Plasma uric acid and plasma albumin in healthy subjects

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SUMMARY In healthy male subjects there was a positive correlation between plasma uric acid and plasma albumin ($r=0.43$, $P<0.005$, $n=49$) when repeated measurements of both variables were used for each subject. Changes in plasma albumin induced by *in vivo* ultrafiltration were not accompanied by changes in plasma uric acid. The correlation of plasma uric acid with plasma albumin cannot be attributed to protein binding of urate. The two variables are probably related indirectly through a common association with an unknown factor or factors.

Studies of protein binding of uric acid have been hampered by problems with *in vitro* methodology, and by difficulty in relating the results of *in vitro* studies to the physiological situation. Reviewing the literature, Gutman and Yu (1972) concluded that binding of urate to plasma proteins *in vivo* was so small as to have negligible physiological implications. However, reports have continued to appear suggesting that up to 20% of uric acid may be protein bound (Campion *et al.*, 1973, 1974; Postlethwaite *et al.*, 1974; Kippen *et al.*, 1974a). A significant development was evidence that uric acid was protein bound *in vivo* and could be displaced by salicylate (Postlethwaite *et al.*, 1974), although Farrell *et al.* (1975) were unable to confirm these findings. The question has assumed added importance after the suggestion that uricosuric agents may act by displacing uric acid from binding sites on protein, thus increasing the renal clearance of urate (Kippen *et al.*, 1974b; Kelley, 1975).

Acheson and Chan (1969) and Roberts (1972) have shown weak positive partial correlations between plasma uric acid and plasma albumin in populations of healthy subjects, and Roberts (1972) thought that this was probably attributable to protein binding of uric acid. The objectives of our first study were to confirm this relationship, and to determine whether it was strengthened by using repeated measurements of the two variables for each

subject. Subsequently we studied changes in plasma uric acid in healthy subjects in whom increases in plasma albumin were induced by *in vivo* ultrafiltration (van Leeuwen, 1964).

Patients and methods

In the first study we examined retrospectively data for 49 males aged 18-55 years who had been judged healthy after medical history, examination, and biochemical and haematological screening. Repeated simultaneous measurements of plasma uric acid and plasma albumin (mean 6.4 estimates per subject, range 3-15 estimates) were available for each subject. Blood was drawn at 9.00 am after the subjects had avoided all forms of medication for at least 5 days, and alcohol for 24 hours. Sampling was usually at one-week intervals. The subjects had been studied as 6 separate groups. The mean of all measurements of plasma uric acid and plasma albumin for each subject was calculated, giving a single estimate for each subject which will be referred to as the 'usual' plasma uric acid and albumin concentrations.

In the second study in 6 healthy subjects *in vivo* ultrafiltration using tourniquet compression of the arm, as described by van Leeuwen (1964), was performed. The subjects were recumbent with both arms resting on the bed throughout the procedure. Sphygmomanometer cuffs were applied to both upper arms and inflated to a pressure midway between the systolic and diastolic blood pressure. Venous blood for measurement of plasma uric acid, albumin, total protein, calcium, and magnesium was

drawn from the first arm after 5 minutes' compression, and the cuff was deflated. A similar sample was taken from the other arm after 15 minutes' compression, and the second cuff was deflated. A third (resting) sample was drawn without venous stasis from the first arm at least 30 minutes after the cuff had been deflated. The resting sample was taken at this time to avoid haematoma formation during tourniquet compression.

LABORATORY INVESTIGATIONS

In the first study plasma uric acid had been measured by a nonspecific colorimetric method (Nishi, 1967) with a coefficient of variation between batches of approximately 4%. In the second study uric acid was measured by a specific uricase method using the Beckman glucose analyser.

Albumin was measured by automated methods (first study: Nishi and Rhodes, 1965; second study: Doumas *et al.*, 1971), and calcium and magnesium by atomic absorption spectrophotometry. In the second study the three samples for each subject were assayed within a single batch to remove any effect of laboratory variation between batches. The statistical procedures used (product moment correlations, comparison of correlation coefficients, calculation of the average correlation coefficient, and Student's *t* test for paired observations) are described by Armitage (1971) and Pearson and Hartley (1966).

Results

URIC ACID AND ALBUMIN: BETWEEN-SUBJECT RELATIONSHIP

In the 49 subjects the usual plasma uric acid concentration ranged from 0.238–0.484 mmol/l (2.5–8.1 mg/100 ml), and the usual albumin from 40.0–48.5 g/l. The two variables correlated weakly, but highly significantly ($r = +0.43$, $P < 0.005$, $n = 49$; Fig.). Comparison of the correlation coefficients for the six subgroups showed that they did not differ significantly, allowing calculation of the average correlation coefficient. This method ensures that combination of the data from different groups had not introduced bias. The correlation was in fact strengthened by this method ($r = +0.58$, $P < 0.001$).

IN VIVO ULTRAFILTRATION

The mean and standard error results for all variables in the 6 subjects are shown in Table 1, and the individual results for plasma uric acid in Table 2. Tourniquet compression for 15 minutes produced highly significant increases in total protein (mean +39%, $P < 0.001$) and plasma albumin (+39%, $P < 0.001$). The range of plasma albumin concentrations observed in this study (39–66 g/l) far exceeded

that in the first study (40–48.5 g/l). Serum calcium (+15%, $P < 0.001$) and magnesium (+17%, $P < 0.001$) also showed significant increases after 15 minutes' compression. In contrast, plasma uric acid concentration did not alter significantly during the experiment, nor was any trend to increase evident.

Fig. Correlation between the usual plasma uric acid (PUA) concentration and usual plasma albumin in 49 healthy males ($r = +0.43$, $P < 0.005$).

Table 1 Mean ($\pm$ SEM) results in 6 subjects for plasma variables measured without tourniquet compression and at 5 and 15 minutes during compression

	Duration of compression (min)		
	0	5	15
Albumin (g/l)	40.5 $\pm$ 0.67	48.8 $\pm$ 1.89†	56.2 $\pm$ 2.26‡
Total protein (g/l)	69.3 $\pm$ 1.35	83.5 $\pm$ 3.00†	96.5 $\pm$ 3.96‡
Uric acid (mmol/l)	0.345 $\pm$ 0.018	0.345 $\pm$ 0.020	0.345 $\pm$ 0.017
Calcium (mmol/l)	2.30 $\pm$ 0.036	2.54 $\pm$ 0.078*	2.65 $\pm$ 0.025‡
Magnesium (mmol/l)	0.78 $\pm$ 0.022	0.90 $\pm$ 0.012†	0.91 $\pm$ 0.015‡

* $P < 0.02$; † $P < 0.01$; ‡ $P < 0.001$ versus zero compression.

Conversion: SI to traditional units—Uric acid: 1 mmol/l $\approx$ 16.8 mg/100 ml. Calcium: 1 mmol/l $\approx$ 4 mg/100 ml. Magnesium: 1 mmol/l $\approx$ 2.4 mg/100 ml.

Table 2 Individual results in 6 subjects for plasma uric acid (mmol/l) during tourniquet compression

Subject	Duration of compression (min)		
	0	5	15
1	0.36	0.36	0.35
2	0.34	0.33	0.36
3	0.31	0.29	0.31
4	0.30	0.31	0.29
5	0.34	0.35	0.35
6	0.42	0.43	0.41
Mean (SEM)	0.345 (0.018)	0.345 (0.020)	0.345 (0.017)

Discussion

The results of our first study confirm the between-subject partial correlations of uric acid and albumin previously reported in healthy populations by Acheson and Chan (1969) and Roberts (1972). The correlation coefficient was considerably higher in the present study presumably because repeated measurements were made in each subject, a procedure tending to minimize the effect of any day-to-day variation within subjects and in the laboratory. From the average correlation coefficient ($r = +0.58$) it can be calculated that 34% ($r^2 \times 100$) of the variation in plasma uric acid between subjects can be related to differences in plasma albumin. If Roberts's (1972) postulate that the correlation arises from protein binding of uric acid was correct, then 34% of the intersubject variance in plasma uric acid could be attributed to protein binding.

To test the hypothesis further we sought a method to study protein binding of uric acid *in vivo*, bearing in mind the controversy which has arisen from *in vitro* studies (Gutman and Yu, 1972; Kippen *et al.*, 1974b). Postlethwaite *et al.* (1974) and Farrell *et al.* (1975) have used haemodialysis for this purpose, but the results from the two groups were not in agreement. The use of haemodialysis also has the disadvantage of being restricted to study of patients with renal failure. The technique of *in vivo* ultrafiltration using tourniquet compression has been thoroughly evaluated by van Leeuwen (1964) and has been used to study the protein binding of various endogenous substances such as calcium and magnesium (Berry *et al.*, 1973; Pain *et al.*, 1975) and thyroid hormones (Judd *et al.*, 1975). We could find no report of measurement of plasma uric acid during *in vivo* ultrafiltration, and adopted this approach to the problem. Measurements of plasma calcium and magnesium were included to give some estimate of the sensitivity of the experiment. Approximately 41% of total calcium and 33% of total magnesium are bound to plasma protein (van Leeuwen, 1964).

The study conditions were sensitive enough to show the expected changes in calcium and magnesium at a high level of significance. In contrast, plasma uric acid showed no change during tourniquet compression, without even a trend to increase. Extrapolating from the between-subject regression of uric acid on albumin in our first study, a rise in plasma uric acid of 0.14 mmol/l would have been anticipated from the changes in plasma albumin observed after 15 minutes' compression. Clearly this did not occur. From their experimental observations Postlethwaite *et al.* (1974) divided subjects into 'binders' and 'non-binders' of uric acid. Inspection of the individual results (Table 2) does not suggest

that any of our 6 subjects were 'binders'. The maximal increase in plasma uric acid in any individual during compression was 6%, and only one subject had a consistent rise (of 3%) at both sampling times. The error of the method can easily explain these variations.

Van Leeuwen (1964) has examined the changes in venous blood during *in vivo* ultrafiltration and, apart from changes in protein and protein-bound substances, the only change of possible relevance in the present context was in pH. Uric acid is a weak acid and its distribution in body fluids may be influenced by the phenomenon of nonionic diffusion (Zweifler and Thompson, 1965). The conditions used in our study are associated with average fall of venous blood pH of 0.04 (van Leeuwen, 1964). Since this change is secondary to intracellular pH change, the intracellular pH will fall to a similar, or greater, degree. If any pH-related shift of uric acid between the intracellular and extracellular compartments does occur, one would expect it to raise plasma uric acid. We conclude that a rise in plasma uric acid due to protein binding would not be disguised by pH changes during compression.

It remains to explain why plasma uric acid and albumin show a significant correlation between subjects, but no relationship during tourniquet ultrafiltration. In the latter study we used the specific uricase method for uric acid, while the uric acid-albumin correlation in the present study and in those of Acheson and Chan (1969) and Roberts (1972) was shown using nonspecific colorimetric methods. It is therefore possible that the between-subject correlation is caused by protein binding of nonurate chromogens, but this seems improbable in a population of subjects taking no medication. If this possibility is discounted then it is clear from the present studies that the between-subject correlation cannot be attributed to protein-binding of uric acid or indeed to any other *direct causal* relationship of the two variables. Rather, plasma uric acid and albumin are related in the population through some other factor in such a way that they can vary independently during ultrafiltration. Recalling that 34% of the variation in plasma uric acid between subjects can be accounted for in terms of plasma albumin, the nature of the factor linking the two variables is clearly of some importance. Acheson and Chan (1969) suggested in general terms that the relationship may depend on nutritional status. A further possibility is that plasma albumin and uric acid may be related through the extracellular fluid volume. In acute situations plasma albumin concentration and plasma uric acid (Steele, 1969) show changes in a similar direction with changes in extracellular volume. It has been suggested that the

usual plasma uric acid concentration in healthy subjects may be determined to some extent by the customary dietary intake of sodium, the latter influencing extracellular fluid volume (Ramsay *et al.*, 1975). If the usual plasma albumin is similarly influenced, then the correlation observed could be explained on this basis.

In conclusion we have shown a moderate correlation between plasma uric acid and plasma albumin in healthy subjects. Data obtained during *in vivo* ultrafiltration do not suggest that they are related through protein binding of uric acid, nor do they support the contention that urate is protein bound to any important degree. Study of the mechanism underlying the relationship between uric acid and albumin might prove helpful in explaining the wide differences in plasma uric acid between healthy subjects.

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