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URIC ACID: BINDING LEVELS OF URATE IONS IN NORMAL AND URAEMIC PLASMA, AND IN HUMAN SERUM ALBUMIN

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Abstract—It has recently been suggested that the binding of urate ions to plasma proteins could be: (i) 20 per cent or more in normal subjects and (ii) as high as 10–15 per cent in some uraemic patients. It has also been suggested that oral aspirin administration may enhance urate ion removal on dialysis. These contentions are in conflict with previously published data. In this paper, the binding of urate in plasma is re-examined *in vivo*, and *in vitro*, at 37° and at pH 7·4. Conventional ultrafiltration techniques showed that the binding of urate ions is 5 ± 1 per cent in normal human serum albumin (HSA), 10 ± 1 per cent in defatted HSA, 6 ± 1 per cent in normal plasma and 2 ± 1 per cent in the plasma of chronic uraemics. *In vivo* urate ion binding was estimated on patients undergoing dialysis. These data were in good agreement with the *in vitro* data. In addition, aspirin administration failed to enhance urate ion clearance on haemodialysis during carefully controlled clinical experiments. Analysis of recent data, suggesting high urate binding levels, indicates that these studies were done under non-physiological conditions. Our data support previous contentions that urate ions are essentially freely filterable at the glomerulus and that binding does not significantly impair urate clearance on dialysis.

The level of urate ion binding in plasma is important in considering both the renal handling of urate (and the consequent action of uricosuric drugs) and urate removal during haemodialysis. However, the actual level of urate binding has been an issue of considerable debate [1–7] and, as three recent reports indicate [1–3], the issue is not yet satisfactorily resolved.

Campion *et al.* [1, 2] and Postlethwaite *et al.* [3] suggest that urate binding may be significant in the plasma of normal and uraemic subjects respectively. These findings appear to refute previously published reports concerning:

(i) urate binding in normal plasma [5, 6],
(ii) binding in uraemic plasma [7], and
(iii) the accepted mode of action of uricosuric drugs [8–10].

In the present study, *in vitro* binding levels were measured by ultrafiltration through Centriflo Cones (Amicon, Lexington, Mass.). Samples (5 ml) of plasma containing C^{14}-labelled urate (> 99% pure, Radiochemical Centre, Amersham, England) were ultrafiltered by centrifugation at 37°, after pH had been adjusted and buffered to 7·4. Urate concentrations were determined by liquid scintillation spectroscopy (Packard Tri Carb, No. 3375), using Instagel (Packard, Downers Grove, Ill.). The integrity of the urate tag was also checked by u.v. spectrophotometry, at 291 nm. Ultrafiltration has been previously used to determine binding [5–7, 11] and found to give an excellent correlation with equilibrium dialysis and gel filtration techniques.

In vivo binding levels were determined indirectly during haemodialysis with Cordis–Dow hollow fibre haemodialysers (Model 4, Cordis–Dow, Miami, FL) and Drake Willock proportioning units (Model 4015, Drake Willock, Portland, OR).

The clearance of a solute by a haemodialyser can be expressed as a function membrane properties and area, blood and dialysate flow rates, and the ratio of free to bound solute [12]. Under controlled conditions this expression can be used to determine *in vivo* binding levels [12].

Ultrafiltration was negligible in all experimental runs. Blood flow rates were measured by bubble times over a known length of tubing and dialysate flow rates by graduated cylinder and stopwatch. Blood and dialysate concentrations of urate were measured in duplicate by auto-analyser (Technicon, New York, NY) and clearances on both blood and dialysate were determined using conventional procedures [12].

In this preliminary investigation, six patients were studied. They were on unlimited diet, except for salt restriction, and were not receiving any coincident drug therapy. Average predialysis uric acid levels were 7 ± 1·4 mg/100 ml; av total protein content and albumin levels were 7·0 ± 0·1 g/100 ml and 4·2 ± 0·1 g/100 ml respectively. Haematocrits ranged from 18 to 29 per cent.

Following measurements of control clearances, 600 mg of aspirin were administered orally and 1 hr later a second clearance measured. Salicylate levels were not measured in this initial study, however, it has been shown that these levels are predictable. Rowland *et al.* [13] showed in an in-depth study that after oral administration of aspirin, salicylate levels reach a plateau of about 4·5 mg/100 ml in the peripheral circulation after 40 min and remain at above 4 mg/100 ml for 120 min following ingestion. At these levels, 50 per cent more salicylate is present in the plasma than is required to reduce *in vitro* urate ion binding by 75 per cent [14].

Table 1 presents binding levels of urate ions in both

Table 1. Ultrafiltration binding data of urate ions in human serum albumin (HSA) at 37° and pH 7·4

Environment*	Retentate concn, C_p (cpm)	Filtrate concn, C_f† (cpm)	Binding level (%) ± σ‡
5g% HSA	58,451	58,097	5·4 ± 0·5%
5g% HSA	30,085	29,740	6·0 ± 0·5%
5g% defatted HSA	31,844	30,064	10·2 ± 0.5%
5g% defatted HSA	166,162	158,700	9·5 ± 0·5%

* Urate carrier conc was 5 mg% in all cases.
† Uncorrected for protein vol exclusion ($\mu = 0{\cdot}95$).
‡ σ is standard error of mean
The binding level (B) of bound solute is given by [6, 7]: $B(\%) = [1 - (\mu C_f/C_p)]100$.

normal HSA solution (5g/100 ml) (Commonwealth Serum Laboratories, Melbourne, Australia) and defatted HSA (Sigma Chemicals, St. Louis, MO); the latter obtained in lyophilized form and made up to 5g/100 ml with sterile saline.

The results show a marked difference in binding levels between normal HSA and defatted HSA. The binding level for defatted HSA (~10%) is less than that reported by Campion *et al.* [2] (~20%) and is due to the purity of the HSA used [1]. Urate ion binding levels are, however, significantly greater for defatted, than for non-defatted HSA (which more closely represents the physiological situation). Normal plasma under resting conditions has a non-esterified fatty acid (NEFA) content of about 3 molecules per albumin molecule, (a figure which may rise to 30 when the need for fatty acid transport is extreme [15]). The binding levels of many solutes, including urate, are depressed in the presence of NEFA molecules [7, 14, 16]. A maximum level of interaction between urate ions and albumin would therefore be expected in defatted HSA, as has been observed [1, 2].

It has been suggested that urate ion binding might be as high as 23 per cent in normal plasma [1]. This latter study was not only done at a non-physiological temperature (22·5°), but the plasma was washed in buffer prior to ultrafiltration until the uric acid concentration was reduced to zero. This hardly represents physiologic plasma, since substantial amounts of other competitive site binding moieties (including NEFA molecules) would be leached out with the uric acid.

In vitro data in Table 2 indicate that under physiological conditions, the binding of urate to plasma proteins in either normal or uraemic subjects is not significant.

Table 3 indicates the difference in urate ion binding level before and after aspirin administration for six chronic uraemic patients on maintenance haemodialysis. With the exception of patient 3, the uric acid clearances, when adjusted for different flow rates [12], are in line with an *in vitro* clearance of 110 ± 4 ml/min, obtained for the Cordis–Dow Model 4 unit in our laboratory at $Q_B = 200$ ml/min, $Q_D = 500$ ml/min and $T = 37°$. The low clearance for patient 3 suggests

Table 2. Ultrafiltration binding of urate ions in fresh human plasma at pH 7·4 and 37°

Environment	Concn, C_p (cpm)	Filtrate concn, C_f* (cpm)	Binding level (%) ± σ†
Heparinised uraemic plasma (Patient 1)	106,414	112,429	0 ± 0·5%
Heparinised uraemic plasma (Patient 2)	109,269	114,021	2 ± 0·5%
Heparinised uraemic plasma (Patient 3)	73,635	76,650	2 ± 0·5%
Heparinised uraemic plasma (Patient 4)	82,480	86,813	1·6 ± 0·5%
Heparinised uraemic plasma (Patient 5)	65,816	68,898	1·7 ± 0·5%
Heparinised uraemic plasma (Patient 6)	103,160	107,441	3·2 ± 0·5%
Heparinised normal plasma (A+)	90,697	90,497	7 ± 0·5%
Citrated normal plasma	74,400	75,137	6 ± 0·5%

* Uncorrected for protein vol exclusion [μ ranged from 0·93 (normal) to 0·95 (uraemic)].
† σ is standard error of mean.
Binding was determined by the procedure shown in Table 1.

Table 3. Binding of urate ions *in vivo*: control compared with after administration of aspirin*

Patient	Blood flow rate (ml/min) ± σ	Dialysate flow rate (ml/min) ± σ	Urate ion clearance K(ml/min) ± σ	Difference in binding level† control treatment ± σ
1 control	167 ± 3	444 ± 5	102 ± 1	0 ± 0·5%
aspirin	175 ± 3	449 ± 5	104 ± 1	
2 control	184 ± 4	516 ± 5	115 ± 4	2 ± 0·5%
aspirin	190 ± 4	509 ± 5	118 ± 1	
3 control	157 ± 3	421 ± 5	48 ± 4	0 ± 0·5%
aspirin	167 ± 3	426 ± 5	44 ± 4	
4 control	157 ± 3	426 ± 5	89 ± 4	4 ± 0·5%
aspirin	157 ± 3	426 ± 5	92 ± 3	
5 control	178 ± 4	250 ± 5	103 ± 2	0 ± 0·5%
aspirin	178 ± 3	240 ± 5	95 ± 5	
6 control	128 ± 3	360 ± 5	80 ± 2	0 ± 0·5%
aspirin	127 ± 3	360 ± 5	80 ± 6	

* σ in the table refers to standard deviation.
† Calculated from mathematical analysis of Farrell *et al.* [12].

probable fibre bundle occlusion by thrombus deposition.

Aspirin can significantly reduce urate ion binding at 4° [3, 14, 17,] where urate ion binding is high (~25%) [5]. Although the dose in this study (600 mg) is less than that used by Postlethwaite *et al.* [3] (1200 mg), a plasma salicylate level of about 4 mg/100 ml is obtained 1 hr after oral administration [13]. Since aspirin has an *in vivo* half-life of about 15 min [13], most of the aspirin will have been hydrolysed to salicylate, which is 2·5 times as effective as aspirin in reducing urate ion binding *in vitro* [14]. Table 3 shows that the decrease in urate ion binding following the oral administration of 600 mg of aspirin is quite small (0–4%) and supports the contention that urate ion binding is small in uraemic patients.

Postlethwaite *et al.* [3] studied 10 patients on haemodialysis and found no *in vitro* bindings of urate ions to plasma proteins at 4° (using equilibrium dialysis) in six cases and levels of from 9 to 17 per cent in the other four cases. These figures are in agreement with earlier reports of urate binding in uraemic plasma at 4° [18]. Both Sheikh and Moller [5] and Klinenberg and Kippen [18] showed that urate ion binding to plasma proteins is a strong function of temperature, the degree of binding dropping by a factor of 5 (from ~25 to ~5%) as the temperature increased from 4 to 37°. When the data of Postlethwaite *et al.* [3] are corrected for temperature effects, the corrected levels (0–3%) are in essential agreement with those found in the present study. The point of contention between this study and that of Postlethwaite *et al.* [3] is the implication by the latter that they measured binding under physiological conditions.

Postlethwaite *et al.* [3] also reported that 1200 mg of orally administered aspirin increased urate ion clearance for those uraemic patients exhibiting binding at 4°. Since their *in vivo* urate clearances were taken under particular conditions (coil dialysis) which prevents the achievement of a true steady state, these data are subject to misinterpretation [19].

In summary, the degree of urate ion binding to plasma proteins has been re-examined using *in vitro* ultrafiltration techniques, and was found to be about 6 per cent in normal subjects, and about 2 per cent in chronic uraemic subjects. Additionally, *in vivo* urate clearances for patients undergoing haemodialysis matched expected *in vitro* values supporting the *in vitro* findings of negligible urate ion binding in the plasma of uraemic patients.

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