

Adjusted calcium conflict resolved?

Differing effects on plasma total calcium of changes in plasma albumin after venous stasis or myocardial infarction

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SUMMARY Others have challenged the concept of adjusting total plasma calcium for albumin concentration on the grounds that after the application of a tourniquet the increase in calcium for a given increase in albumin differs significantly between normal individuals. We have confirmed this finding.

In contrast, we have found that after myocardial infarction the fall in calcium for a given fall in albumin does not differ significantly between patients. Thus the adjustment of calcium for albumin using a single equation remains valid in patients with changes in albumin due to disease.

We recommend that for consistent results blood should be taken with the minimum of venous stasis even though the patient's calcium is to be adjusted for albumin.

Ideally, plasma ionised calcium should be measured directly if a disturbance of calcium homeostasis is suspected. However, there are practical difficulties: direct measurement requires the rapid analysis of an anaerobic blood sample, and the rate of analysis with instruments at present available is slow, so that most hospital laboratories are unable to make direct measurements on each of the large number of patients with a potential calcium problem. Thus some form of screening is needed.

Screening tests for alterations in ionised calcium are based on making an allowance for protein-bound calcium (Berry *et al.*, 1973; Payne *et al.*, 1973) and are applied only to patients with no abnormality of blood pH. Normally, less than half of total calcium is protein bound, principally to albumin. An assumption inherent in methods which adjust total calcium according to albumin concentration is that, in the individual in whom ionised calcium remains constant, there is a linear relationship between total plasma calcium and albumin when albumin changes. A further assumption is that the slope of the regression of calcium on albumin does not differ significantly between individuals so that a single equation can be used for all patients.

Phillips *et al.* (1977) have demonstrated that individuals may show significant differences between

the slopes of their regression lines of calcium on albumin when albumin is increased after venous occlusion by the application of a tourniquet. We discuss later our reasons for believing that the increase in albumin resulting from venous occlusion does not affect plasma calcium in the same way as the fall in albumin in disease states. We have therefore compared the regressions of calcium on albumin after venous occlusion in normal subjects with the regressions obtained in patients who have a progressive fall in plasma albumin after myocardial infarction.

Patients and methods

Changes in plasma calcium and albumin after the application of a tourniquet were studied in six normal volunteers. After an overnight fast cannulae were inserted into the antecubital veins of both arms of the subjects, who were seated throughout the experiment. Heparin, which was used to prevent clotting within the cannulae, was withdrawn with 5 ml of blood and discarded before the blood samples were collected into lithium heparin tubes. Samples were taken from both arms simultaneously at 0, 5, 10, and 15 minutes after occlusion. The veins of one arm were occluded with a sphygmomanometer cuff immediately after the collection of the initial sample, and the pressure was maintained at

10 mm Hg below the systolic pressure until after the last sample had been collected. During occlusion both fists were clenched briefly every 10 seconds.

Changes in plasma calcium and albumin after myocardial infarction were studied in 15 patients. Each had a typical history, demonstrated Q-waves on the electrocardiograph, and had typical rises in serum aspartate transaminase (AST;SGOT). Blood samples were collected with minimum venous occlusion at 0800 hours after an overnight fast for seven consecutive days after admission to hospital. Plasma was separated within one hour of collection and stored deep frozen.

Calcium was measured by an atomic absorption method—plasma was diluted 1:50 with 0.1% lanthanum in 0.1 M hydrochloric acid (Dawson and Walker, 1969),—and albumin was measured by a bromocresol green method (Spencer and Price, 1977). The analytical within-batch coefficients of variation at normal plasma concentrations were less than 1.0% for calcium and less than 2.0% for albumin.

Results

PLASMA ALBUMIN

The plasma albumin concentrations after the tourniquet experiment and in the patients with myocardial infarction are shown in Figure 1. There was a rapid rise in the mean plasma albumin after venous occlusion from 43.9 ± 0.11 (SEM) g/l to 55.8 ± 0.38 g/l after 15 minutes. The mean plasma albumin in the 15 patients with myocardial infarction was 38.2 ± 1.15 g/l on the day of admission, and during

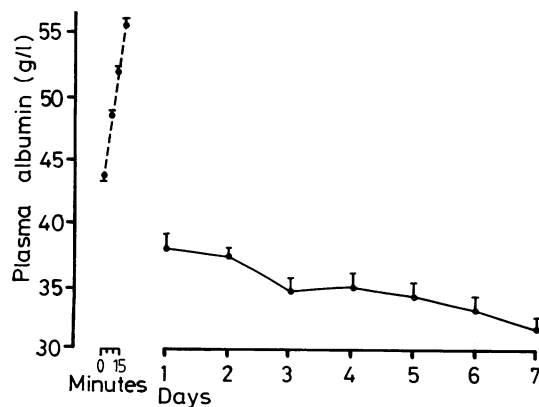


Fig. 1 Changes in plasma albumin concentration (mean \pm SE):
 ----- 5 normal volunteers after venous occlusion;
 ————— 12 patients after myocardial infarction.

the seven days of study it fell progressively to 31.7 ± 1.9 g/l.

REGRESSION ANALYSIS OF CALCIUM ON ALBUMIN

The individual regression lines for calcium on albumin whose slopes were significantly different from zero are shown in Figure 2. In one of the normal subjects after venous occlusion and in three of the patients with myocardial infarction there were only small changes, and the slopes of the individual regression lines were not significantly different from zero; they have been excluded from analysis. The apparent differences between the slopes of the regression lines after venous occlusion (Fig. 2) were shown by analysis of variance to be unlikely to be due to chance ($P < 0.01$). In contrast, the slopes of regressions of calcium on albumin after myocardial infarction differed little (Fig. 2), and analysis of variance confirmed that the small differences could well have occurred by chance ($P \approx 0.25$). The common slope for the tourniquet subjects was 0.012 ± 0.0021 (SE) mmol/g (0.48 ± 0.084 mg/g) and for the infarct patients 0.023 ± 0.0016 mmol/g (0.91 ± 0.064 mg/g).

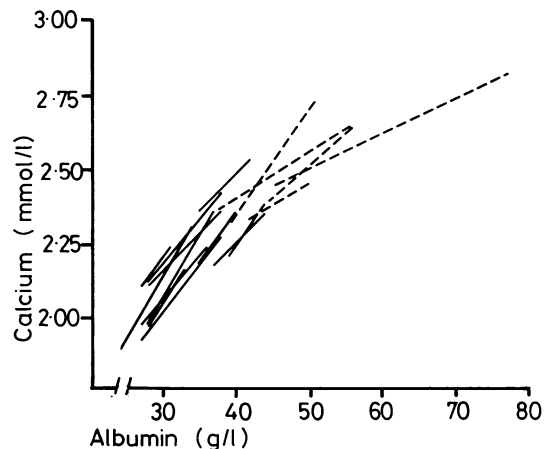


Fig. 2 Individual regression lines of calcium on albumin:
 ----- 5 normal volunteers after venous occlusion;
 ————— 12 patients after myocardial infarction.

Discussion

We have previously shown that in patients with no suspicion of a disturbance of calcium homeostasis but with albumin concentrations ranging from normal to very low values the overall regression of single measurements of total calcium on albumin was 0.025 ± 0.0010 (SE) mmol/g (0.99 ± 0.041

mg/g) and the correlation coefficient was high (0.867). This regression coefficient was used as the basis of a method for adjusting total calcium for changes of albumin in disease (Payne *et al.*, 1973). If individual patients had differed markedly in the way calcium fell with albumin during the course of their illness it is very unlikely that such a close relationship would have been found. Pain *et al.* (1975) used tourniquet data to challenge the concept that a single regression equation could be used to 'correct' calcium, but subsequent correspondence concluded that the authors had paid insufficient attention to the effect of analytical variation and had not shown statistically significant differences between individual regressions (Hodkinson, 1976; Payne *et al.*, 1976; Ramsay and Shelton, 1976). However, the same group subsequently published a letter describing the results of tourniquet experiments on 17 normal individuals (Phillips *et al.*, 1977). The median regression coefficient was 0.028 mmol/g (1.12 mg/g) with a range from 0.013 to 0.44 mmol/g (0.52 to 1.76 mg/g), and analysis of variance showed that the differences between individual regressions made a significant contribution to the total variance ($P < 0.05$). The present work has confirmed that there are significant differences between individual regression coefficients after venous occlusion with forearm exercise.

To study changes in disease we chose patients who were admitted to hospital with acute myocardial infarction. Albumin concentration is known to fall during the first week or so after infarction (Owen, 1967), and it is unlikely that there are major changes in ionised calcium because changes in total calcium are associated with simultaneous changes in albumin (Wiener, 1977). Our patients showed no significant difference between the slopes of their individual regression lines, and the common regression coefficient (0.023 mmol/g) was not significantly different from the value of 0.025 mmol/g previously described in single observations on patients with a wide range of albumin concentrations (Payne *et al.*, 1973). Thus, the effect on total calcium of the rapid rise in plasma albumin after venous occlusion with forearm exercise differs between subjects and cannot be compared with the much more consistent effect of the slow fall in albumin due to disease.

There are several possible explanations for the variable changes in calcium and albumin after venous occlusion. The possibility that there is a difference in filtration of calcium-binding macromolecules through the capillary wall during occlusion with a proportionally greater escape of albumin can be excluded because proteins of different molecular weight increase in parallel after occlusion (B. E. Walker *et al.*, in preparation). A factor that would

contribute to the differences between individuals is the decrease in plasma water as plasma protein concentration increases; this would vary according to the initial plasma water. However, our sera were not lipaemic, and calculation indicates that this effect alone would be too small to account for the observed differences. An additional source of variation would be the concentration in individuals of globulins, largely α -globulins, which, like albumin, bind calcium although to a smaller extent. In tourniquet experiments the increase is in total protein-bound calcium, and significant differences between individuals in the proportions of calcium bound to albumin and to globulins would result in differing regressions of calcium on albumin after occlusion (Hodkinson, 1976). Finally, clenching the fist for as short a period as 1 minute during venous occlusion has been shown to cause an increase in lactate of 3.0-3.5 mmol/l and a decrease in venous pH to 7.10-7.15 (van Leeuwen *et al.*, 1961), and intermittent fist-clenching once a second for 3 minutes during occlusion increases blood lactate by a mean of 4.5 mmol/l (Braybrooke *et al.*, 1975). Direct measurement has shown that serum ionised calcium increases during exercise on a bicycle ergometer (Nielsen *et al.*, 1977). Variation in forearm exercise, which causes hypoxia and dissociation of calcium bound to protein due to a fall in pH and to an increase in undissociated calcium lactate, might cause a variable decrease in the change of total calcium as albumin increases. It is notable that Berry *et al.* (1973), who found much more consistent changes in calcium and albumin after venous occlusion than those reported by Phillips *et al.* (1977), observed no change in directly measured ionised calcium after venous stasis. This may well be because of differences in the amount of forearm exercise during occlusion. A combination of these mechanisms may account for the differences between individuals.

Our results support the use of a common regression coefficient derived from patients with normal and low albumin concentrations but without a disturbance of calcium metabolism to adjust calcium values in patients who are thought to have such a disturbance (Payne *et al.*, 1973; Payne *et al.*, 1979). However, a single coefficient cannot be used to allow for the variable relations between calcium and albumin which follow venous occlusion. The message that emerges from our investigation is important if precise results are to be obtained: blood specimens should be taken with the minimum of venous stasis even though the patient's calcium is to be adjusted for albumin.

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