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Variation in Plasma Calcium with Induced Changes in Plasma Specific Gravity, Total Protein, and Albumin

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Summary

The relationship of plasma calcium levels to changes in plasma specific gravity, total protein, and albumin induced by venous stasis was investigated. Factors were derived for adjusting calcium results to offset the effects of variation in protein concentration and thus to make them of increased discriminatory value to the clinician. The validity of an existing specific gravity correction has been substantiated, but a more exact adjustment of 0·23 mg/100 ml of calcium for every 0·001 change in specific gravity is proposed. We recommend for automated laboratories that the factor based on albumin be used: 0·09 mg/100 ml of calcium should be subtracted from the total calcium value for every increase of 0·1 g in albumin above 4·6 g/100 ml, and a corresponding addition should be made for values of albumin below 4·6 g/100 ml.

Using a calcium specific electrode, it has been shown that the ionized calcium concentration does not alter with prolonged venous stasis.

Introduction

Calcium is present in three forms in the plasma—ionized, complexed, and protein-bound. The physiologically important fraction is the ionized calcium, but hitherto its measurement has been difficult and impracticable for a routine laboratory. Total plasma calcium is easier to determine but may not give a true index of the ionized component, because the protein-bound calcium varies independently and to a greater extent. Normally between 30% and 50% of the calcium is protein-bound (Toribara *et al.*, 1957; Loken *et al.*, 1960), depending on the method of determination. This proportion varies when serum protein concentration is altered as a result of disease (Prasad and Flink, 1958), change in posture (Pedersen, 1972), or prolonged venous occlusion (Philpot, 1958). At University College Hospital we have for some years used a factor based on plasma specific gravity to correct for protein variation (Philpot, 1958; Dent, 1962). The original work on which this correction was based was derived from relatively few patients, however, and has not been

published in detail (C. E. Dent, personal communication). Specific gravity varies mainly with total protein concentration but is also influenced by abnormally high sugar, urea, and lipid levels. The usual manual technique employed for its measurement is not very sensitive because a change of one unit in the third decimal place corresponds to a change in total protein of between 0·3 and 0·4 g/100 ml (Varley, 1967). This compares unfavourably with the precision of analyses achieved by automated procedures for the determination of serum total protein and albumin, which can be measured to within 0·1 g/100 ml.

There is a linear relation between total calcium and total protein (Gutman and Gutman, 1937; van Leeuwen *et al.*, 1961) and between total calcium and albumin (Moore, 1971). It follows that there must be a similar linear relation with specific gravity. We have tried here to derive and compare factors for correcting calcium results based on specific gravity, total protein, and albumin measurements.

Methods

Blood was taken from 25 healthy male doctors and medical students, aged between 21 and 35, both before and after venous stasis. The venous return was obstructed for 15 minutes by applying a sphygmomanometer cuff to the arm and maintaining a pressure of 90 mm Hg. Heparinized plasma was separated within five minutes for calcium and specific gravity estimations. Total protein and albumin were determined on serum samples.

Total calcium was measured by an E.G.T.A. (1, 2-Bis-2-aminotheoxyethane-NNN'N'-tetra-acetic acid) Zincon method (Halse, 1967) with a within-batch coefficient of variation of 1·6%. Specific gravity was measured by the copper sulphate method (Phillips *et al.*, 1950). Total protein was determined by a modified biuret reaction,* with a coefficient of variation of 1·2%. Albumin was measured by a method using bromcresol green (Northam *et al.*, 1967) with modifications to sample volume and buffer concentrations and with a coefficient of variation of 2·0%. For the last two procedures purified human albumin (Behringwerke) was used for preparing the standards. In seven of the subjects ionized calcium was also measured with a calcium specific electrode (Orion calcium-selective, flow-through electrode, model 98/20) with a coefficient of variation of 2·8%. For each subject both the specimens taken before and those taken after venous stasis were analysed in the same batch.

Methods of Statistical Analysis and Results

The change in total calcium with change in specific gravity, total protein, and albumin concentration induced by venous stasis is shown by a series of lines in figs. 1, 2, and 3. The data were analysed assuming there was a series of parallel lines which described each subject's change in calcium concentration with

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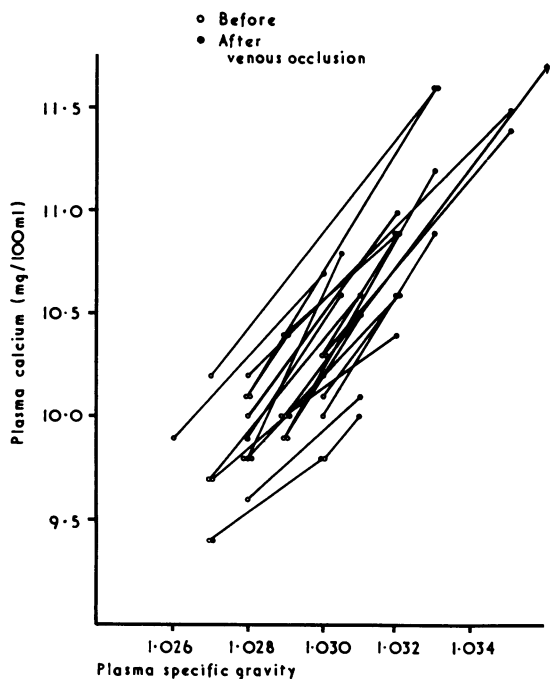*Technicon AutoAnalyzer II, Methodology AAII-14.

FIG. 1—Variation in plasma calcium with induced changes in specific gravity.
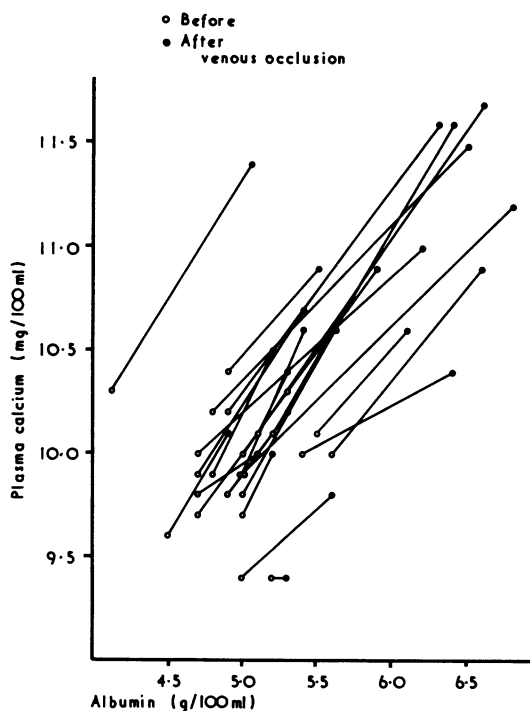

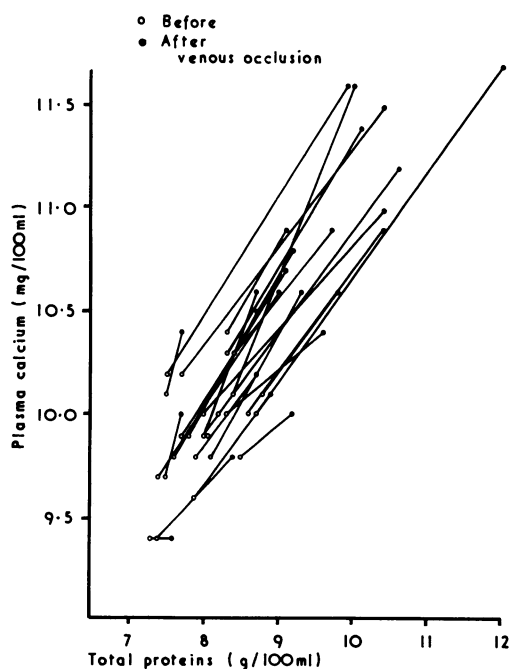FIG. 3—Variation in plasma calcium with induced changes in albumin.

FIG. 2—Variation in plasma calcium with induced changes in total protein.

change in the other value. The analysis of covariance technique was used and the consequent F-tests showed in each case that the average slope was significantly different from zero (P <0.001) —that is, there was a genuine correlation between total calcium levels and specific gravity, total protein, and albumin. Furthermore, there were significant differences in the positions of the lines for each subject (P <0.001)—that is, the data were better described by a series of parallel regression lines (method A) than by a single regression line (method B). We also employed method B to compare our results with other published data. The average slope of the series of parallel regression lines was calculated with its standard error (S.E.). The results were expressed in the form

$$Y = mX + C$$

where Y = total calcium, X = specific gravity, total protein, or albumin, m = slope or regression coefficient, and C = constant denoting the position of the line. The results with the slope calculated by the two methods mentioned above are shown in table I: 95% confidence limits of these slopes (m) are approximately given by m ± 2 × S.E. The results of the ionized calcium measurements with the total calcium values both before and after venous stasis are compared in table II. It is apparent that there was no significant difference between the ionized calcium levels, while the total calcium increased between 0·1 and 0·8 mg/100 ml. Comparison between the regression coefficients of calcium on total protein and albumin derived by other workers and by ourselves are shown in table III.

TABLE I—Relation between Total Calcium (y) and Plasma Specific Gravity, Total Protein, and Albumin (x) in the Form Y = mX + C

y	x	Slope m		S.E. of m		Accuracy of Estimation of m(%) $\frac{2 \times \text{S.E.}}{m} \times 100$	
		A	B	A	B	A	B
Total calcium (mg/100 ml)	Specific gravity	0·23	0·23	0·010	0·008	8·7	7·1
Total calcium (mg/100 ml)	Total protein (g/100 ml)	0·54	0·50	0·025	0·019	9·3	7·6
Total calcium (mg/100 ml)	Albumin (g/100 ml)	0·91	0·71	0·045	0·035	9·9	9·8

A = Data described by a series of parallel regression lines, n = 25.
B = Data described by a single regression line, n = 50.
m = Slope or regression coefficient.
C = Constant denoting the position of the line.

TABLE II—*Plasma Total Calcium and Ionized Calcium Concentrations (mg/100 ml) in Seven Subjects Before and After Venous Stasis*

Subject	Before Stasis		After Stasis	
	Total	Ionized	Total	Ionized
1	9·4	4·4	10·1	4·4
2	9·8	4·3	10·6	4·3
3	9·9	4·3	10·0	4·3
4	9·1	4·1	9·3	4·1
5	10·0	4·4	10·5	4·4
6	9·7	4·1	10·5	4·2
7	9·6	4·3	10·0	4·3

TABLE III—*Comparison of Values Found for the Regression Coefficient (m) of Total Plasma Calcium on Total Protein and Albumin (Data Converted to Units of Calcium mg/100 ml and of Total Protein and Albumin g/100 ml)*

Y	X	m	Reference
Calcium	Total Protein	0.75	McLean and Hastings (1935)
,,	,,	0.59	Hopkins et al. (1952)
,,	,,	0.53	Toribara et al. (1957)
,,	,,	0.52	Fanconi and Rose (1958)
,,	,,	0.60	van Leeuwen et al. (1961)
,,	,,	0.60	Breen and Freeman (1961)
,,	,,	0.52	Hanna et al. (1964)
,,	,,	0.49, 0.57*	Pedersen (1972)
,,	,,	0.50, 0.54*	Present Study
Calcium	Albumin	0.7–0.9	Gutman and Gutman (1937)
,,	,,	0.70	Fourman and Royer (1968)
,,	,,	0.64–0.75	Orrell (1971)
,,	,,	0.76	Moore (1971)
,,	,,	0.73, 0.93*	Pedersen (1972)
,,	,,	0.71, 0.91*	Present Study

*Data analysed by method B (see text).

Discussion

During venous stasis ionized calcium, being diffusible, should not alter appreciably in concentration; this was confirmed in seven subjects using the calcium-specific electrode. The protein-bound non-diffusible calcium fraction, however, becomes more concentrated, causing a corresponding increase in the total calcium concentration. The slope and length of the lines connecting the plasma calcium values (figs. 1, 2, and 3) reflect the permeability of the subjects' capillaries to plasma proteins during the test procedure and the binding of calcium to the retained proteins. Prolonged venous obstruction was used in this study to obtain a wide separation of the initial and final calcium and protein values. This would be expected to reveal the full extent of the within-individual variation and also to minimize the effect of analytical error.

In the derivation of the average slopes (table I, method A) we have assumed that though the positions of the lines were different they were in fact parallel. Nevertheless, analysis of table V from the paper of van Leeuwen et al. (1961) suggested a small but genuine difference in the slopes and possibly no difference in the positions of the lines for calcium against total protein. There is no comparable data for specific gravity or albumin.

It can be seen from table I that the regression coefficient (m) was of the same magnitude for specific gravity and total protein whether the results were analysed as paired or single values (methods A and B respectively). The albumin correction factor however, was 0·71 or 0·91 depending on the method of statistical analysis. This discrepancy was due to the wider spread of the albumin regression lines.

The regression coefficients for total protein and albumin found by other workers are shown in table III. Our value of 0·54 for total protein compares well with that calculated by others, but most of these derive a figure of around 0·7 for albumin. The study by Pedersen (1972) on the postural changes in serum calcium, total protein, and albumin provides a set of graphs similar to our figs. 2 and 3. Pedersen's data, on replotting, may be treated in exactly the same way as our own. The regression coefficient by analysis A is 0·93 (allowing for a change in units) and by analysis B is 0·71. Pedersen does not state how he analysed his data, but gives a value of 0·73 for his regression

coefficient of calcium to albumin. The calculations used by other workers to obtain a value of 0·7 were based on one reading per patient. We consider the higher regression factor of 0·91 to be more relevant as it gives the within-subject relation between calcium and albumin.

Justification for accepting our figure of 0·91 can also be derived as follows: assuming that albumin forms a relatively constant proportion of total protein and that the binding of calcium to protein does not vary, then the following relationships may be obtained:

$$Y = m \text{ (total protein)} + C$$
$$Y = m' \text{ (albumin)} + C$$

Therefore m (total protein) = m' (albumin).

The mean total protein and albumin concentrations in our series were 7·7 g/100 ml and 5·0 g/100 ml respectively. Substituting these figures together with our m values we find:

$$m \text{ (total protein)} = 0·54 \times 7·7 = 4·16$$
$$m' \text{ (albumin)} = 0·91 \times 5·0 = 4·55.$$

In a much larger series of 500 men and 500 women blood donors aged between 18 and 65 the mean total protein and albumin concentrations were 7·5 g/100 ml and 4·6 g/100 ml respectively (Flynn et al., unpublished). If these figures are substituted then the values are even closer:

$$0·54 \times 7·5 = 4·05$$
$$0·91 \times 4·6 = 4·19$$

As these two products are very similar it follows that our correction factor of 0·91 based on albumin must be valid. From our results the following correction factors may be derived:

calcium changes by 0·23 mg/100 ml for every 0·001 change in specific gravity;

calcium changes by 0·054 mg/100 ml for every 0·1 g/100 ml change in total protein;

calcium changes by 0·091 mg/100 ml for every 0·1 g/100 ml change in albumin.

The previous correction factor for specific gravity of 0·25, as proposed by Dent (1962), has now been accurately recalculated to 0·23. The accuracy of the estimations of the mean slopes, and thus the correction factors in table I, was given by 2 × S.E., expressed as a percentage of m. Using method A analysis the specific gravity correction was slightly more precise than that for total protein and albumin. This difference was accentuated with method B analysis. For the reasons stated above, method A was preferred and we felt that the figures (between 8·7% and 9·9%) were of the same magnitude and therefore no one factor could be recommended as the best.

For routine use the factors based on total protein and albumin are more attractive because these tests can be automated and are therefore less time consuming. They are also inherently capable of at least three to four times the precision of the manual specific gravity method. When automated methods for protein and albumin determinations are not available, however, the specific gravity correction is easy to apply.

We suggest that a factor based on albumin be used to correct plasma calcium. Calcium is thought to bind predominantly with albumin (Moore, 1971; Fourman and Royer, 1968) and consequently the factor is likely to remain valid even when there is pathological alteration of the globulins (Moore, 1971). Further, as more information is obtained about the functions and the methods of assay of specific blood proteins total protein estimations are likely to become of less clinical importance. In order to apply the calcium correction factor it is necessary to have an arbitrary reference point for albumin concentration with which all individual values can be compared. We have chosen the mean level of 4·6 g/100 ml as found by Flynn et al. (1973) in the survey quoted above. We recommend that calcium should be corrected to an albumin concentration of 4·6 g/100 ml, subtracting 0·09 mg/100 ml from the plasma calcium value for every increase in albumin of 0·1 g/100 ml and adding 0·09 mg/100 ml for every decrease in albumin of 0·1 g/100 ml. Such a correction could be applied automatically if a computer was used to process the results. Further work is needed to assess the usefulness of the correction in clinical practice.

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Interpretation of Serum Calcium in Patients with Abnormal Serum Proteins

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Summary

Two hundred consecutive specimens received in this laboratory for "liver function tests" showed a wide range of abnormal protein concentrations. Calcium concentration correlated closely with albumin (r = 0·867) but less closely with total protein (r = 0·682). A simple formula for adjusting calcium concentration was derived from the regression equation of calcium on albumin. Adjusted calcium = calcium − albumin + 4·0, where calcium is in mg/100 ml and albumin in g/100 ml.

Low calcium concentrations were found in 49 (24·5%) and raised concentrations in six (3%) of the 200 blood specimens taken for liver function tests. After adjustment, the 95% limits of the observed range were identical with the 95% limits of the normal range determined in this laboratory. Unlike adjustments based on total protein or specific gravity, the adjustment on albumin in 39 specimens which showed hypergammaglobulinaemia on electrophoresis gave normal calcium concentrations.

Introduction

The total calcium of serum or plasma is nearly all accounted for as calcium bound to protein and as calcium ions in roughly equal proportions, and it is the latter which are clinically important (McLean and Hastings, 1935). Measurement of ionized or diffusible calcium is at present technically complex (Rose, 1972; Rushton et al., 1973), so that a screening procedure to detect patients with abnormal protein concentrations and to allow for the effect of changes in protein-bound calcium on the measurement of total calcium is desirable (Dent, 1962).

There are statistically significant correlations between calcium and total protein and between calcium and albumin in normal subjects, but the correlation coefficients are low (less than 0·4) and because of the limited ranges of the observed values the regression lines cannot be extrapolated with confidence to low protein concentrations (Keating et al., 1969; Williams et al., 1973). For this reason we have examined the relation between calcium and total protein and between calcium and albumin in patients who show a wide range of abnormalities of protein concentration but who are likely to have a low prevalence of disorders of calcium homoeostasis.

Material and Methods

Two hundred consecutive serum specimens received in the laboratory with a request for "liver function tests" but with no request for calcium estimation were analysed for calcium in addition to total protein and albumin on a Technicon S.M.A. Plus using cresolphthalein complexone for calcium, biuret for total protein, and bromocresol green in succinic acid buffer pH 4·2 for albumin. Duplicate analyses were not done. Measurements (in mg/100 ml and g/100 ml) were made to one decimal place. The specimens included all those received from wards, outpatient departments, and general practitioners, but excluded those from the department of renal medicine in this hospital because many of their patients were receiving therapy which would affect calcium concentration. No account was taken of age, sex, or provisional diagnosis. The between-batch coefficients of variation at normal serum concentrations during the period of the investigation were 1·2% for calcium,

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