Uric Acid Binding to Serum Proteins: Differences Among Species (36196)

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An area of long standing controversy in renal physiology has been reopened by *in vitro* demonstrations of uric acid binding to human plasma proteins. Although significant binding had been suggested in the past, this possibility was largely discounted after the negative ultrafiltration experiments of Gutman and Yu in 1953 (1). Recent studies have now shown that 20% or more of plasma uric acid may be protein-bound, although low temperature and hypotonic buffer strength have been required for this demonstration (2–4). The question is an important one since protein-bound uric acid might escape filtration at the glomerulus in contrast to the present concept of free filtration with subsequent bidirectional transport in the renal tubule. The additional demonstration of competitive inhibition of urate binding by drugs (5, 6) also raises the possibility that filtration may be increased by pharmacologic dissociation of uric acid from protein binding sites. Systems for uric acid excretion are clinically relevant in man since renal retention of uric acid can lead to supersaturation of body fluids. The secondary precipitation of crystals which may then ensue is thought to be the initiating event in acute and chronic gouty arthritis.

In order to assess the possible physiologic significance of protein binding, an investigation was undertaken in a number of animal species.[1] Previous work has shown a confusing degree of species variation in renal transport of uric acid. Thus, probenecid is thought to inhibit tubular reabsorption of uric acid in man, Cebus monkey and rat but to inhibit tubular secretion in the Dalmatian dog, rabbit, goat, and guinea pig. Similar disparities have been found in the effects of pyrazinoic acid, lactate, aspirin and a number of other agents. Species differences in protein binding which correlated with these known pharmacologic variations could have great significance in reinterpreting the mechanisms of renal transport. To evaluate this possibility protein binding of uric acid was studied in eleven animal species using an isotopic modification of the method of Klinenberg *et al.* (4).

Methods. Duplicate 1 ml samples of serum were tied in cellophane dialysis bags (Visking dialysis casing, flat width 8/32 in.) and predialyzed for 24 hr at 4° with one bath change against large volumes of 0.05 *M* sodium phosphate buffer pH 7.0 containing 5 mg% uric acid and 20 mg% sodium azide. The bags were then dialyzed an additional 18 hr at 4° against the same buffer with trace amounts of uric acid-2-^{14}C (Amersham/Searle Corp.) added to the bath. 0.1 ml aliquots of both bath and sample were counted in duplicate and the degree of binding expressed as the ratio of the counting rate in serum divided by the counting rate in the dialysate.

Binding ratios greater than 1.0 indicate concentrations of labeled uric acid within the dialysis bag which are higher than that of the surrounding bath. This implies an interaction between the urate ion and the nondiffusable proteins within the bag. When there is no binding, the ratio will be somewhat less than 1.0 as a result both of Gibbs–Donnan effects on anion distribution and also of the specific volume of the proteins within the bag.

Results. Binding of uric acid to serum proteins was clearly shown for man, Rhesus monkeys, rabbits, rats, guinea pigs and chickens. In marked contrast, little or no binding could be found in the serum of Cebus monkeys, frogs, snakes and both mongrel and Dalmatian dogs (Table I). The differences

[1] The term "species" is used broadly since the comparisons drawn are at a number of different levels by proper biologic classification.

TABLE I. Uric Acid Binding Ratios in Selected Animal Species.

Species	n	Binding ratio ± SD
Man	27	1.21 ± 0.04
Rhesus monkey	5	1.25 ± 0.06
Cebus monkey	4	0.98 ± 0.04
Mongrel dog	11	0.99 ± 0.06
Dalmatian dog	5	0.92 ± 0.02
Rabbit	4	1.28 ± 0.06
Rat	7	1.20 ± 0.04
Guinea pig	4	1.19 ± 0.10
Frog	4	0.91 ± 0.03
Snake	2	0.95 ± 0.04
Chicken	3	1.18 ± 0.04

among groups were significant as indicated by an F-test showing $p < 0.05$. Differences between individual species were more significant when examined by the t-test. Duplicate specimens agreed well with each other having an overall standard deviation of 0.03 for 80 serum samples studied.

Discussion. The marked differences in protein binding of uric acid observed between species do not correlate with known features of the physiology and pharmacology of uric acid excretion. This is well shown in the contrast between man and Cebus monkeys. These New World monkeys do not bind uric acid in our *in vitro* system, but have been thought to represent one of the closest parallels to human uric acid transport of any mammalian species studied (7, 8). Similarly, there is no apparent correlation between the known variations in the renal effects of probenecid (or other drugs) and the uric acid binding differences found among species.

The renal excretion of uric acid in the Dalmatian coach hound is of special interest since, in contrast to other mammals, this dog commonly exhibits a renal clearance of uric acid greater than its glomerular filtration rate. This unique characteristic is inherited as an autosomal recessive trait. Studies of these animals have suggested that they have normal tubular secretion but diminished reabsorption of filtered uric acid (9). An alternative explanation could be advanced, however, based on the possibility of complete filtration of unbound uric acid in Dalmatians with limited filtration of protein-bound uric acid in other dogs. The binding ratio found in five Dalmatians was extremely low at 0.92 and is significantly less ($p < .005$) than the mean value of 0.99 obtained in eleven mongrel dogs. The difference found is small, however, and the value for mongrel dogs is so low that there can be no evidence from this study of physiologically important protein binding in the mongrel. Certainly binding of this small degree, were it present *in vivo,* would have no significant effect on glomerular filtration of uric acid.

Binding ratios of uric acid in frogs and snakes are also of particular interest since these are the only animals in which filtration of uric acid has been examined *in vivo* by micropuncture studies of glomerular filtrate. In elegant studies reported in 1933, Bordley and Richards found uric acid to be filtered fully at the glomeruli of both frogs and snakes (10). If these animals were shown to bind uric acid *in vitro,* it would be clear that the binding studied is a laboratory phenomenon without physiologic effects at the glomerulus. Neither frogs nor snakes, however, showed significant binding of uric acid in the present studies.

Further study of uric acid filtration across living biological membranes seems important. Investigations of uric acid binding have employed equilibrium dialysis, ultrafiltration or gel filtration techniques. The membranes and resins employed in such studies, however, will themselves adsorb uric acid at low temperatures and may present a poor analogy to the glomerular membrane *in vivo.* Micropuncture studies of glomerular filtrate in an animal which does bind uric acid such as the rat will be necessary to definitively establish whether filtration of uric acid is limited by protein binding in the living animal.

Several investigators (2–4) have pointed out that albumin is the principal human protein involved in uric acid binding. Electrophoretic surveys of serum proteins in various animal species have found relatively little variation in albumin levels (11). The differences observed in the present study, therefore, cannot be attributed to differences in the amount of albumin available. The studies of Bluestone *et al.* (5, 6) showing inhibition of binding by probenecid and other drugs suggest that binding occurs at a specific site

or sites on the albumin molecule. In that context, the present studies may be useful in pointing to interspecies differences in the structure of albumin.

Summary. Binding of labeled uric acid to serum proteins of eleven animal species was studied using an equilibrium dialysis system. Significant differences were found among species with serum of man, rhesus monkey, rabbit, rat, guinea pig, and chicken demonstrating binding but with little or no binding found in serum of other animals studied. The physiologic significance of these variations is uncertain since they do not correlate with known differences in uric acid excretion by these animals, and binding is demonstrated only at low temperature in hypotonic buffers. The findings do indicate significant differences in the binding site on albumin.

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