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The effect of diurnal variation on total plasma calcium concentration in normal subjects

M. R. WILLS[1]

From the Department of Chemical Pathology, Bristol Royal Infirmary, Bristol

SYNOPSIS In 12 normal subjects plasma calcium and phosphorus concentrations were estimated during a normal working day. In the individual subjects fluctuations from the fasting values occurred during the period of study, but at no time during that period did the mean values differ significantly from the fasting plasma calcium or phosphorus concentrations. In three of the subjects the study was repeated while fasting during a normal working day. In these subjects the fluctuations in both plasma calcium and phosphorus concentrations were less than during a normal day while taking meals, and there was evidence of a diurnal variation in the plasma phosphorus concentration.

The alternation of daily activity and nocturnal sleep is associated with a circadian rhythm in the concentration of some blood constituents and in the renal output of water and electrolytes (Mills, 1966). Studies of circadian variation in total plasma calcium concentration are conflicting. In fasting subjects some have reported that no fluctuations occur (Greenberg and Gunther, 1930 and 1932; Philpot, 1958), while others have reported changes of 2 or 3 mg per 100 ml during a 24-hour period (Nicolaysen, 1932). Similarly in subjects on a normal diet it has been reported that there are no appreciable variations (Watchorn, 1929; Carruthers, Copp, and McIntosh, 1964), while others have reported marked fluctuations (Philpot, 1958; Chaptal, Jean, Guillamot, and Morel, 1962). Farquharson and Tibbetts (1931), in a study of two healthy adults during ordinary work, found that the plasma calcium concentration was constant in one and varied in the other. In view of these conflicting reports, and as the estimation of total plasma calcium concentration is, at present, one of the most valuable indices of disorders of calcium homeostasis, the study reported here was undertaken in an attempt to define the effect of diurnal variation on total plasma calcium concentration in normal subjects.

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Subjects Studied

Nine males and three females were studied. All were healthy members of the laboratory staff and their ages ranged from 22 to 50 years. During the day of study they followed their normal daily work routine and received a standard diet. The diet was prepared to give a normal content of fat, carbohydrate, and protein with a fixed calcium intake of 1,100 mg/day; this approximately equalled their usual diet and the normal daily adult intake (National Food Survey, 1963). The distribution of the calcium intake (mainly as milk) through the study day was 260 mg at 0915, 140 mg at 1030, 245 mg at 1230, 220 mg at 1530, and 235 mg at 1830 hours.

Routine of Specimen Collection

After an overnight fast blood was collected at 0900 hours and thereafter at approximately three-hourly intervals with a final blood specimen at 2000 hours in nine of the subjects. In the other three subjects blood specimens were col-

[1]Present address: Department of Chemical Pathology, Royal Free Hospital, Lawn Road, London, NW3.

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lected at 1030 and 1300 hours and thereafter at the same time as in the other subjects. The timing of the blood specimens in relation to meals (which were taken at 0915, 1030, 1230, 1530, and 1830 hours) was such as to provide specimens at varying intervals after meals.

Blood samples were collected, venous and without stasis, into heparin containers (sodium heparin 10 units per ml).

Methods

Plasma calcium concentration was estimated by the plasma-Corinth B (Corinth calcium) AutoAnalyzer method of Wills and Gray (1964). The standard deviation of replicate estimations was ±0·05 mg per 100 ml. The specific gravity of the plasma samples was estimated by the copper sulphate drop method of Phillips, van Slyke, Dole, Emerson, Hamilton, and Archibald (1945). The estimated plasma calcium values were corrected to a standard specific gravity of 1·027 as proposed by Dent (1962).

Plasma phosphorus concentration was estimated by the method of Fiske and Subbarow (1925).

Results

The full results for the 12 subjects studied are detailed in Table I, together with the values for the mean and standard deviation (SD) at the times studied. The results through the day have also been compared by Student's t index with the fasting calcium and phosphorus concentrations, and the P values determined. At no time during the day of study did the mean values for plasma calcium or phosphorus differ significantly from the fasting levels. In only two subjects (nos. 2 and 9) were the fasting plasma calcium

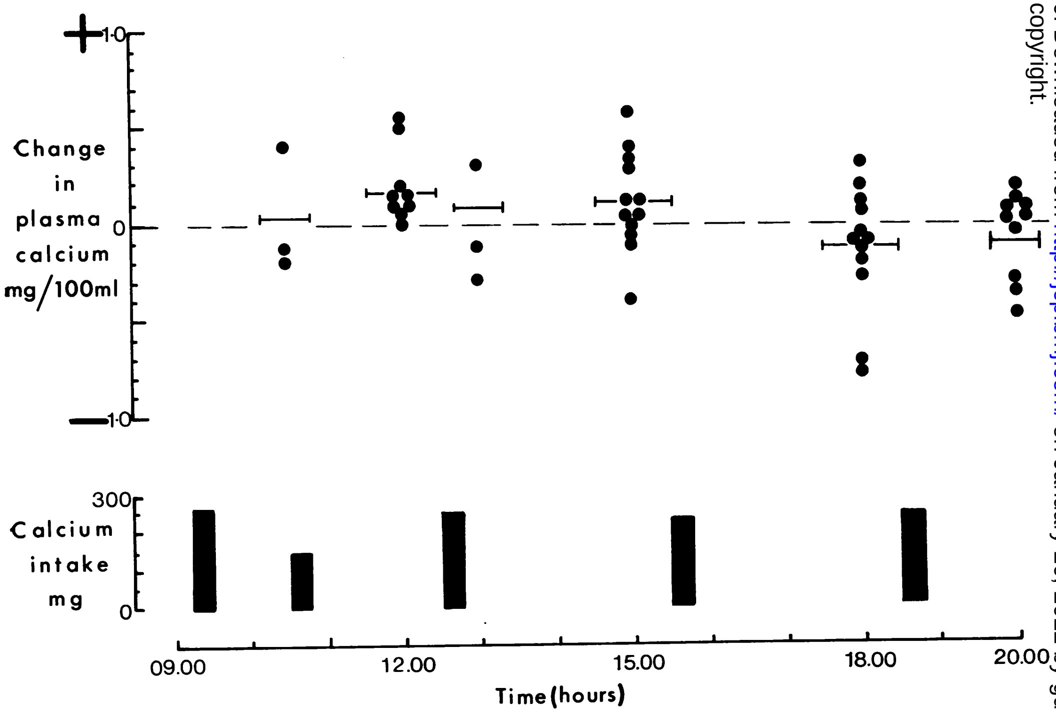

Fig. 1 *Changes in plasma calcium concentration, in 12 normal subjects, during a normal working day plotted as the difference in mg per 100 ml from the 0900 hour fasting concentration ⊢⊣. The mean difference from the fasting concentration at each time is also plotted (—). The dietary calcium intake in mg (black areas) and times taken are also shown.*

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Subject	Sex	Age	Time (hours)														Mean ± SD	
			0900[1]		1030		1200		1300		1500		1800		2000		Ca	P
			Ca[2]	P[2]	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P		
1	F	26	8·70	2·5	—	—	8·70	2·1	—	—	8·70	2·5	8·48	2·5	8·70	2·6	8·66 ± 0·10	2·44 ± 0·19
2	M	36	8·70	3·4	—	—	9·22	4·1	—	—	8·80	4·0	8·98	4·0	8·85	3·5	8·91 ± 0·20	3.80 ± 0·32
3	F	22	9·10	3·8	—	—	9·22	4·1	—	—	9·48	4·5	8·98	4·5	9·18	3·0	9·19 ± 0·19	3·98 ± 0.62
4	F	22	9·42	3·2	—	—	9·45	3·1	—	—	9·70	3·3	9·30	4·2	8·92	5·1	9·36 ± 0·28	3·78 ± 0·86
5	M	29	9·45	3·8	—	—	9·55	4·5	—	—	9·32	4·2	8·70	3·8	9·47	4·5	9·30 ± 0·34	4·16 ± 0·35
6	M	32	9·05	3·3	—	—	9·55	3·6	—	—	9·00	4·1	9·22	4·0	8·72	4·6	9·11 ± 0·31	3·92 ± 0·50
7	M	50	9·62	4·5	—	—	9·72	4·5	—	—	9·72	3·9	9·32	4·6	—	—	9·60 ± 0·19	4·38 ± 0·32
8	M	28	9·32	4·2	—	—	9·45	4·4	—	—	9·32	4·3	9·22	4·6	9·32	4·0	9·33 ± 0·08	4·30 ± 0·22
9	M	26	9·10	4·3	—	—	9·27	3·6	—	—	9·65	3·9	9·15	3·9	9·15	4·5	9·26 ± 0·22	4·04 ± 0·36
10	M	22	9·65	2·8	9·52	3·1	—	—	9·42	3·2	9·62	3·3	9·52	3·8	9·25	3·6	9·50 ± 0·15	3·30 ± 0·36
11	M	23	9·22	3·6	9·62	3·2	—	—	9·52	4·0	9·52	4·5	9·30	5·0	9·15	4·2	9·39 ± 0·19	4·08 ± 0·64
12	M	26	9·60	2·8	9·40	3·6	—	—	9·30	—	9·18	—	8·80	3·4	—	—	9·26 ± 0·30	3·27 ± 0·41
Mean	—	—	9·24	3·52	9·51	3·30	9·35	3·78	9·41	3·60	9·33	3·86	9·08	4·03	9·07	3·96		
±SD	—	—	±0·33	±0·64	±0·11	±0·26	±0·30	±0·79	±0·11	±0·57	±0·35	±0·61	±0·30	±0·65	±0·26	±0·78		
P[3]	—	—	—	—	—	—	>0·40	>0·40	—	—	>0·50	>0·10	>0·20	>0·05	>0·10	>0·10		

Table I *Plasma calcium and phosphorus concentrations in 12 normal subjects during a normal working day*

[1]Fasting specimen.
[2]Concentrations of plasma calcium (Ca) and phosphorus (P) in mg per 100 ml.
[3]Significance of difference of mean at any one time from fasting mean.

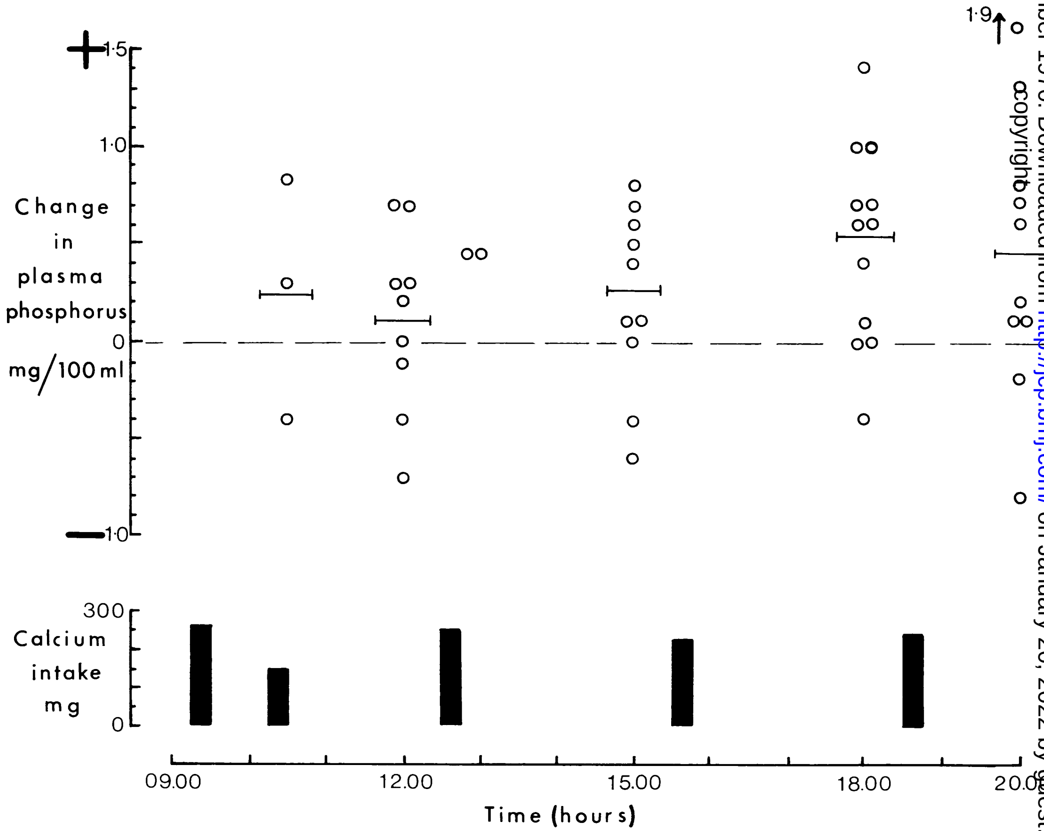

Fig. 2 *Changes in plasma phosphorus concentration in 12 normal subjects during a normal working day plotted as the difference in mg per 100 ml from the 0900 hour fasting concentration. The mean difference from the fasting concentration at each time is also plotted. The dietary calcium intake in mg (black areas) and times taken are also shown.*

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concentrations the lowest values obtained, and in three (nos. 1, 10, and 12) the fasting values were the highest values obtained. In only four of the subjects (nos. 5, 6, 10, and 12) were the fasting plasma phosphorus concentrations the lowest values obtained. In each subject the difference, or change in the concentrations of both calcium and phosphorus from the fasting value were also calculated, and these are shown plotted in Figures 1 and 2 respectively together with the mean difference at each of the times studied.

The study was repeated in three subjects (nos. 1, 2, and 6) who fasted from the preceding evening throughout a normal working day. Owing to hunger symptoms the study was stopped in all the subjects at 1700 hours. The full results are detailed in Table II. The changes in the concentrations of both calcium and phosphorus from the 0900 hour value are shown in Figure 3. In these subjects there were virtually no fluctuations in plasma calcium concentration, although fluctuations did occur in plasma phosphorus concentration. In view of the small number of subjects these values were not compared statistically.

Subject	*Time (hours)*								*Mean*	
	0900		*1200*		*1500*		*1700*		*Ca*	*P*
	Ca[1]	*P*[1]	*Ca*	*P*	*Ca*	*P*	*Ca*	*P*		
1	8·72	2·5	8·62	2·7	8·85	2·6	8·82	2·7	8·75	2·63
2	9·65	4·8	9·65	4·1	9·80	4·5	9·80	5·0	9·73	4·60
6	9·22	3·7	9·12	3·5	9·22	3·3	9·22	4·1	9·20	3·65
Mean	9·20	3·67	9·13	3·43	9·29	3·47	9·28	3·93		

Table II *Plasma calcium and phosphorus concentrations in three normal subjects while fasting during a normal working day*

[1]Plasma concentrations of calcium (Ca) and phosphorus (P) in mg per 100 ml.

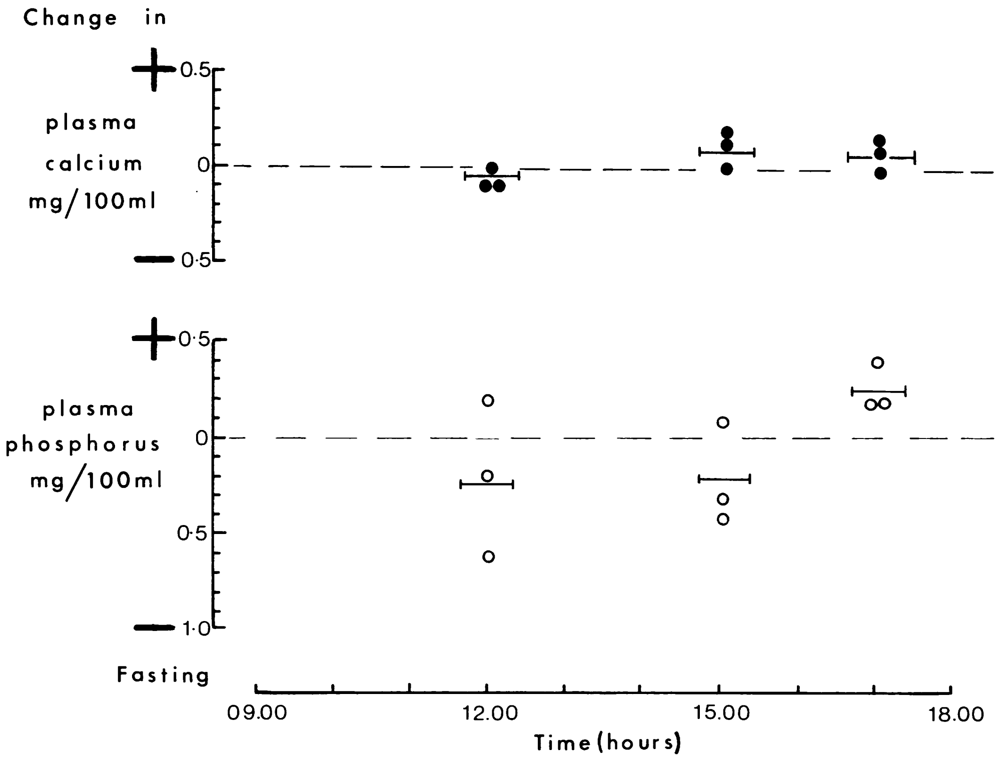

Fig. 3 *Changes in plasma calcium and phosphorus concentrations in three normal subjects while fasting during a normal working day. The changes are plotted as the difference in mg per 100 ml from 0900 hour concentrations.*

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Discussion

In all the normal subjects studied some degree of fluctuation occurred in both the plasma calcium and phosphorus concentrations during a normal working day. At no time during the day of study did the mean values for all the subjects differ significantly from the mean fasting values for either the plasma calcium or phosphorus concentration. In the individual subjects the fluctuations in plasma calcium concentration from the fasting value ranged from a maximum of +0·55 mg per 100 ml (1500 hours in subject 9) to −0·80 mg per 100 ml (1800 hours in subject 12). These fluctuations were presumably due to the oral intake of calcium in the diet as in the three subjects who were studied while fasting during a normal working day the fluctuations in plasma calcium concentration relative to the 0900 hour concentration ranged from +0·15 to −0·10 mg per 100 ml. The individual fluctuations from the fasting value in plasma phosphorus concentration, during a normal working day, ranged from +1·9 mg per 100 ml (subject 4 at 1800 hours) to −0·8 mg per 100 ml (subject 3 at 1800 hours). These fluctuations were not as markedly reduced by fasting as were the fluctuations in calcium concentration. The fluctuations in the plasma phosphorus concentration that did occur during both the normal and the fasting days were relatively large when compared with the total plasma concentration. The results suggest that the time of day at which blood samples are collected for the estimation of total plasma calcium concentration does not cause an important difference in the estimated value when compared with the fasting concentration. As dietary calcium intake caused fluctuations in the total plasma calcium concentration in any one individual subject, fasting samples should always be collected when the therapeutic effects of different regimens are being studied.

The findings reported here are in accordance with some but not all of the previous reports in the literature. Greenberg and Gunther (1930, 1932) reported that there were no circadian changes in plasma calcium concentration in fasting subjects. Watchorn (1929) found no appreciable variation in plasma calcium concentration throughout a day, and they concluded that it was 'not necessary to ensure that the subject is fasting when blood is taken'. Farquharson and Tibbetts (1931), in a study of two healthy adults during ordinary work and on a normal diet, reported that the plasma calcium concentration was constant in one, while in the other the values varied between 9·4 and 10·4 mg per 100 ml during the day. Nicolaysen (1932) reported fluctuations of 2 to 3 mg per 100 ml in fasting subjects during a 24-hour period. Philpot (1958), in one of his subjects, reported fluctuations in the plasma calcium concentration between 10·1 and 11·0 mg per 100 ml during a normal day with a variation of 9·7 to 10·0 mg per 100 ml during a day of fasting. Chaptal *et al* (1962) in one normal subject, on a normal diet, reported considerable fluctuations in the plasma calcium concentration over a 24-hour period. In 12 normal subjects Wesson (1964) reported that there was a 'poorly defined but statistically significant cycle' in the plasma calcium concentration. Carruthers *et al* (1964), in a study of circadian variations in five normal individuals under standardized conditions of calcium intake, reported that 'a remarkably constant plasma calcium level was observed in all subjects'. In four normal subjects Hodgkinson and Heaton (1965) reported that there was no consistent change in serum calcium or inorganic phosphorus concentrations during fasting. Briscoe and Ragan (1966) in a study of seven patients over three days reported that there was a 'slight but consistent' diurnal variation in serum calcium concentration with lower values in the morning. In the subjects reported here, although fluctuations did occur in plasma calcium concentrations, the mean values did not differ significantly from the fasting concentration during the study period.

Circadian variations in plasma phosphorus concentration have been well established with low values in the morning followed by an increase in the afternoon and evening (Wesson, 1964; Carruthers *et al*, 1964; Mills, 1966). The results in the three fasting normal subjects (Fig. 3) are in accordance with these earlier reports.

Plasma calcium concentration in man is regulated in a precise manner primarily by parathyroid hormone and calcitonin probably under a feedback control mechanism (Copp, 1969), although the physiological significance of calcitonin in normal homeostasis is not at present well established (Copp, 1969). It has been reported that the absolute concentration at which the plasma calcium concentration is maintained is related to total dietary calcium intake, with significantly lower values in normal subjects on a low calcium diet compared with a normal calcium diet (MacFadyen, Nordin, Smith, Wayne, and Rae, 1965). Recently however, Phang, Berman, Finerman, Neer, Rosenberg, and Hahn (1969) have reported results in disagreement with these observations, in that they found no significant difference in serum calcium concentrations in normal subjects while taking normal and either high or low calcium intakes. The precise control mechanisms of total plasma calcium concentration and the 'rigidity' of the control shown in the study reported here requires further investigation. The recent hypothesis of Nordin and Peacock (1969) that this control is undertaken through the action of parathyroid hormone on the kidneys is of particular interest in this respect.

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