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SEASONAL VARIATIONS IN SERUM INORGANIC PHOSPHATE AND CALCIUM WITH SPECIAL REFERENCE TO PARATHYROID ACTIVITY

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Seasonal variation in the inorganic phosphate concentration of blood was first noted by Hess & Lundagen (1922) in children and by Havard & Reay (1925) in adults. Since the level of inorganic phosphate in the blood of infants could be raised by frequent exposure to ultra-violet light (Hess & Gutman, 1921), it was concluded that the increase in the blood-phosphate level in summer and the decrease in winter were related to seasonal variations in the intensity of ultra-violet light. More recently de Rudder (1952) has suggested that this seasonal variation in the blood-phosphate level in infants is intimately connected with corresponding variations in rate of growth.

The present work is an extension of the previous investigation of Yoshimura (1958) on climatic adaptation in water and salt metabolism, and concerns the seasonal variations in the blood levels of both inorganic phosphate and calcium. Effects of alterations in environmental temperature have in particular been studied, since seasonal variations in temperature profoundly affect physiological activity. Since phosphate and calcium metabolism are largely regulated by the parathyroid, seasonal variations in the activity of this gland have also been investigated.

METHODS

Experimental procedure for investigating seasonal effects. These experiments were carried out between February 1954 and January 1955 on four male adults aged from 26 to 33 years. The subjects stayed in the laboratory for 2 or 3 days towards the end of each month, except at the end of February, May, August and October when they stayed for about a week. The food given in these experimental periods varied very little from month to month, and especially in the experiments of one-week duration, when the P and Ca intakes were kept strictly constant (2·44 and 0·76 g per day respectively). During these periods of constant ration, the urine was collected daily and inorganic P and Ca determined by Gomori's (1942) and Shohl & Pedley's methods (1922) respectively (see also Hawk, Oser & Summerson, 1951). Blood samples were collected in the morning, when the subjects were in a basal state, two or three times during each month. Serum inorganic phosphate was determined according to the method of Gomori (1942) and total Ca by Clark & Collip's method (1925) (see also Hawk *et al.* 1951). The specific gravity and the Na, Cl and K contents of the serum measured have been reported previously (Yoshimura, 1958).

Procedure for investigating effects of heat. Two subjects who had been living in cold rooms during the winter were kept in a thermostatically controlled hot room at 30° C for about 3 weeks, except for school attendance for 8 hours each day. The food intake was kept constant, the daily P and Ca intakes being, respectively, 1·74 and 0·96 g. Blood was collected in the morning at intervals of 2 or 3 days before and during their stay in the hot room. Urine was collected every other day and analysed as described above.

Determination of parathyroid activity. According to Crawford, Osborne, Talbot, Terry & Morrill (1950), the ratio of phosphate reabsorbed by renal tubules (T.R.P.) to that filtered through the glomerulus (G.F.P.) is very useful as an inverse index of parathyroid activity. This method has been used to compare the parathyroid activities of the same subjects in summer (August) and winter (February). To accentuate the seasonal changes in parathyroid activity phosphate was administered orally to fasting subjects and the change in T.R.P./G.F.P. after this phosphate load was determined. The experiment was repeated twice in summer and in winter on the same subjects. To estimate G.F.P. the glomerular filtration rate (G.F.R.) was determined by measuring the endogenous creatinine clearance, and this was then multiplied by the serum concentration of inorganic P. By subtracting the amount of inorganic P excreted per minute in the urine from G.F.P., the renal phosphate reabsorbed, i.e. T.R.P., was calculated. Creatinine was measured in serum and urine by Folin's method (Hawk *et al.* 1951). Further details of these experiments are given in the Results section.

RESULTS

Seasonal variations in serum inorganic phosphate and calcium

The concentrations of serum inorganic P and total Ca in four subjects at monthly intervals are presented in Fig. 1. The mean atmospheric temperature during the last 10 days of each month is also shown. It is evident that the concentration of inorganic P in serum runs parallel with the atmospheric temperature, increasing in summer and decreasing in winter. Serum concentrations of Ca on the other hand are inversely related to seasonal variations in atmospheric temperature. The mean values in summer and winter are presented in Table 1.

The concentration of serum inorganic P shows a seasonal variation from the annual mean, of +27·6 % in summer to −17·9 % in winter, whereas the total Ca concentration shows a variation of from −3·1 to +3·5 % from the mean. An inverse relationship between serum Ca and inorganic P has already been demonstrated by many authors; and Peters & Eiserson (1925) derived the following equation from clinical data to correlate this relationship between the concentrations of total Ca and inorganic P in serum (mg/100 ml.) and serum protein (g/100 ml.) (see Peters & Van Slyke, 1931).

$$\mathrm{Ca} = 0{\cdot}255\mathrm{P} + 0{\cdot}556\ \mathrm{Protein} + a,$$

where '*a*' is a constant which equals 7, according to Peters & Eiserson (1925). When the present results for inorganic P and total Ca were applied to the above equation, the serum protein being calculated from serum specific gravity determined by the $CuSO_4$ method, the correlation was better when 8·09 rather than 7 was used for the value of constant '*a*'.

Urinary outputs of inorganic phosphate and Ca are summarized in Table 2.

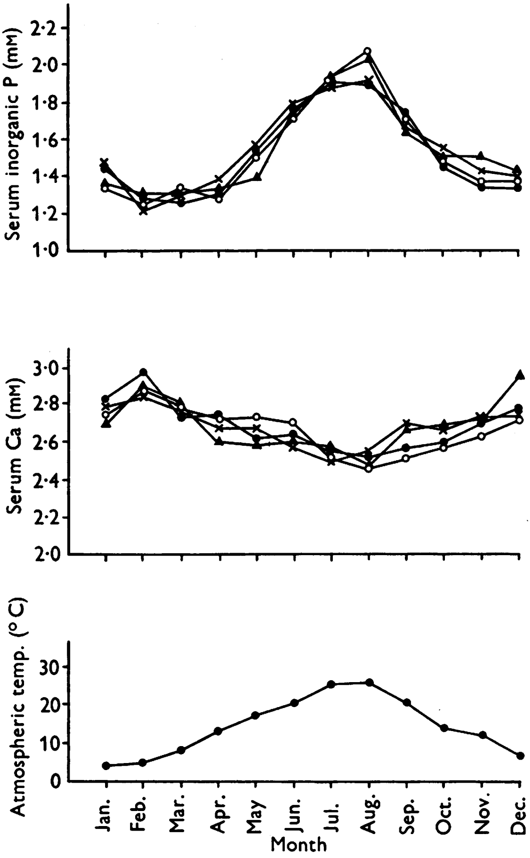


Fig. 1. Seasonal variations in serum inorganic phosphate and calcium. Subjects: T.Y. ▲—▲, J.K. ●—●, M.I. ×—×, S.O. ○—○.

TABLE 1. Seasonal variations in serum calcium and inorganic phosphate concentrations

Serum inorganic phosphate (mM)			Serum calcium (mM)		
Whole year	Summer	Winter	Whole year	Summer	Winter
1·56 ±0·30	1·99 ±0·13	1·28 ±0·10	2·59 ±0·13	2·51 ±0·09	2·68 ±0·13

The values in summer and winter are, respectively, the mean values for June, July and August, and for December, January and February.

Values are means ±S.D. for nine subjects, four participating in the present investigation and five in previous experiments (Yoshimura, 1958). Differences between summer and winter are statistically significant at the 1% level.

Although no consistent seasonal variation could be found in phosphate excretion, Ca excretion in summer was significantly lower than in the other seasons. McCance & Widdowson (1943) found that the absorption of Ca from the gut and its excretion in the urine was greater in summer than in winter in England. In our work we did not measure the amounts of Ca absorbed, but the intake of Ca by our subjects was fairly constant throughout the year. The reduction in urinary Ca in August cannot, therefore, be explained by a decreased intake or absorption of Ca and must be due to increased excretion elsewhere. As an additional route of Ca output in the hot summer of Japan, sweating is probably the most important. According to Kuno (1956), sweat contains 1·3–4·0 mg Ca/100 ml. and according to St John Lyburn (1956) 5–6 mg Ca/100 ml. Ca excretion in sweat may thus amount to 1 m-mole or more per day in summer, which could account for the reduction in urinary output of Ca.

TABLE 2. Mean urinary excretion of inorganic phosphate and calcium

Subjects	February	May	August	October	Mean
		Inorganic phosphate excretion (m-moles/24 hr)			
M.I.	19·50	19·35	19·08	19·79	19·44
T.Y.	21·53	20·46	15·70	19·85	19·39
J.K.	20·11	18·88	17·98	20·50	19·37
S.O.	17·62	17·20	18·53	18·88	18·06
Mean	19·69	18·98	17·82	19·76	19·07
S.D.	1·40	1·17	1·29	0·58	0·58
		Calcium excretion (m-moles/24 hr)			
M.I.	4·92	4·32	4·12	4·77	4·53
T.Y.	4·84	4·47	4·37	4·39	4·52
J.K.	5·14	4·17	4·04	4·77	4·53
S.O.	4·69	4·34	4·22	4·62	4·47
Mean	4·90	4·33	4·19	4·64	4·51
S.D.	0·16	0·11	0·12	0·16	0·02

Effect of heat on serum and urinary inorganic phosphate and calcium

The effect of heat was examined by comparing the inorganic P and total Ca concentrations in serum and urine before and after residential change from a cold to a hot environment. The results for serum are presented in Fig. 2, and indicate that the concentration of inorganic P rises gradually after entering the hot room, while that of Ca falls slightly. Since the room was illuminated with an ordinary Mazuda lamp, which is somewhat deficient in ultra-violet intensity, the rise in serum phosphate cannot be attributed to increased ultra-violet light; but it may be due to a change in phosphate metabolism produced by heat adaptation. The change in total Ca concentration seems to be a secondary effect, since it appears later than the change in inorganic P.

Alterations in the urinary excretion of these two constituents are illustrated in Figs. 3 and 4. It is apparent from Fig. 3 that phosphate excretion decreases after entering the hot room. Calcium excretion (Fig. 4) did not change appreciably during the first two weeks in the hot room but later tended to increase.

Since Ca is also excreted in the sweat, the total output in urine and sweat is probably significantly increased on exposure to heat. These changes in P and Ca excretion cannot be attributed to altered salt intake, since the food intake was kept constant throughout the experimental period. The reduction in

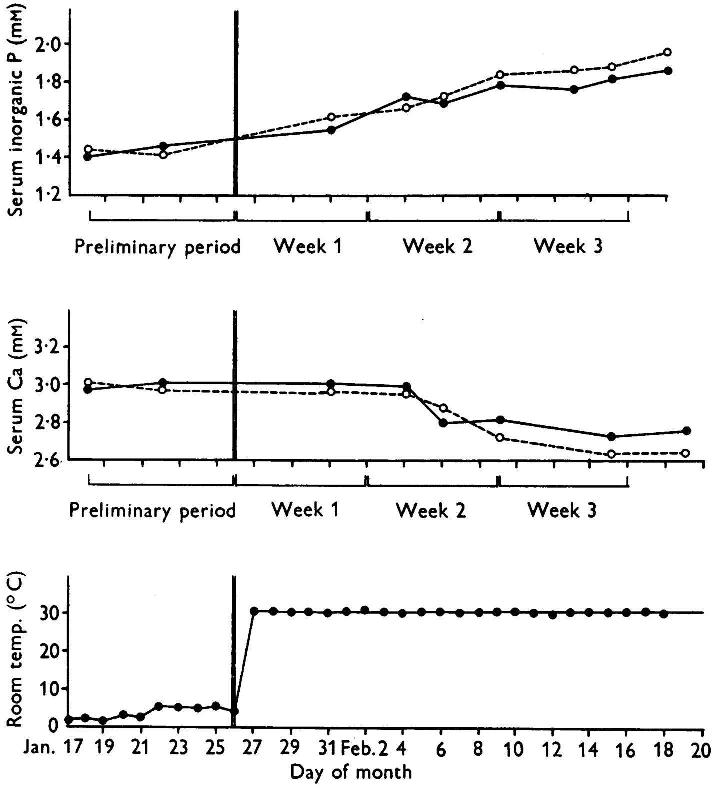
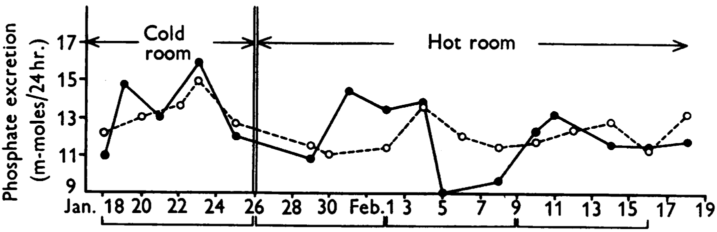


Fig. 2. Effect of heat adaptation on serum inorganic phosphate and total calcium concentrations. The double vertical lines indicate when the subjects entered the hot room. Subjects: M.F. ●—●, H.K. ○--○.



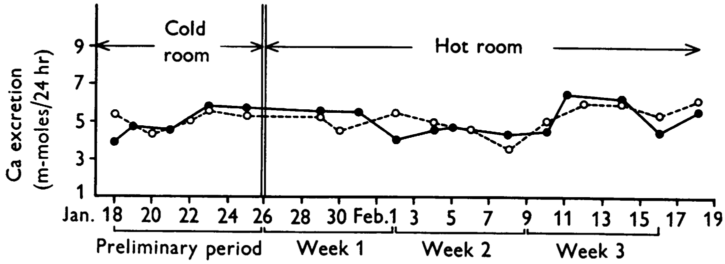
	Preliminary period	Week 1	Week 2	Week 3
Mean for M.F.	13·5	13·0	10·9	12·3
Mean for H.K.	13·3	11·3	12·4	12·1

Fig. 3. Urinary excretion of inorganic phosphate. Subjects: M.F. ●—●, H.K. ○-- ○.

phosphate excretion is presumed to be due to increased reabsorption of phosphate by the kidney, which may be induced by some adaptative mechanism to heat, as suggested above. The change in calcium excretion lags somewhat behind that of phosphate and consequently may be a secondary effect of the increased level of phosphate in serum.

Seasonal changes in parathyroid activity

The parathyroid activity of the same four subjects was examined both in summer and winter under the same phosphate load. The subjects were kept on a standard diet of low phosphate content (0·225 g/day) for a preliminary period of 3 days in order to induce a constant level of phosphate metabolism.



	Preliminary period	Week 1	Week 2	Week 3
Mean for M.F.	4·88	5·03	4·53	5·49
Mean for H.K.	5·08	4·99	4·37	5·59

Fig. 4. Urinary excretion of calcium. Subjects: M.F. ●—●, H.K. ○--○.

Beginning in the morning, each subject then drank a solution of phosphate (7 mM-Na_2HPO_4 and 4·5 mM-NaH_2PO_4, pH 7) at half-hourly intervals for 9–12 hr, at a rate of 0·03 m-moles phosphate/kg/30 min. During this experimental period the subject stayed in bed and both urine and blood were collected at hourly intervals. Calcium, inorganic phosphate and creatinine were determined in urine and serum and from this the glomerular filtration rate, the rate of glomerular filtration of phosphate (G.F.P.) and the tubular reabsorption of phosphate (T.R.P.) were calculated as explained in the Methods section. Results from one subject are illustrated in Fig. 5.

It is evident that the serum phosphate concentration before the phosphate load is higher in summer than in winter. It commences to rise immediately after phosphate administration and reaches a maximum level after several hours. The peak is attained earlier at a lower level in winter than in summer, indicating that the regulation of serum phosphate after phosphate loading is accomplished more effectively in winter. Serum calcium concentration decreases after administration of phosphate, but this is probably a secondary effect of increased phosphate. Urinary excretion of phosphate increases as glomerular filtration of phosphate is accelerated by the rise of serum phos-

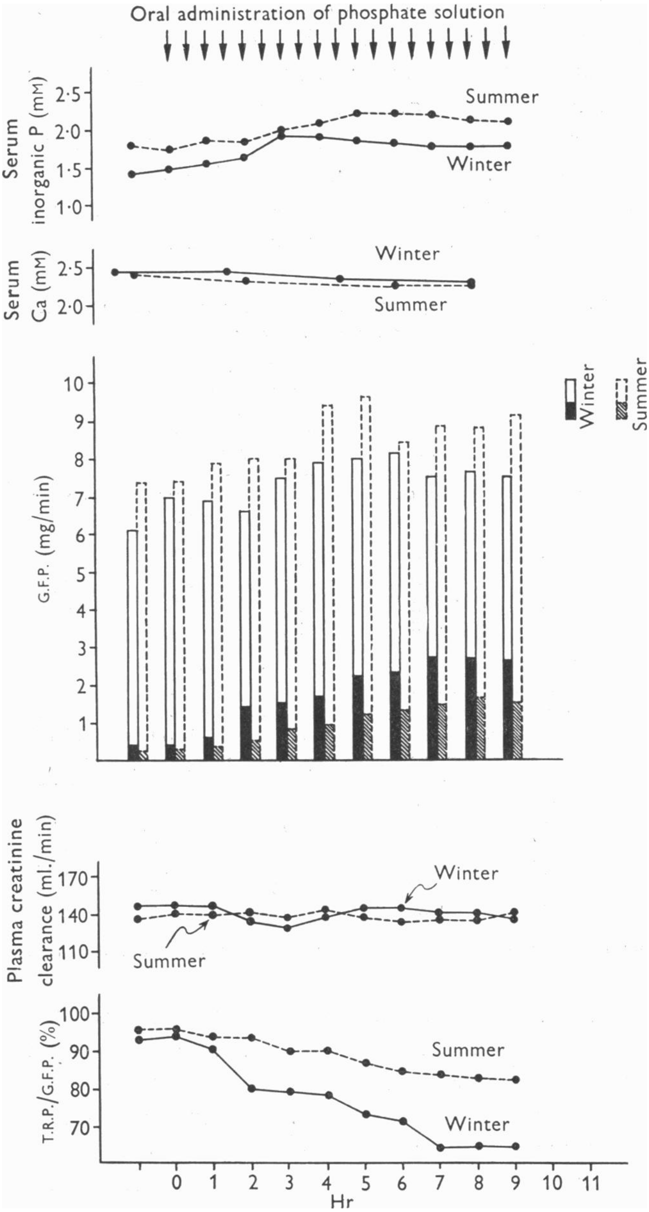


Fig. 5. Phosphate metabolism after a phosphate load. Subject M.I. Starting at 0 hr, he drank a phosphate solution mixture (see text) at 30 min intervals, as indicated by the arrows, at a rate of 0·03 m-moles phosphate/kg/30 min. Histograms of rate of glomerular filtration of phosphate (G.F.P.): these columns are divided into shaded areas representing the rate of urinary excretion of phosphate and unshaded areas representing the rate of tubular reabsorption of phosphate (T.R.P.). The total height of each column represents the glomerular filtration rate of phosphate.

phate, and reaches a maximum value where the urinary excretion tends to balance the amount administered. This increase in urinary phosphate is effected by a reduction in the rate of reabsorption of phosphate by the kidney. Thus T.R.P./G.F.P. declines gradually after phosphate administration and tends to reach a minimum value after 7–10 hr. According to Crawford *et al.* (1950), the value of T.R.P./G.F.P. is an inverse index of parathyroid activity and is accentuated by phosphate loading. Since the parathyroid promotes the excretion of urinary phosphate by inhibiting tubular reabsorption, the serum phosphate level can be regulated within certain limits. It is clear from Fig. 5 that the fall in T.R.P./G.F.P. after phosphate loading is more pronounced in winter than in summer, indicating that parathyroid activity is more sensitive to phosphate loading in winter. Results for the four subjects examined for parathyroid activity are summarized in Table 3, where values of T.R.P./G.F.P. before and after phosphate loading are presented, together with the inorganic phosphate concentration of serum. The value of T.R.P./G.F.P. before phosphate loading shows a tendency to decrease more in winter than in summer, but this difference is not statistically significant. The values after phosphate loading, however, show marked seasonal changes and the decrease in T.R.P./G.F.P. is more rapid and its minimum level is lower in winter than in summer.

TABLE 3. Seasonal changes in serum inorganic phosphate and parathyroid activity

	Basal condition					
	Serum P (mM)		Plasma creatinine clearance (ml./min/m^2)		T.R.P./G.F.P. (%)	
Subjects	Summer	Winter	Summer	Winter	Summer	Winter
M.I.	1·75	1·45	76·8	84·7	95·0	93·7
T.Y.	1·66	1·41	75·7	75·9	95·2	92·3
T.U.	1·76	1·47	63·5	69·4	92·2	90·6
S.O.	1·76	1·36	73·7	81·1	93·3	94·0
Mean	1·73	*1·42	72·4	77·7	93·9	92·7
S.D.	0·07	0·03	5·3	5·8	1·3	1·4

	After phosphate load					
	Maximum serum P (mM)		Rate of decrease of T.R.P./G.F.P. (%/hr)		Minimum value of T.R.P./G.F.P. (%)	
Subjects	Summer	Winter	Summer	Winter	Summer	Winter
M.I.	2·16	1·80	1·66	4·25	82·1	64·2
T.Y.	2·16	1·95	1·69	4·45	83·2	60·9
T.U.	2·21	1·92	1·69	5·53	80·4	61·5
S.O.	2·24	1·85	1·94	3·94	77·2	62·6
Mean	2·19	*1·88	1·74	*4·54	80·7	*62·3
S.D.	0·04	0·05	0·11	0·60	2·3	1·3

* Difference between summer and winter is statistically significant at 1 % level.

DISCUSSION

Seasonal variations in serum inorganic phosphate concentration have been attributed previously to an effect of ultra-violet light (Hess & Lundagen, 1922). Another important contributory factor is high environmental temperature in summer, as has been demonstrated in the present investigation, although the mechanism of this increase is at present unknown. However, the results of experiments carried out in a hot room suggest that some adaptative alteration in phosphate metabolism on exposure to heat initiates changes in the serum phosphate level. Since parathyroid activity undergoes a seasonal change and can control the level of serum phosphate by altering phosphate metabolism (Albright, Bauer, Ropes & Aub, 1929), alterations in parathyroid activity could be one of the most important factors producing seasonal variations in serum phosphate. Parathyroid hormone is known to promote the excretion of phosphate in urine and to reduce the level of serum inorganic phosphate. Since parathyroid activity is higher in winter than in summer, serum inorganic phosphate tends to decrease in winter and to increase in summer. The results of our hot-room experiments support this view. The decrease in urinary phosphate excretion in the hot room may be due to a reduction in parathyroid activity accompanied by increased renal reabsorption of phosphate. This could account for the observed gradual rise of the concentration of phosphate in serum. Since the maximum level of serum phosphate was not attained until about 3 weeks after entering the hot room, the equilibrium state could not be established in this experiment, as is seen in Fig. 2. The fact that the reduction in phosphate excretion continued even after the end of the experimental period (Fig. 3) accords with this view. However, no consistent seasonal alteration could be found in phosphate excretion (Table 2). This can probably be attributed to the fact that the equilibrium state was already established in these experiments.

A similar argument may be applied in the case of calcium. Since Ca excretion in urine is, however, influenced by sweating it may be lower after attainment of the equilibrium state in the hot environment than the control value. This was indeed found to be the case in summer (Table 2).

The reason the parathyroid gland undergoes such a marked seasonal variation is quite obscure. Since we have demonstrated a change in phosphate metabolism as a result of adaptation to heat, it is suggested that alterations in the activity of the parathyroid gland may also be produced by some temperature-adaptive mechanism.

With respect to the physiological significance of the seasonal variations in serum phosphate concentration, little is known except that growth of bone also undergoes seasonal changes in childhood (de Rudder, 1952). Seasonal changes in blood phosphate might also be expected to affect carbohydrate metabolism and energy production.

Seasonal variations in serum calcium seem to be a secondary result of the inverse relationship with serum phosphate and in any case are very small. Changes in the volume of extracellular fluid might be partly responsible for these changes, since this volume increases in summer and decreases in winter (Yoshimura, 1958).

SUMMARY

1. It has been demonstrated on human adult subjects that the serum inorganic phosphate concentration increases by 27·6% in summer and decreases by 17·9% in winter from the annual mean value, whereas the total Ca concentration of serum presents small seasonal changes in the opposite direction.

2. Experiments conducted in a hot room at 30° C have shown that adaptation to heat raises the level of serum inorganic phosphate under conditions where the effect of ultra-violet light can be excluded. To clarify this adaptative change, the activity of the parathyroid gland has been determined in the same subjects in summer and winter, by measuring the ratio of the tubular reabsorbed phosphate to the glomerular filtered phosphate (T.R.P./G.F.P.) after a phosphate load. The results indicated that the activity of the gland is higher and more sensitive to phosphate loading in winter than in summer.

3. It is suggested that the activity of the parathyroid gland undergoes seasonal variations by adapting to heat and cold and that this causes seasonal changes in serum inorganic phosphate.

4. Seasonal variations in the total Ca concentration of serum are considered to be secondary to various other changes.

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