accompanying immunosuppressive drugs prevents the development of allergy. A few patients experienced transient confusion, nausea, and subjective difficulty in breathing after the *first* injection only of colaspase in every course of CART treatment. These reactions are unpleasant and potentially dangerous, but they did not occur when the initial injection was administered subcutaneously. They are unlikely to be allergic but might be due to the rapid deamination of a normal plasma content of asparagine with a transient rise in blood ammonia when the colaspase is administered intravenously.

Our results suggest that the COAP/POMP/CART regimen merits further study, particularly in adults with ALL and in patients of any age with high blast cell counts at presentation. Its value in cases of documented T-cell ALL is as yet uncertain. It is not significantly superior when given to children with ALL who have no adverse prognostic features, though our data do not exclude the possibility that such superiority may exist.

## References


[1] George, P, *et al*, *Journal of Pediatrics*, 1968, **72**, 399.
[2] Pinkel, D, *et al*, *Pediatrics*, 1972, **50**, 246.
[3] Pinkel, D, *Eighth Annual Guest Lecture*. London, Leukaemia Research Fund, 1972.
[4] Medical Research Council Working Party, *British Medical Journal*, 1973, **2**, 381.
[5] Clarkson, B D, and Fried, J, *Medical Clinics of North America*, 1971, **55**, 561.
[6] Spiers, A S D, *Clinics in Haematology*, 1972, **1**, 127.
[7] Spiers, A S D, *Lancet*, 1972, **2**, 473.
[8] WHO/IARC Workshop, *Scandinavian Journal of Immunology*, 1974, **3**, 521.
[9] Johnston, P G B, *et al*, *British Medical Journal*, 1973, **3**, 81.
[10] Whitecar, J P, Bodey, G P, and Freireich, E J, *Proceedings of the American Association of Cancer Research*, 1970, **11**, 83.
[11] Frei, E, and Freireich, E J, *Advances in Chemotherapy*, 1965, **2**, 269.
[12] Spiers, A S D, and Firth, J L, *Lancet*, 1972, **1**, 433.
[13] Spiers, A S D, and Booth, A E, *Lancet*, 1973, **1**, 1263.
[14] Galton, D A G, and Dacie, J V, *Blood Cells*, 1975, **1**, 17.
[15] Catovsky, D, Frisch, B, and Van Noorden, S, *Blood Cells*, 1975, **1**, 115.
[16] Till, M M, Hardisty, R M, and Pike, M C, *Lancet*, 1973, **1**, 534.
[17] Clarkson, B D, *Cancer*, 1972, **30**, 1572.
[18] Haghbin, M, *et al*, *Proceedings of the American Association of Cancer Research*, 1972, **13**, 22.
[19] Spiers, A S D, *Lancet*, 1974, **1**, 674.
[20] Morley, A A, Carew, J P, and Baikie, A G, *British Journal of Haematology*, 1967, **13**, 719.
[21] Kay, H E M, *et al*, *Archives of Disease in Childhood*, 1972, **47**, 344.
[22] Aur, R, *et al*, *Proceedings of the American Association of Cancer Research*, 1975, **16**, 92.
[23] Aur, R, *et al*, *Proceedings of the American Association of Cancer Research*, 1974, **15**, 144.


# Current "corrected" calcium concept challenged

R W PAIN, K M ROWLAND, P J PHILLIPS, B McL DUNCAN

*British Medical Journal*, 1975, **4**, 617-619


## Summary

**There is wide individual variation in the number of millimoles of calcium bound per gram of albumin in the serum. Individual regression coefficients for serum calcium concentration on serum albumin concentration have been determined in 62 people (25 of our own patients and 37 reported by others). The 95 percentile range was 0·007-0·053 mmol/g, with a median value of 0·025 mmol/g. Accordingly, it is not valid to "correct" a person's measured serum total calcium concentration for variations in serum albumin concentration using an average regression coefficient. Rather, the individual's own regression coefficient must be used. A tourniquet test seems to be the simplest technique for determining this value. Even then, precise interpretation of an individual's corrected serum calcium concentration is possible only when an appropriate reference range for corrected serum calcium concentration has been established. Such an appropriate reference range must be determined from an adequate number of normal people using the individual's own correction factor in each case.**



**Division of Clinical Chemistry, Institute of Medical and Veterinary Science, Adelaide, South Australia 5000**

R W PAIN, MB, FRCPA, senior clinical chemist (medical)
K M ROWLAND, MB, MRCPATH, clinical chemist (medical)
P J PHILLIPS, MB, MRACP, clinical chemist (medical)
B McL DUNCAN, BSC, DIPCOMPSC, biochemist


## Introduction

Most routine laboratories can estimate the serum total calcium concentration very accurately, but since total calcium is the sum of ionised (50%), protein bound (40%), and complexed (10%) calcium fractions, and it is only the ionised fraction that is physiologically important, the estimation falls short of clinical requirements. Since albumin is the main (or sole) protein to which calcium is bound changes in serum albumin concentration will change the serum total calcium concentration. Hence numerous correction factors, relating measured serum calcium concentration to specific gravity, total protein, and serum albumin concentrations, have been developed.[1-5]

We report here the wide individual variation in the regression coefficients of serum calcium concentration on serum albumin concentration found in 25 hospital inpatients and in a further 37 patients reported by others. We consequently question the validity of "correcting" an individual's measured serum calcium concentration by an average correction factor. Alternative approaches to the problem are discussed.

## Patients and methods

Concentrations of both serum total calcium and serum albumin were measured by dye-binding methods on the Technicon SMA 12/60. Serum total calcium was measured using the standard Technicon cresolphthalein complexone method with a typical between-batch coefficient of variation of 1·8%. Serum albumin estimations were performed by a modified bromocresol green method with increased linearity[6] and with a typical between-batch coefficient of variation of 2·0%. Purified human albumin (Dade) was used as a standard.

*Mean regression coefficient of calcium on albumin*—Blood was collected without stasis from 163 fasting healthy laboratory personnel aged from 17 to 65 years. These ambulant subjects were not on any medication and blood was collected without stasis and separated within two hours. Data were also collected from 163 hospital inpatients with a variety of medical and surgical conditions but without renal impairment (serum creatinine concentration $<150$ μmol/l

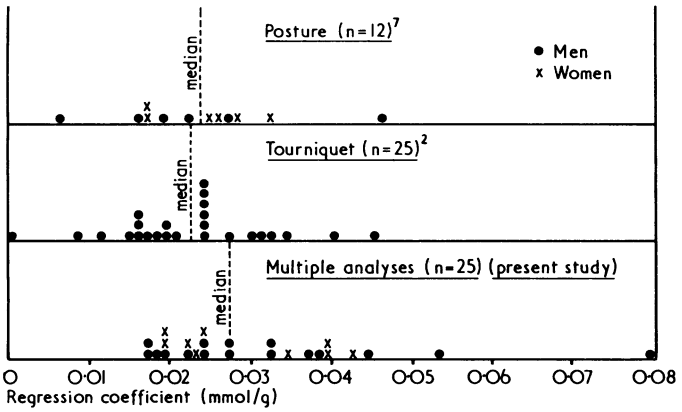
(1·7 mg/100 ml)), electrolyte disturbance, or abnormal phosphate concentration. The regression coefficient for serum total calcium concentration on serum albumin concentration was obtained for both sets of data with a computer.

*Individual regression coefficients of calcium on albumin*—The case records of hospital inpatients selected as above but also without known malignancy or bone disease were examined and patients with multiple recent simultaneous estimations of both serum total calcium and serum total albumin were selected. Thirty-four patients with a significant change in albumin concentration were examined further. A satisfactory individual regression line could be fitted in only 25 cases, and the regression coefficients were determined. There were nine women and 16 men aged from 39-86 years (mean 63 years). Multiple consecutive estimations (3-17, mean 8) for these 25 patients were collected for periods of 2-39 days (mean 15), and the change in serum albumin ranged from 5-18 g/l (mean 11 g/l). Individual regression coefficients were also calculated from two sets of published data. In the first,[7] blood was taken from 12 healthy laboratory staff (six men and six women aged 29-42 years) when ambulant and a second specimen was taken after they had been supine for 60 minutes. The mean change in serum albumin concentration was 3·5 g/l (range 1·5-5·0 g/l). In the second[2] two blood samples were analysed from 25 healthy male doctors and medical students aged 21-35 years, one before and one after 90-mm Hg tourniquet pressure for 15 minutes. The mean change in serum albumin concentration was 7·4 g/l (range 1·0-18·0 g/l).

## Results

The 95 percentile limits for healthy staff members were 43-53 g/l (median 48 g/l) for serum albumin concentration and 2·20-2·55 mmol/l (8·8-10·2 mg/100 ml) (median 2·38 mmol/l (9·5 mg/100 ml)) for serum total calcium concentration. The distribution of serum albumin concentration in hospital inpatients was trimodal with a 95 percentile range of 26-48 g/l and an overall median of 38 g/l. The 95 percentile range for serum total calcium concentration in this latter population was 1·90-2·50 mmol/l (7·6-10·0 mg/100 ml) (median 2·20 mmol/l (8·8 mg/100 ml)). The equations for the regression lines were $y = 0{\cdot}0221x + 1{\cdot}31$ for healthy staff and $y = 0{\cdot}0218x + 1{\cdot}39$ for inpatients where y and x were total calcium and total albumin respectively. Thus the mean regression coefficient (correction factor) for adjusting calcium for albumin was 0·022 mmol/g for both populations.

The individual regression lines for the 25 hospital inpatients were not always parallel, with individual regression coefficients varying from 0·018-0·080 mmol/g and a median coefficient of 0·028 mmol/g (see fig). The median value (the middle value of a set of results) is quoted rather than the mean value (0·032 mmol/g) because of the small number of subjects and the skewness of the distribution of results. There was no apparent difference between results for men and women nor any apparent relation with age. A similar wide scatter of individual regression coefficients was calculated from the published data[2 7] (see fig). Combining these two sets of published data with our own, 62 individual regression coefficients were obtained with an absolute range of 0-0·080 mmol/g, a 95 percentile range of 0·007-0·053 mmol/g, and an overall median value of 0·025 mmol/g.




Individual regression coefficients for calcium on albumin calculated for our patients (using multiple analyses) and for those of Pedersen[7] (using a posture technique) and Berry *et al*[2] (using a tourniquet method).

## Discussion

It is a popular concept that the measured serum total calcium should be routinely corrected for variation in the serum albumin concentration by using an average correction factor.[2-5 8-15] Since, however, we have shown that the variation of individual regression coefficients (correction factors) is wide (covering a sevenfold range) the routine application of an average regress on coefficient might easily lead to diagnostic errors, as shown in table I. Depending on the correction factor used, a given measured total calcium concentration could be corrected to a value that is low, normal, or high. Furthermore, an individual's correction factor might vary, depending on such influences as hydrogen ion concentration, medication, disease states, and infusions of albumin or blood. For example, in one patient with probable disseminated lupus erythematosus we observed a sudden change in regression coefficient from 0·043 to 0·058 mmol/g after she had begun prednisolone treatment.

TABLE I—*Variation in "corrected calcium" in three patients depending on regression coefficient used*

| Case No | Measured | | Corrected calcium (mmol/l) using following regression coefficients | | | |
|---|---|---|---|---|---|---|
| | | | 95 Percentile range | | | Median coefficient on 163 healthy staff and 163 inpatients |
| | Albumin* (g/l) | Total calcium (mmol/l) | Lower limit 0·007 | Median 0·025 | Upper limit 0·053 | 0·022 |
| 1 | 20 | 1·65 | 1·85 | 2·35 | 3·13 | 2·27 |
| 2 | 30 | 1·80 | 1·93 | 2·25 | 2·75 | 2·20 |
| 3 | 54 | 2·70 | 2·66 | 2·55 | 2·38 | 2·57 |

*All corrections made to an albumin of 48 g/l, the median of the reference range.
*Conversion: SI to traditional units*—Calcium: 1 mmol/l ≈ 4 mg/100 ml.

Accordingly, if the measurement of serum ionised calcium concentration is unavailable and if it is wished to correct the serum total calcium concentration for variations in serum albumin concentration then that individual's own regression coefficient for calcium on albumin must be determined. Of the three possible ways of doing this referred to in fig 1 the use of a tourniquet test[2 16] seems to be the simplest and most accurate. If more than two timed samples are collected then the likelihood of analytical error causing an erroneous regression line becomes negligible. One of us (PJP) is currently investigating this approach, collecting four blood samples over 15 minutes with a sphygmomanometer cuff kept half-way between the systolic and diastolic pressures. In a limited preliminary study the variation of the regression coefficient between individuals has been confirmed and the individual's regression coefficient has been shown to be reasonably reproducible on three occasions over two weeks (table II). We plan to establish a valid normal reference range for corrected serum calcium concentration from individual regression coefficients obtained by this means on a sufficiently large number of people and then investigate the diagnostic usefulness of this manoeuvre in patients suspected of having disorders of calcium homoeostasis.

TABLE II—*Reproducibility of individual regression coefficients in three normal subjects over two weeks*

| Subject | Age and Sex | Calculation of regression coefficient | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| A | 24 F | 0·018 | 0·018 | 0·022 |
| B | 30 F | 0·026 | 0·031 | 0·031 |
| C | 44 F | 0·024 | 0·021 | 0·028 |

We thank Dr D W Thomas, assistant director of clinical chemistry, for permission to use the data on healthy staff members and Mr T D Geary for helpful discussion.

## References

[1] Dent, C E, *British Medical Journal*, 1969, **2**, 1419.
[2] Berry, E M, *et al*, *British Medical Journal*, 1973, **4**, 640.
[3] Payne, R B, *et al*, *British Medical Journal*, 1973, **4**, 643.
[4] Marshall, R W, and Nordin, B E C, *British Medical Journal*, 1974, **2**, 729.
[5] Parfitt, A M, *British Medical Journal*, 1974, **1**, 520.
[6] Prior, M, and Peake, M, *Australian Journal of Medical Technology*, 1975, **6**, 5.
[7] Pedersen, K O, *Scandinavian Journal of Clinical and Laboratory Investigation*, 1972, **30**, 321.
[8] Payne, R B, *Lancet*, 1973, **2**, 375.
[9] Berry, E M, *et al*, *British Medical Journal*, 1974, **2**, 53.
[10] Hodkinson, H M, *British Medical Journal*, 1974, **2**, 223.
[11] Payne, R B, *et al*, *British Medical Journal*, 1974, **1**, 393.
[12] Payne, R B, *et al*, *British Medical Journal*, 1974, **2**, 504.
[13] Payne, R B *et al*, *British Medical Journal*, 1974, **3**, 345.
[14] Christiansen, C, *et al*, *Clinica Chimica Acta*, 1975, **62**, 65.
[15] Husdan, H, *et al*, *Clinical Chemistry*, 1974, **20**, 529.
[16] van Leeuwen, A M, Thomasse, C M, and Kapteyn, P C, *Clinica Chimica Acta*, 1961, **6**, 550.


# Effect on adrenal function of topically applied clobetasol propionate (Dermovate)

C F ALLENBY, R A MAIN, R A MARSDEN, C G SPARKES

*British Medical Journal*, 1975, **4**, 619-621


## Summary

**Thirty-nine patients (15 outpatients and 24 inpatients) with a variety of skin diseases affecting variable areas of the body surface were treated with clobetasol propionate ointment (Dermovate). Before and after treatment the adrenal response to an intramuscular injection of tetracosactrin was tested and additional 9 am plasma cortisol levels were measured at intervals during treatment. A satisfactory initial therapeutic response was achieved in almost all cases during the trial period. When more than 50 g of ointment a week was used a significant number of patients developed adrenal suppression. When less than 50 g per week was used any suppression tended to be transient, and cortisol levels recovered as treatment progressed.**


## Introduction

It is well recognised that when corticosteroids are applied to the skin there may be sufficient percutaneous absorption to affect hypothalamic-pituitary-adrenal (HPA) function. This effect seems to parallel topical anti-inflammatory activity and is more likely to occur when large quantities of cream or ointment are applied to extensive areas of diseased skin.

Clobetasol propionate in a strength of 0·05% (Dermovate) is a highly effective corticosteroid preparation.[1] Walker *et al* have shown that it has little effect on the HPA axis of outpatients when applied topically.[2] They assessed adrenal function by estimating plasma cortisol levels at the beginning and end of treatment. But cortisol values during the early treatment period, when maximum absorption is most likely, were not measured. Furthermore, inpatients, whose lesions are usually more extensive, require larger amounts of steroid preparation than outpatients. For these reasons we investigated the effects of treatment with clobetasol propionate in both inpatients and outpatients.


**Department of Dermatology, Lister Hospital, Hertfordshire**
C F ALLENBY, MB, MRCP, consultant dermatologist

**Department of Dermatology, Aberdeen Royal Infirmary, Aberdeen**
R A MAIN, MB, FRCP, consultant dermatologist and honorary senior lecturer
R A MARSDEN, MB, MRCP, registrar in dermatology (present address: Slade Hospital, Headington, Oxford)

**Clinical Research Unit, Glaxo Laboratories Ltd, Greenford, Middlesex**
C G SPARKES, BSC, PHD, clinical pharmacologist


## Patients and methods

Twenty-three patients with psoriasis, 15 with dermatitis, and one with lichen planus were included. Children and patients who had received systemic steroid treatment during the previous six months were excluded. Ointment was applied to lesions up to twice daily without the use of occlusion (except in case 11). At the end of treatment the amount of ointment used was calculated.

HPA function in all patients was investigated using the short tetracosactrin test described by Wood *et al*,[3] and 9 am plasma cortisol levels were assessed. Cortisol levels were considered abnormal if they were below 166 nmol/l (6 μg/100 ml). An abnormal response to an injection of 250 μg of tetracosactrin was recorded if after 30 minutes plasma cortisol levels had not risen by at least 193 nmol/l (7 μg/100 ml) to a level of 552 nmol/l (20 μg/100 ml) or more. Plasma cortisol estimations were carried out in the same laboratory using a fluorimetric method described by Spencer-Peet *et al*.[4]

The extent of the disease was graded as follows: grade 1, small circumscribed lesions—for example, on elbows, knees, and scalp; grade 2, under 50% of body surface affected; grade 3, over 50% of body surface affected; grade 4, almost confluent lesions.

## Results

The results are given in table I. The ages of the 39 patients ranged from 15 to 79 years (median 48 years). There were 19 women and 20 men. Fifteen were treated as outpatients and 24 were inpatients. In most cases about half of the skin surface was treated. Only one patient (case 12) failed to improve. During treatment two patients developed an acneform rash.

### ADRENAL FUNCTION

Adrenal function remained normal throughout the trial in only 14 patients (10 outpatients and 4 inpatients). Nineteen patients with initial normal 9 am plasma cortisol levels had abnormal levels for a variable period during treatment. In seven of these the plasma cortisol levels recovered, and in six of the seven the final tetracosactrin test was also normal. Of the 12 patients with depressed cortisol levels at the end of the trial half had an abnormal response to tetracosactrin.

Eight patients had a poor response to tetracosactrin at the beginning of the trial, and in four the 9 am plasma cortisol level was also abnormal. Evidence of improving adrenal function was present in three patients at the end of the trial (cases 17, 18, and 34). These eight patients had probably used large amounts of topical corticosteroids before starting the trial.