Ultrafiltrability of Plasma Urate in Man.* (20528)

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The urate clearance in normal man is of the order 7-10 cc/min. at adequate urine flows and therefore only some 5-8% of the inulin clearance. Opinion as to the significance of this urate clearance deficit is still divided(1). If the plasma urate is wholly filtrable at the glomerulus, it would follow that more than 90% of the filtered urate is reabsorbed in the tubules. If the plasma urate is partly or wholly non-filtrable, due to firm binding to plasma proteins or to formation of large molecular polymers, there would be correspondingly less tubular reabsorption; indeed, tubular secretion of urate might be postulated. The available data concerning the degree of filtrability of the plasma urate are conflicting. The older literature indicates complete diffusibility upon dialysis and variable results, generally 70-100% diffusibility, by ultrafiltration. Some of these records are difficult to evaluate because of sparse detail and use of now obsolete methods. More recently, Adlersberg, Grishman, and Sobotka(2) confirmed the complete diffusibility of plasma urate by compensation dialysis but, in an extensive study by ultrafiltration technics, found a mean of 16% "bound"/total uric acid (range 4-24%) in normal human subjects; higher percentages of non-filtrable urate (25-65%) were noted in 6 of 10 cases of gout, in some cases of liver disease and in a case of multiple myeloma. Bauer and Klemperer (3) stated that they could not confirm the findings of Adlersberg *et al.* but gave no details. Our own earlier experience, using the Simms-Sanders ultrafiltration apparatus in a small number of sera, indicated virtually complete filtrability of the plasma urate(4,5). On the other hand, Wolfson *et al.*(6), using a modification of the ultrafiltration apparatus devised by Lavietes(7), reported 27 and 19% nonfiltrable plasma urate, corrected for non-urate chromogen, in 2 normal human subjects studied by them.

Nonfiltrability of plasma urate of the degree reported by Adlersberg *et al.* and Wolfson *et al.* would account for only a fraction of the urate clearance deficit but the point involved is critical in respect to prevailing concepts of the renal mechanisms for urate excretion. Further study therefore seems indicated.

Methods. Two methods of ultrafiltration were employed. The first utilized the principle of the Simms-Sanders "surge" ultrafiltration apparatus(8) by which positive pressures of 200-300 mm Hg are applied to the serum samples (approximately 8 ml) contained in a specially prepared collodion tube. A rocking device containing mercury provides an alternating pressure gradient of 10 mm Hg. The resulting "surge" periodically removes the serum protein film which would otherwise occlude the filtration surface of the collodion membrane. The rate of ultrafiltration is thus greatly accelerated, 3-4 ml of ultrafiltrate being obtained in 20-30 minutes, and adsorption of urate on the protein film is minimized. Since the "surging" involves agitation of the serum, the possibility of partial denaturation of the serum proteins and liberation of loosely bound complexes was investigated by ultrafiltering serum obtained from a patient injected with 5 ml T-1824 dye. The ultrafiltrate contained no dye. The second ultrafiltration apparatus employed was that previously used by Adlersberg, Grishman, and Sobotka(2) and kindly made available to us by these investigators. This method involves filtration through "600" cellophane discs resting on metal sieve plates, using constant positive pressures of nitrogen (80 lb per sq. in.). Approximately 2 ml of ultrafiltrate are obtained in 24 hours by this method. All ultrafiltrates were tested with 10% trichloracetic

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TABLE I. Urate Content of Serum Water ($U_{s.w.}$) and of Corresponding Ultrafiltrates (U_{ul}) in 10 Non-gouty Subjects (1A), 16 Gouty Subjects (1B) and 16 Gouty Subjects Receiving Benemid (1C). Ultrafiltration carried out in Simms-Sanders apparatus.

	No.	$U_{s.w.}$ (mg %)	U_{ul} (mg %)	$\frac{U_{s.w.}-U_{ul}}{U_{s.w.}} \times 100$ (%)
1A	1	6.1	6.1	0
	2	5.2	5.2	0
	3	5.8	5.8	0
	4	5.0	4.9	+2.0
	5	5.5	5.3	+3.6
	6	4.4	4.2	+4.5
	7	4.1	3.9	+4.9
	8	7.1	6.7	+5.6
	9	7.6	7.1	+6.6
	10	19.0	17.7	+7.3
				Mean = +3.5%
				S.D. = ±2.7
1B	1	11.2	11.6	—3.6
	2	8.6	8.8	—2.3
	3	8.4	8.5	—1.2
	4	10.0	9.7	+3.0
	5	11.0	10.6	+3.6
	6	7.9	7.6	+3.8
	7	8.3	8.0	+3.8
	8	10.9	10.4	+4.6
	9	10.8	10.4	+4.6
	10	8.0	7.5	+6.3
	11	11.9	11.0	+7.6
	12	8.5	7.8	+8.2
	13	11.7	10.7	+8.5
	14	9.6	8.7	+9.4
	15	12.9	11.4	+11.6
	16	10.0	8.8	+12.0
				Mean = +5.0%
				S.D. = ±5.2
1C	1	5.8	6.1	—5.2
	2	9.6	10.0	—4.2
	3	5.6	5.6	0
	4	8.9	8.9	0
	5	6.9	6.9	0
	6	7.4	7.3	+1.4
	7	5.8	5.7	+1.7
	8	6.6	6.4	+3.0
	9	6.6	6.4	+3.0
	10	8.5	8.2	+3.5
	11	8.3	8.0	+3.6
	12	6.0	5.7	+5.0
	13	10.2	9.6	+5.9
	14	6.4	5.8	+9.4
	15	6.3	5.6	+11.1
	16	5.6	4.8	+14.3
				Mean = +3.3%
				S.D. = ±5.4

acid for protein and with biuret reagent for polypeptides to exclude the possibility of membrane leakage. Urate was determined by a modification of the Buchanan, Block, and Christman method(9) incorporating the use of uricase, arsenophosphotungstic acid and urea cyanide-carbonate. The reproducibility of this method in our laboratory is in the range 5-10%. Ultrafiltrates were treated exactly the same way as serum, including addition of tungstic acid and filtration through Whatman No. 40 filter paper even though no protein was present. Uric acid concentrations in serum are expressed in terms of serum water for comparison with ultrafiltrates. The serum water content was calculated by the formula of McLean and Hastings(9a), $W_s = 99.0 - 0.75\ P_s$, a serum protein concentration of 6 g % being assumed.

Results. Table I 1A summarizes the results obtained with the Simms-Sanders ultrafiltration apparatus in 10 non-gouty subjects. The concentrations of urate in serum water are all within normal limits except for the last 3 values listed which are elevated because of azotemia associated with renal impairment. The mean difference between the concentrations of urate in serum water and those of the corresponding ultrafiltrates was +3.5%, S.D. ± 2.7%.

Table 1B summarizes the results obtained with the Simms-Sanders apparatus in 16 gouty subjects all showing distinct elevations in serum urate; 3 were in the midst of an acute attack, none were receiving drugs. There was no significant deviation in filtrability from the normal. The mean difference between the concentrations of urate in serum water and in the corresponding ultrafiltrates was +5.0%, S.D. ± 5.2%. Table 1C summarizes data on 16 additional gouty subjects, all receiving Benemid, a potent uricosuric agent(5). The increase in urinary uric acid excretion caused by such uricosuric agents as Benemid is generally believed to reflect suppression of tubular reabsorption of urate(5) but it has been suggested that they act by converting serum urate from colloidal polymer aggregates to an ultrafiltrable form(10). This hypothesis is not borne out by our data.

When the ultrafiltration apparatus employed by Adlersberg *et al.*(2) was used, however, appreciable quantities of serum urate could not be recovered in the ultrafiltrates (Table II), thus confirming their findings. The data in Table II also substantiate their

TABLE II. Urate Content of Serum Water ($U_{s.w}$) and of Corresponding Ultrafiltrates (U_{ul}) when Ultrafiltration was Performed in Apparatus of Adlersberg *et al.* for Varying Periods of Time.

No.	$U_{s.w.}$ (mg %)	U_{ul} (mg %)	$\frac{U_{s.w.}-U_{ul}}{U_{s.w.}} \times 100$ (%)	Hr of ultrafiltration
1*	5.4	4.3	20.4	12
2†	9.3	7.9	15.6	12
3†	10.7	8.3	22.4	12
4*	4.5	3.7	16.9	18
5†	10.7	9.1	15.1	24
6†	10.5	8.5	18.5	24
7*	5.4	4.3	20.4	24
8*	4.8	2.1	55.5	30
9*	5.4	3.4	37.9	30
10*	5.1	2.1	39.5	60

* Non-gouty subject.
† Gouty subject.

TABLE III. Urate Content of Serum Water before ($U^1_{s.w.}$) and after ($U^2_{s.w.}$) Contact with Metal Sieve.

No.	$U^1_{s.w.}$ (mg %)	$U^2_{s.w.}$ (mg %)	$\frac{U^1_{s.w.}-U^2_{s.w.}}{U^1_{s.w.}} \times 100$ (%)	Hr of contact
1	5.3	4.6	13.3	6
2	5.4	4.6	15.7	12
3	7.5	6.8	9.1	14
4	7.7	6.4	17.5	18
5	7.8	6.8	12.9	18
6	7.1	5.5	22.8	24
7	5.4	4.6	15.7	24
8	7.7	6.4	22.1	24
9	6.2	5.5	15.7	24
10	7.0	1.6*	76.9	36
11	6.8	.7*	89.0	48

* Heavy bacterial growth occurred in these two sera.

notation that the discrepancy increased as the duration of ultrafiltration was prolonged. This latter observation suggested that the apparent nonfiltrability of urate might be factitious since the composition of ultrafiltrates does not ordinarily change with the time of ultrafiltration. It occurred to us that protracted contact with the supporting metal sieve could lead to degradation of uric acid, which is notoriously susceptible to alteration by a wide variety of oxidizing and reducing substances. Accordingly, samples of serum were allowed to remain in the apparatus in contact with cellophane membranes resting on the metal sieve, but without ultrafiltration by application of pressure; then the serum was withdrawn and the uric acid content redetermined. As indicated in Table III, losses in uric acid of the same order of magnitude as observed after ultrafiltration were noted; moreover, the discrepancy increased with prolongation of the experiment.† Sera kept in Erlenmeyer flasks at room temperature, without contact with the metal sieves, showed a change in urate content from +3.4% to —6.5% after 24-48 hours.

Discussion. Our results are consistent with the view that the plasma urate in man, whether present in normal quantities or in the higher concentrations encountered in gouty or uremic subjects, may be assumed to be virtually wholly filtrable at the glomerulus, and that the urate clearance deficit may properly be ascribed to some 90% reabsorption of filtered urate in the tubules. The Simms-Sanders ultrafiltration technic employed does, however, leave open the possibility that the plasma urate may be so loosely bound to plasma proteins as to be liberated in the course of ultrafiltration. As already indicated, this possibility could be excluded in the case of protein-bound T-1824 dye. Additional evidence to the contrary was obtained in the course of study of a patient with glomerulo-tubular imbalance of the Fanconi type(5). As a consequence of defective tubular transport mechanisms, this patient's kidneys were incapable of reabsorbing urate and his urate clearance was found to be equivalent to his glomerular filtration rate: $C_{urate}/C_{In} = 0.98$, as compared with a mean urate clearance ratio for normal man of 0.067. If a substantial proportion of the plasma urate were in a nonfiltrable state, this could not occur unless through the coincidence of precisely proportionate tubular secretion of urate; and the absence of any uricosuric response to Benemid in this subject makes such a coin-

† It should be pointed out, however, that there is probably more contact between the serum urate and the metal sieve in the simulated ultrafiltration experiment than when pressure is applied, since pressure would flatten out the interposed cellophane membrane. Also, the explanation offered for the discrepancy would not apply to the Lavietes apparatus in which there is no contact with a metallic support.

cidence even more improbable. In the Dalmatian hound, equivalence of the urate clearance and the glomerular filtration rate occurs regularly(11) and accounts for the excess urinary urate excretion characteristic of this species; moreover, the Dalmation hound, unlike other dogs, does not respond to uricosuric agents(12,13). In the snake and frog, Bordley and Richards(14) demonstrated equivalent concentrations of urate in plasma and capsular urine obtained by direct puncture.

The possibility that some urate may be present in the plasma in aggregates which are filtrable or readily depolymerized is not excluded by our data. Such polymers presumably would be more likely to form at the higher plasma urate concentrations found in gout or nephritis. Our data do not indicate a significant deviation from the normal diffusibility under these circumstances. In fact, gouty subjects with azotemia due to significant diminution in glomerular filtration rate often show higher C_{urate}/C_{In} ratios(15,16).

Wolfson and his collaborators(6,17) have pointed to the low urate content of cerebrospinal fluid as indicating that a substantial part of the plasma urate must be in nonfiltrable form. This does not necessarily follow since the composition of the cerebrospinal fluid does not in many respects conform to that of a plasma ultrafiltrate(18).

Summary and conclusions. 1. Sera of nongouty and gouty human subjects were subjected to ultrafiltration by 2 different procedures. The urate concentration of ultrafiltrates obtained by means of a Simms-Sanders apparatus did not differ significantly from that of the corresponding sera, expressed as serum water. Low values obtained by the second method employed were found to be factitious. 2. The available data appear to justify the assumption that the plasma urate in man is virtually wholly filtrable at the glomerulus and that some 90% of the filtered urate normally is reabsorbed in the tubules.

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