and early morning changes occurred. Serum calcium levels were highest at 8:00 p.m. and reached a nadir between 2:00 and 4:00 a.m. Serum albumin levels were parallel to those of serum calcium. PTH levels began to rise after 8:00 p.m., reached the highest levels between 2:00 and 4:00 a.m., and fell to baseline values by 8:00 a.m. The nocturnal fall in serum calcium levels appears to be secondary to dilution of serum proteins by increasing blood volume. The nocturnal rise in PTH levels appears to be independent of serum calcium levels within the normal range but it can be abolished by induced hypercalcemia.

Serum phosphate levels were lowest between 8:00 a.m. and 10:00 a.m. and highest between 2:00 a.m. and 4:00 a.m. The data presented suggest that circadian changes in serum phosphate levels are not mediated *in toto* by parathyroid hormone but they are exaggerated when the secretion of this hormone is inhibited. They are independent of growth hormone levels and activity but they are greatly modified during a prolonged fast.

INTRODUCTION

The existence of predictable circadian changes in the levels of many hormones and electrolytes in blood and

urine has been well documented (1). Urinary calcium excretion has been found to be highest during the day (2-5), but several investigators have failed to demonstrate a rhythm in serum calcium levels (2, 5, 6). Serum phosphate levels are higher during the night than during the day (2). Total serum protein concentration falls during the night and early morning hours (6).

In their initial studies, Berson and Yalow observed no circadian variation in serum parathyroid hormone (PTH)¹ levels in four subjects studied (7), Dube, Goldstein, Riggs, and Arnaud (8), and Arnaud, Tsau, and Littledike (9) reported a gradual rise in immunoassayable serum PTH in normal subjects between 8:00 a.m. and 8:00 p.m. and a decline by the following morning. However, individual values were not recorded and no nighttime samples were drawn.

We have presented a preliminary report of the existence of circadian variation of serum calcium and PTH (10). This paper is an expansion of our studies regarding the circadian changes in serum calcium, albumin, and PTH levels in normal subjects, the correlation with serum phosphate and growth hormone (GH) levels and the alterations of these rhythms in disease states.

METHODS

Studies were conducted in normal subjects, eight men and two women, ages 22-32. All were engaged in normal activi-

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¹Abbreviations used in this paper: GH, growth hormone; PTH, parathyroid hormone; TSH, thyroid stimulating hormone.

ties and were eating a self-selected diet. Venous blood (15 ml) was drawn every 2 hr for 24 hr beginning at 8:00 a.m. The subjects slept in the hospital with an indwelling catheter in the antecubital vein to facilitate the blood drawing without disturbing them during the night. The serum was separated within 30 min and kept frozen at -20°C until assayed. Serum calcium and phosphate were measured on each sample from all subjects, serum albumin in eight, and PTH and GH in four. All of the subjects went to sleep around 11:00 p.m.

The effect of an i.v. calcium infusion on serum PTH was studied in four normal subjects. In two, calcium was given from 7:00 p.m. to 8:00 a.m. as an infusion of calcium gluconate in 5% dextrose in water at a rate of 1 mg Ca^{++}/kg each hour. Both subjects had previously been studied without calcium infusion. Two other subjects were studied during two consecutive 24 hr periods, first without a calcium infusion and then with calcium being infused from 8 p.m. to midnight at a rate of 4 mg Ca^{++}/kg each hour. Blood for calcium and PTH was collected every 2-4 hr for 48 hr, including a period of time before and after the calcium infusion.

To study the effect of inactivity on serum calcium, PTH, and albumin levels, these measurements were performed in two normal subjects who were kept in bed for 24 consecutive hours.

The effect of fasting on serum calcium, PTH, GH and phosphate concentrations was investigated in two normal subjects. They were fasted for 24 hr but were allowed to engage in normal activities. No fluids, including water, were permitted during this period.

Two patients with primary hyperparathyroidism, each caused by a solitary parathyroid adenoma, and one patient with surgical hypoparathyroidism off vitamin D therapy for 2 wk were studied. Finally we studied two patients with pituitary insufficiency. One (M. S.) had a complete ACTH deficiency, partial thyroid stimulating hormone (TSH) and gonadotropin deficiency, and intact growth hormone secretion. The other (M. W.) had panhypopituitarism secondary to hypophysectomy for chromophobe adenoma.

Serum calcium was measured by the method of Clark and Collip (11). Normal range in our laboratory is 9-11 mg/100 ml. All samples were assayed in duplicate in 2 ml portions of serum. For a given sample the intra-assay coefficient of variation ranged from 0-1.8% and the interassay coefficient of variation was 3%. Serum phosphate was estimated by the method of Fiske and SubbaRow (12). Serum albumin was determined by Reinhold's method (13). PTH was measured by radioimmunoassay (14, 15) and is expressed in units by reference to the potency of an arbitrarily selected standard hyperparathyroid serum. Intra-assay coefficient of variation was 8% and interassay coefficient of variation 13%. Normal range is 10-60 μl equivalent/ml. GH also was measured by radioimmunoassay (16). The lower limit of sensitivity of our assay is 1 $\mu\text{g}/\text{ml}$. Intra-assay coefficient of variation is 10% and interassay coefficient of variation is 15%.

RESULTS

Individual serum calcium, phosphate and albumin levels in the ten normal subjects are shown in Table I.

Sequential serum PTH, calcium, albumin, phosphate, and GH determinations in four of the subjects are shown in Fig. 1. Serum PTH concentration was remarkably constant during the day, began to rise after

FIGURE 1 Serum PTH, calcium, albumin, phosphate, and GH concentration in four normal subjects throughout one 24 hr period.

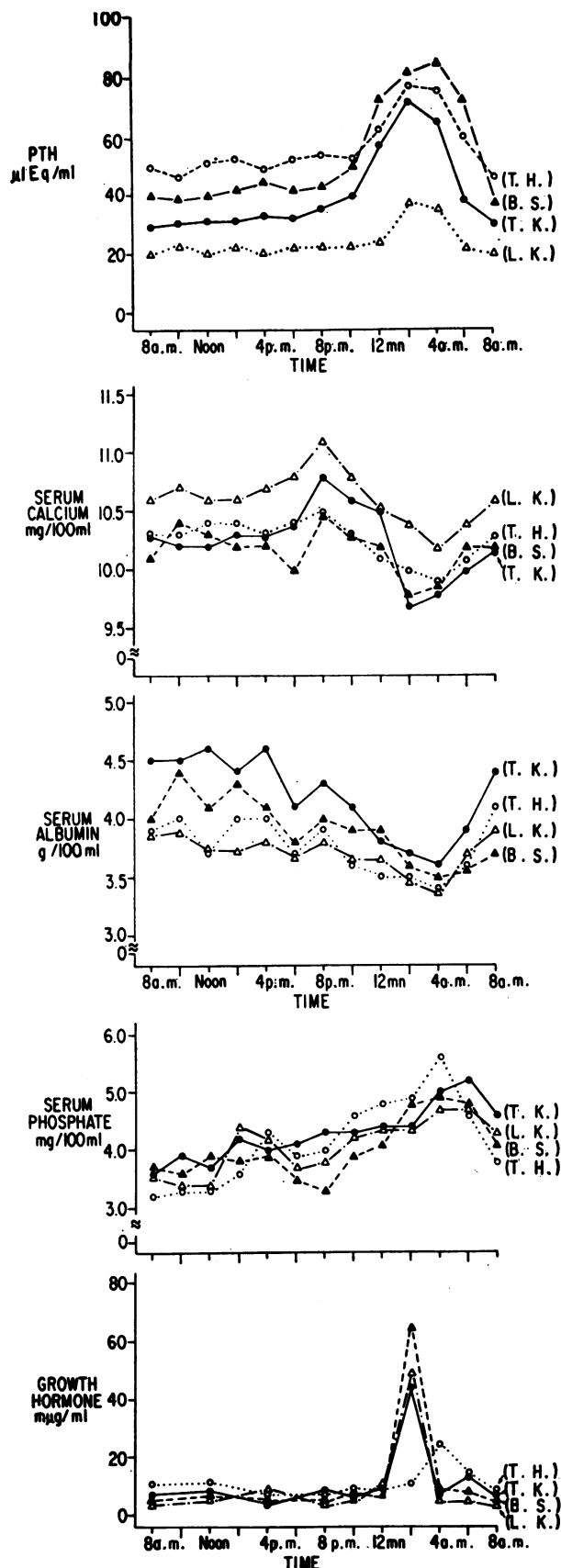


TABLE I

Individual Serum Calcium, Phosphate, and Albumin Concentration

Name	8 a.m.			10 a.m.			Noon			2 p.m.			4 p.m.			6 p.m.		
	Ca	PO ₄	Alb	Ca	PO ₄	Alb	Ca	PO ₄	Alb	Ca	PO ₄	Alb	Ca	PO ₄	Alb	Ca	PO ₄	Alb
R. G.	10.2	3.1	4.6	10.8	2.9	4.5	10.7	4.1	4.6	10.6	3.5	4.6	10.6	4.1	4.5	10.7	4.5	—
G. J.	10.4	3.4	4.1	10.3	3.3	4.0	10.5	3.7	4.1	10.5	4.3	3.9	10.3	4.6	4.0	10.4	4.5	4.0
H. C.	10.4	3.9	4.1	10.8	3.1	—	10.5	3.7	4.0	10.3	3.9	4.0	10.4	4.2	4.1	10.7	4.5	4.2
L. S.	10.0	4.1	3.9	10.1	3.9	3.8	10.0	4.1	3.9	10.1	4.6	4.2	10.0	4.3	4.2	10.2	4.4	3.8
T. H.	10.3	3.2	3.9	10.3	3.3	4.0	10.4	3.3	3.7	10.4	3.6	4.0	10.3	4.3	4.0	10.4	3.9	3.7
B. S.	10.1	3.7	4.0	10.4	3.6	4.4	10.3	3.9	4.1	10.2	3.8	4.3	10.2	3.9	4.1	10.0	3.5	3.8
T. K.	10.3	3.6	4.5	10.2	3.9	4.5	10.2	3.7	4.6	10.3	4.2	4.4	10.3	4.0	4.6	10.4	4.1	4.1
L. K.	10.6	3.6	3.9	10.7	3.4	3.9	10.6	3.4	3.7	10.6	4.4	3.7	10.7	4.2	3.8	10.8	3.7	3.7
C. N.	10.5	3.7	—	—	—	—	10.5	3.3	—	—	—	—	10.4	3.7	—	—	—	—
G. N.	10.7	3.2	—	—	—	—	10.8	4.1	—	—	—	—	10.8	4.3	—	—	—	—
Mean	10.4	3.6	4.1	10.5	3.4	4.2	10.5	3.7	4.1	10.4	4.0	4.1	10.4	4.2	4.2	10.5	4.1	3.9
SD	0.2	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.2	0.4	0.3	0.2	0.2	0.3	0.3	0.4	0.2

Serum calcium and phosphate are expressed in milligrams per 100 ml and serum albumin in grams per 100 ml.

8:00 p.m. and attained a peak value between 2:00 a.m. and 4:00 a.m. PTH then decreased to baseline levels by 8:00 a.m. The change of PTH from baseline to peak values ranged from 50 to 150%, a statistically significant difference ($P < 0.05$).

Serum calcium levels were remarkably constant during the day time. Consistent changes were observed

during the evening and early morning hours. The serum calcium level was highest at 8:00 p.m. in each subject, decreased progressively to lowest levels between 2:00 a.m. and 4:00 a.m., and then returned to the same levels that were obtained at 8:00 a.m. the previous day. These changes are highly significant (P value 8:00 a.m. vs. 8:00 p.m. < 0.001 , 8:00 p.m. vs. 2:00 a.m. < 0.001). Serum albumin levels were parallel to those of serum calcium except for the absence of the 8:00 p.m. peak.

Serum phosphate levels were lowest between 8:00 a.m. and 10:00 a.m. Then a biphasic pattern was observed with increasing values up to 6:00 p.m., a transient decrease at 8:00 p.m. and a steady rise to highest levels between 2:00 a.m. and 4:00 a.m. Values subsequently decreased at 8:00 a.m. although they were not completely back to baseline levels in some of the subjects. Similar calcium, albumin and phosphate results were obtained in the remaining subjects (see Table I). GH levels were relatively constant except at 2:00 a.m. when a significant sharp peak occurred. The highest GH levels coincided with or preceded the highest serum phosphate and PTH values.

The response of serum calcium and PTH to a low dose calcium infusion in two normal subjects (T. H. and L. K.) is presented in Fig. 2. Both subjects had demonstrated a normal circadian PTH rhythm without calcium infusion (see Fig. 1). The amount of calcium given (1 mg Ca^{++} /kg each hour) not only obliterated the usual nocturnal fall in serum calcium levels but increased the serum calcium to the upper limits of normal. Despite this, the nocturnal PTH spike persisted and the pattern was remarkably similar to that observed without the calcium infusion (see Fig. 1). When the amount of calcium infused was increased to 4 mg Ca^{++} /kg each hour, marked hypercalcemia was

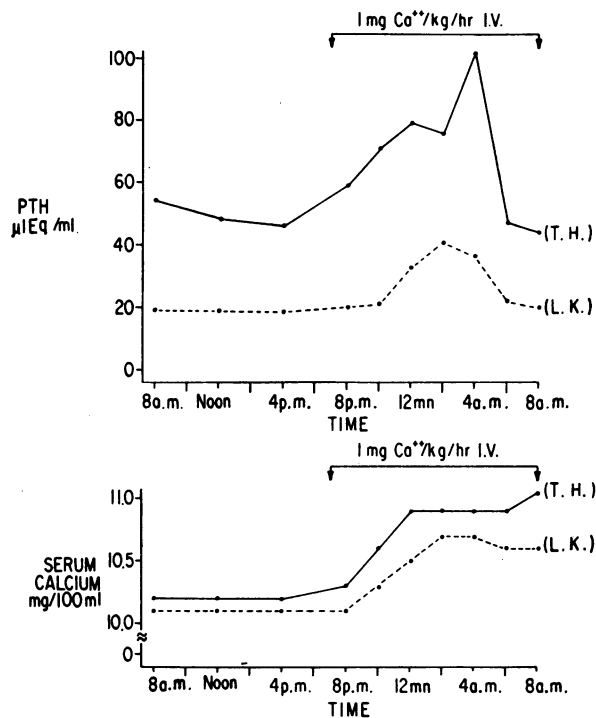


FIGURE 2 Serum PTH and calcium concentration in two normal subjects throughout one 24 hr period. A continuous calcium infusion at a rate of 1 mg Ca^{++} per kg body weight each hour was given from 7:00 p.m. to 8:00 a.m.

in 10 Normal Subjects throughout One 24 hr Period

8 p.m.			10 p.m.			Midnight			2 a.m.			4 a.m.			6 a.m.			8 a.m.		
Ca	PO ₄	Alb	Ca	PO ₄	Alb	Ca	PO ₄	Alb	Ca	PO ₄	Alb	Ca	PO ₄	Alb	Ca	PO ₄	Alb	Ca	PO ₄	Alb
10.7	4.0	4.3	10.8	4.3	—	10.3	4.8	4.1	10.2	4.9	3.8	10.2	5.3	4.0	10.3	5.0	—	10.3	4.0	4.1
10.9	4.6	4.1	10.5	4.5	3.9	10.5	4.5	3.8	10.4	4.6	3.6	10.2	4.2	3.5	10.1	4.5	3.6	10.3	3.5	3.8
10.9	4.3	4.4	10.6	4.5	4.2	10.5	4.6	4.1	10.4	5.0	4.1	10.3	5.4	3.9	10.2	4.8	3.8	10.5	4.4	3.9
10.4	4.4	4.0	10.3	4.3	3.9	10.2	4.6	3.8	10.2	4.8	3.6	10.1	4.8	3.7	10.1	4.6	3.6	10.0	4.3	3.4
10.5	4.0	3.9	10.3	4.6	3.6	10.1	4.8	3.5	10.0	4.9	3.5	9.9	5.6	3.4	10.1	4.6	3.6	10.3	3.8	4.1
10.5	3.3	4.0	10.3	3.9	3.9	10.2	4.1	3.9	9.8	4.8	3.6	9.9	4.9	3.5	10.2	4.8	3.6	10.2	4.1	3.7
10.8	4.3	4.3	10.6	4.3	4.1	10.5	4.4	3.8	9.7	4.4	3.7	9.8	5.0	3.6	10.0	5.2	3.9	10.2	4.6	4.4
11.1	3.8	3.8	10.8	4.3	3.7	10.5	4.4	3.7	10.4	4.4	3.5	10.2	4.7	3.4	10.4	4.7	3.7	10.6	4.3	3.9
11.2	2.9	—	—	—	—	10.1	4.7	—	10.0	5.3	—	10.0	5.0	—	10.3	4.5	—	10.5	3.6	—
11.0	4.0	—	—	—	—	10.2	4.5	—	10.4	4.6	—	10.4	4.1	—	—	3.5	—	10.7	4.0	—
10.8	4.0	4.1	10.5	4.3	3.9	10.3	4.5	3.8	10.2	4.8	3.7	10.1	4.9	3.6	10.2	4.6	3.7	10.4	4.0	3.9
0.3	0.5	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.2	0.2	0.5	0.2	0.1	0.4	0.1	0.2	0.5	0.3

induced. This not only abolished the nocturnal PTH rise but resulted in suppression of basal secretion (Fig. 3).

The study of two normal subjects at complete bed rest for 24 consecutive hours is shown in Fig. 4. One of them (B. S.) had been studied before (see Fig. 1).

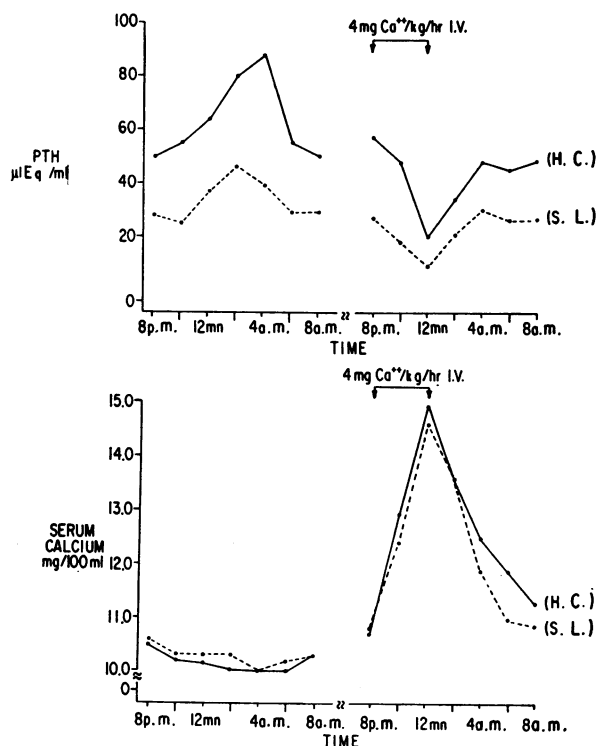


FIGURE 3 Serum PTH and calcium concentrations in two normal subjects from 8:00 p.m. to 8:00 a.m. on each of two successive days. A continuous calcium infusion at a rate of 4 mg Ca⁺⁺ per kg body weight each hour was given on the second day from 8:00 p.m. to midnight.

Neither serum albumin nor serum calcium levels exhibited the nocturnal fall but the peak of PTH was unaffected.

In response to induced hypercalcemia, the rhythm in serum phosphate concentration persisted in the two subjects that we studied.

In two patients with pituitary insufficiency and growth hormone deficiency the circadian variation in serum calcium and phosphate persisted. The early morning spike in PTH was exaggerated in one patient (M. S.) who had ACTH and TSH deficiency but intact

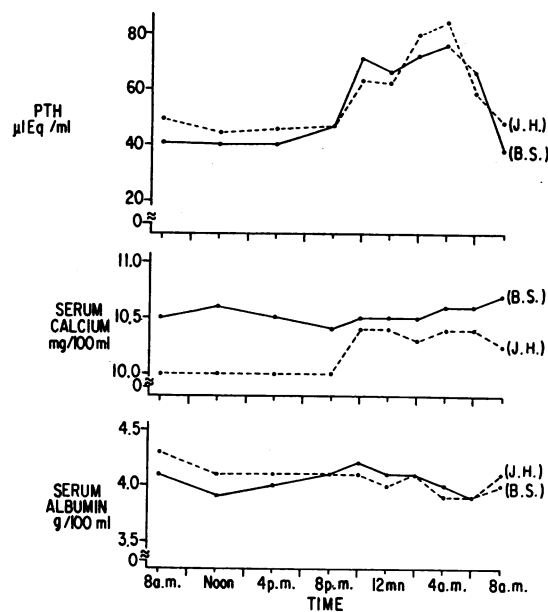


FIGURE 4 The PTH, calcium and albumin concentrations in two normal subjects at complete bed rest for 24 consecutive hours.

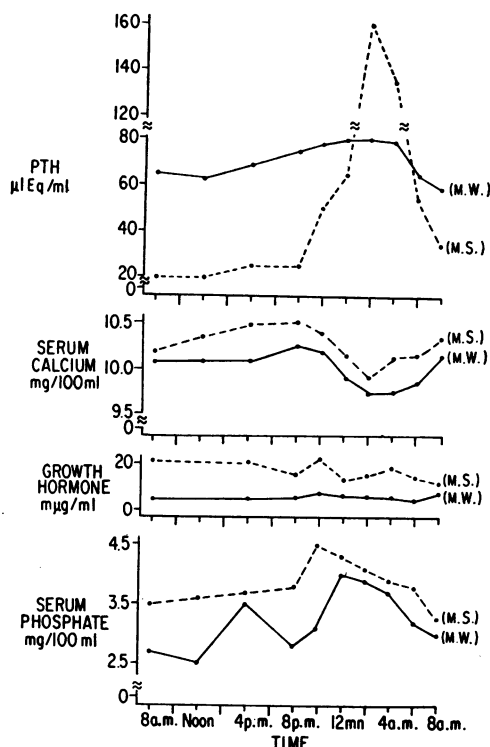


FIGURE 5 Serum PTH, calcium, GH, and phosphate concentration in two patients with pituitary insufficiency. MS had ACTH and TSH deficiency but intact GH secretion. MW had panhypopituitarism.

GH secretion and blunted in the other (M. W.) who had panhypopituitarism (Fig. 5).

Both patients with primary hyperparathyroidism (R. C. and R. M.) were hypercalcemic and exhibited persistently elevated PTH levels without the usual circadian pattern. PTH levels in the patient with hypoparathyroidism (R. Y.) were immeasurably low throughout. Nonetheless, the circadian variation in serum phosphate concentration persisted in the three patients (Fig. 6).

Prolonged fasting abolished the circadian phosphate changes without altering the serum calcium, PTH or GH changes (Fig. 7). In fact, serum phosphate concentration fell during the night, the time when values are normally highest.

DISCUSSION

This study, in contrast to earlier reports, indicates that serum calcium levels undergo significant circadian changes in normal subjects. The failure of other investigators to observe the rhythm in serum calcium (2, 5, 6) could be the result of infrequent sampling. It is known that circulating calcium is bound to plasma protein, mainly albumin. The good correlation between

serum calcium and albumin levels (coefficient of correlation 0.7), especially at night, suggests that these changes in serum calcium are secondary to changes in serum proteins. Non-protein-bound calcium estimated from the nomogram of Hanna, Nicholas, and Chamberlin (17) showed slightly higher values at night than during the day but the difference was not significant. In normal subjects, blood volume is lower during the day than during the night, presumably as a result of fluid shift from the vascular to the extravascular compartment associated with the upright posture (18). The fall in serum albumin and therefore in calcium levels during the early morning hours presumably is secondary to hemodilution. The fact that the changes in serum calcium and albumin levels disappeared in the subjects who were maintained in bed for 24 hr gives support to this interpretation.

Serum calcium levels decreased as PTH levels increased. Since hypocalcemia is an established stimulus for PTH secretion, the most obvious interpretation of

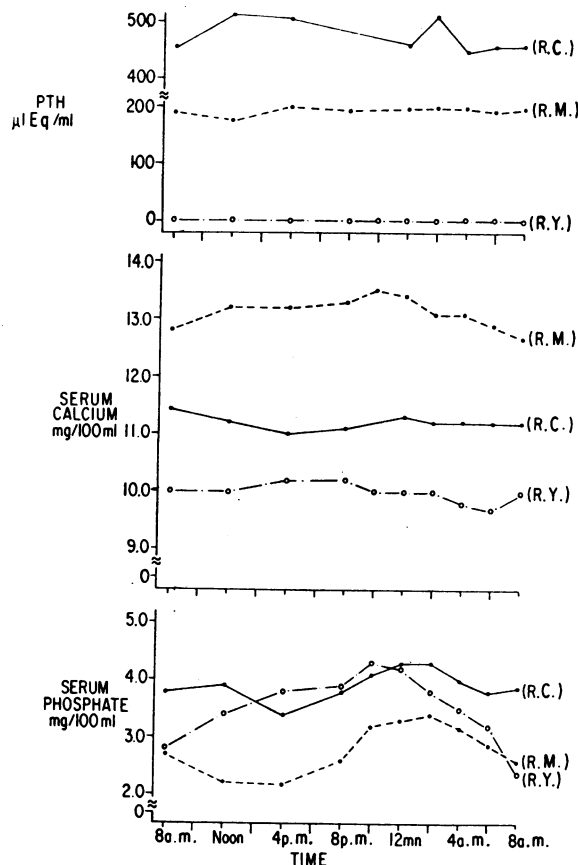


FIGURE 6 Serum PTH, calcium, and phosphate in two patients with primary hyperparathyroidism (RC and RM) and in one patient with surgical hypoparathyroidism (RY).

the data would be that nocturnal hypocalcemia triggers the release of PTH. However, as indicated above, the present data do not suggest any decrease of non-protein-bound calcium at night. In addition, the circadian rhythm of PTH persisted after a low dose calcium infusion that increased total calcium to the upper limits of normal. Moreover, the subjects on prolonged recumbency did not show nocturnal hypocalcemia, yet maintained the PTH rhythm. Hence, nocturnal hypocalcemia does not appear to account for the circadian variation in PTH levels.

At present, there is considerable doubt about the precise interpretation of data derived by immunoassay of PTH. Berson and Yalow first reported immunochemical heterogeneity of circulating PTH (19). More recently, Arnaud and Sherwood have independently reported data showing distinct immunologic differences between PTH extracted from glands and circulating PTH (20, 21). The assay system used in this study is characterized by remarkable sensitivity, clear cut differentiation between normal and abnormal sera and excellent clinicopathologic correlation (15). Preliminary data from our own laboratory suggest that our antibody recognizes not only native hormone but also smaller peptides, possibly metabolites of the hormone. The uncertainties of interpretation have been recently discussed in detail (22). In spite of these problems the large changes observed suggest changing rates of secretion.

We have confirmed previous reports concerning the existence of circadian changes in serum phosphate levels in normal subjects and have performed additional studies to try to elucidate the mechanism of those changes. From the results obtained we can make the following observations: (a) The circadian variation in serum phosphate levels seems to be independent from GH since we have shown that the changes in the former persist in the absence of changes in the latter. Patients with pituitary insufficiency and growth hormone deficiency represent examples of this. (b) Changes in serum phosphate are not mediated in toto by PTH. On one hand, the variations in serum phosphate concentration persist in patients with hyper or hypoparathyroidism who do not exhibit a circadian rhythm in PTH levels. On the other hand, inhibition of the PTH spike by induced hypercalcemia caused an exaggeration of the usual phosphate changes. This suggests that although the circadian variation in serum phosphate concentration is not controlled directly by PTH, it can be exaggerated by suppressing its secretion during the early morning hours. (c) The diurnal variation in serum phosphate concentration is independent of physical activity since it persists in normal individuals during prolonged bed rest. (d) Our studies suggest that the

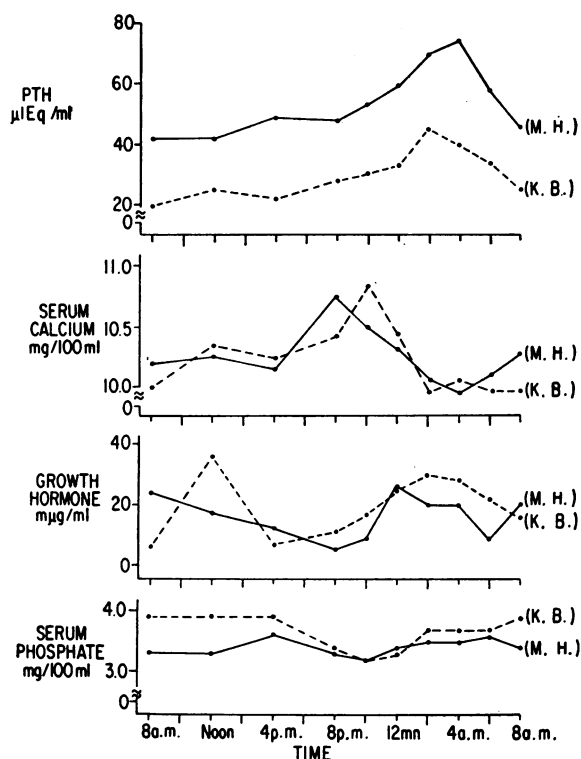


FIGURE 7 Serum PTH, calcium, GH, and phosphate concentration in response to a prolonged fast in two normal subjects.

circadian changes in serum phosphate are in large part secondary to food ingestion since prolonged fasting was the only maneuver that abolished them. The mechanism whereby food ingestion induces the circadian changes in serum phosphate levels is not clear. It is known that serum insulin levels rise shortly after each meal but that these changes do not take place during a prolonged fast (23). Serum phosphate levels fall in response to insulin administration (24). It is conceivable that the changes in serum phosphate levels are secondary to the changes in insulin secretion which accompany the ingestion of food. The continuous elevation of serum phosphate levels after 8:00 p.m. may result from the fact that we do not usually eat after that hour and therefore insulin release is not stimulated.

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