Renal Clearance and Serum Protein Binding of Acetaminophen and Its Major Conjugates in Humans

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Abstract □ The renal clearances of acetaminophen, acetaminophen glucuronide, and acetaminophen sulfate were determined in eight healthy adults 2 h after administration of 1.5 g of acetaminophen. The renal clearance ratios (relative to creatinine) were 0.058 ± 0.026, 0.890 ± 0.153, and 1.43 ± 0.250 (mean ± *SD*), respectively. The renal clearance of acetaminophen increased with increasing urine flow rate, and that of acetaminophen sulfate decreased with increasing serum concentration of the conjugate. A strong positive correlation was found between the renal clearances of acetaminophen glucuronide and acetaminophen sulfate, possibly due to blood perfusion rate-dependent renal tubular secretion of the two conjugates. The serum protein binding of acetaminophen (≃20%) and acetaminophen glucuronide (<10%) are minor. Acetaminophen sulfate is >50% protein bound, as determined by equilibrium dialysis and ultrafiltration. The results of these studies are (*a*) consistent with previous reports of animal studies, indicating that renal excretion of acetaminophen involves glomerular filtration and passive reabsorption and that acetaminophen sulfate is subject to active renal tubular secretion, and (*b*) compatible with the reported occurrence of renal tubular secretion of acetaminophen glucuronide in animals.

Keyphrases □ Acetaminophen—renal clearance, serum protein binding, glucuronide and sulfate metabolites □ Renal clearance—acetaminophen, serum protein binding glucuronide and sulfate conjugates □ Protein binding—acetaminophen, ligand and albumin concentration

Acetaminophen, the widely used nonnarcotic analgesic and antipyretic agent, is eliminated primarily by formation of the glucuronide and sulfate conjugates (1–3). These metabolites are excreted as such in the urine. While the renal clearance of the acetaminophen conjugates has been studied extensively in experimental animals (4–6), only limited information is available concerning their renal clearance in humans (7, 8). We have, therefore, determined the renal clearances of acetaminophen, acetaminophen glucuronide, and acetaminophen sulfate in normal humans of both sexes and examined the concentration dependence of, and the interrelationship between, these processes. Since proper interpretation of renal clearance data requires knowledge of the plasma or serum protein binding characteristics of the cleared substance, we have also determined the serum protein binding of acetaminophen and its two major metabolites, and the effect of ligand and albumin concentration, respectively, on the protein binding of these compounds.

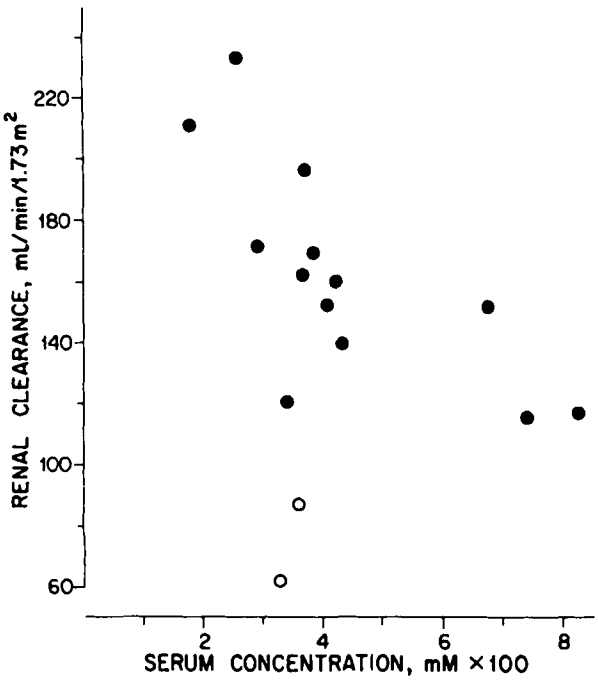

Figure 1—*Relationship between the apparent renal clearance of acetaminophen sulfate (normalized to a body surface area of 1.73 m²) and the serum concentration of acetaminophen sulfate; r = 0.718, p < 0.01. The open symbols are the data points of one outlier, which were not included in the statistical analysis; 0.01 mM acetaminophen = 1.51 μg/mL.*

EXPERIMENTAL SECTION

Eight adults in apparent good health (two females and six males, weighing 46–98 kg, 26–35 years old), received 1.5 g of acetaminophen in solution[1] orally, after an overnight fast. Seven of these subjects received acetaminophen a second time, 8 months later, after a light, low-fat breakfast (as part of another study). The subjects were drug-free for at least 4 d prior to the study. Urine was collected from 1 to 3 h after administration of the drug, and a blood sample was obtained at the midpoint of the urine collection period. Urine and serum samples were stored at −20°C until assayed. The concentrations of acetaminophen and its conjugates were determined by a slightly modified version of the HPLC assay of Adriaenssen and Prescott (9). A C_{18} column[2] with a mobile phase of 10% methanol and 1% acetic acid in 0.1 M potassium dihydrogen phosphate solution was used. The assay has a sensitivity limit of ~0.1 μg/mL (in terms of acetaminophen) for acetaminophen, acetaminophen sulfate, and acetaminophen glucuronide. Serum and urine were also assayed for creatinine by a modified picrate method[3].

Renal clearance values were determined by dividing the urinary excretion rate of acetaminophen, its conjugates, or creatinine by the midpoint serum concentration of the respective compound. Clearance ratios were calculated by dividing the clearance of acetaminophen or its conjugates by the creatinine clearance. Body surface area was estimated by means of a nomogram (10) based on body weight and height.

Multiple regression analysis was used to determine the relationships between the renal clearances of acetaminophen, or its conjugates, and urine flow rate, creatinine clearance, and serum concentration (11). Bivariate linear regression equations were determined by the perpendicular least-squares method, in which it is assumed that both variables are subject to error (12).

Serum protein binding measurements were made by equilibrium dialysis at 37°C against an equal volume, 0.3 mL, of 0.13 M phosphate buffer (pH 7.4) and by ultrafiltration. In the equilibrium dialysis studies, acetaminophen and its conjugates were added to the buffer phase in amounts designed to produce serum concentrations similar to those found in the study samples. The buffer solutions were dialyzed against pooled blank human serum or solutions of crystalline human albumin[4]; also, blank buffer was dialyzed against samples of pooled serum obtained in this study. Preliminary experiments showed that the optimal equilibration time for acetaminophen, acetaminophen glucuronide, and acetaminophen sulfate was 6–8 h. The protein binding of acetaminophen, acetaminophen glucuronide, and acetaminophen sulfate over the concentration ranges of 0.10–1.15 mM, 0.04–0.52 mM, and 0.11–1.22 mM, respectively, in human albumin solutions of varying concentrations (1.57–4.79 g/dL) was determined. Tris buffer (0.16 M) was used instead of phosphate buffer in some of these experiments.

[1] Tylenol Elixir; McNeil Lab, Inc., Fort Washington, Pa.
[2] μ-Bondapak C_{18}; Waters Associates, Milford, Mass.
[3] Creatinine Kit (No. 555-A); Sigma Chemical Co., St. Louis, Mo.
[4] Calbiochem-Behring, La Jolla, Calif.

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Table I—Renal Clearance of Acetaminophen, Acetaminophen Glucuronide, and Acetaminophen Sulfate in Normal Adults [a]

Excretion Product	Renal Clearance, mL/min/1.73 m²	Clearance Ratio[b]
Acetaminophen	6.32 ± 1.93 (3.26-8.92)	0.058 ± .026 (0.030-.103)
Acetaminophen glucuronide	100 ± 13.6 (80.9-120)	0.890 ± .153 (0.770 ± 1.16)
Acetaminophen sulfate	161 ± 30.6[c] (116-200)	1.43 ± .250[c] (1.05-1.86)

[a] Results expressed as mean ± *SD* (range), $n = 8$, based on clearance values for each subject, which are the average of two determinations made on separate occasions. The average renal clearance of creatinine ranged from 75.5 to 135 mL/min/1.73 m², and average urine flow rates ranged from 15 to 75 mL/h/1.73 m². [b] Relative to creatinine. [c] One outlier with an average renal clearance of 74.9 mL/min/1.73 m² and an average clearance ratio of 0.628 was excluded.

Ultrafiltration studies were performed with solutions of acetaminophen and its conjugates in pH 7.4 isotonic phosphate buffer (to examine the possibility of membrane binding) and with serum obtained following the administration of acetaminophen. Approximately 2 mL of buffer solution or serum was placed in cellulose tubing[5] (0.83 mm o.d., molecular exclusion limit of 12,000–14,000 Da) which was mounted in stoppered plastic centrifuge tubes. The tubes were centrifuged at 750×*g* for 30 min at 37°C, and 0.10–0.15 mL of ultrafiltrate was collected (13).

Acetaminophen sulfate was synthesized in our laboratory[6] by the method of Feigenbaum and Neuberg (14), and acetaminophen glucuronide was supplied[7] (15). Total protein concentrations were determined by the biuret method (16), and the albumin fraction was determined by electrophoresis[8].

RESULTS

The creatinine clearance values of the subjects in this study ranged from 74 to 139 mL/min/1.73 m². Urine flow rates ranged[9] from 15 to 110 mL/h/1.73 m². The renal clearance values for acetaminophen, acetaminophen glucuronide, and acetaminophen sulfate and the corresponding renal clearance ratios relative to creatinine are summarized in Table I[10]. The renal clearance ratio of acetaminophen sulfate was consistently larger than unity, indicative of net renal tubular secretion. The renal clearance ratio of acetaminophen was quite small, reflecting extensive renal tubular reabsorption since (as will be shown) the serum protein binding of the drug is very limited. The body surface area-normalized renal clearance of acetaminophen increased with increasing normalized urine flow rate ($r = 0.577$, $p < 0.05$), but the urine flow rate had no apparent effect on the renal clearances of acetaminophen glucuronide or acetaminophen sulfate.

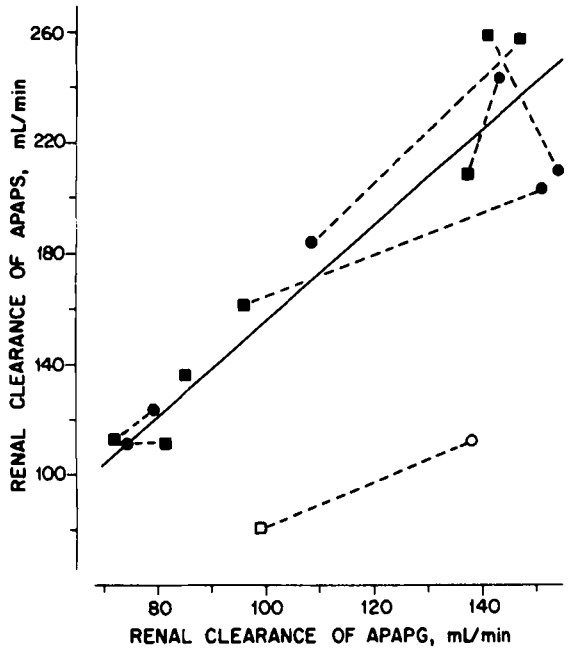

Figure 2—*Relationship between the apparent renal clearances of acetaminophen sulfate (APAPS) and acetaminophen glucuronide (APAPG); r = 0.928, p < 0.001. Seven of the eight subjects received acetaminophen on two separate occasions [first dose (■), second dose (●)], and the two data points for any one subject are connected by a dashed line. The open symbols are the data points for one outlier, which were not included in the statistical analysis.*

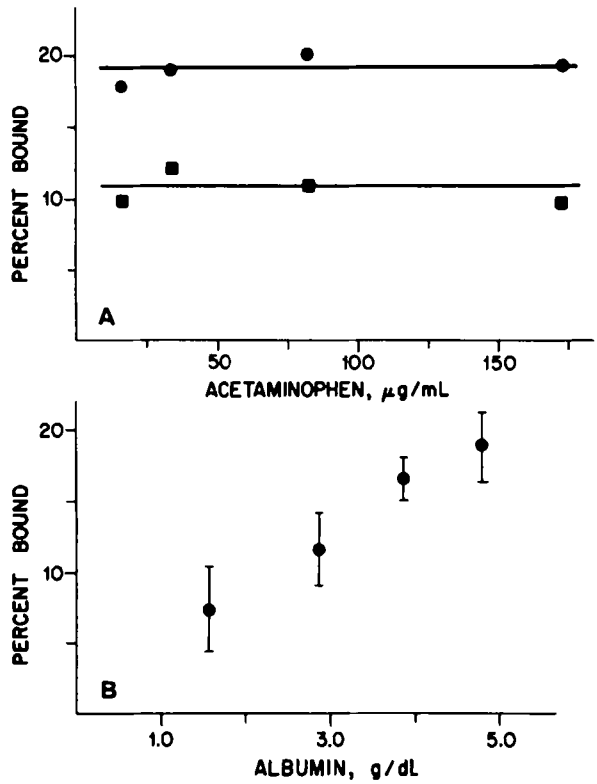

Figure 3—*Protein binding of acetaminophen in human albumin solution, determined by equilibrium dialysis. (A) Binding of acetaminophen over the concentration range of 15–174 μg/mL in 4.79 g/dL (●) and 2.88 g/dL (■) albumin solution. (B) Binding (mean ± SD, n = 9) of acetaminophen over the same drug concentration range in albumin solutions of 1.57, 2.88, 3.88, and 4.79 g/dL.*

The normalized renal clearance of acetaminophen sulfate decreased with increasing serum concentration of this conjugate (Table II and Fig. 1). A similar negative correlation ($r = -0.771$, $p < 0.01$) was found with respect to the acetaminophen sulfate-to-creatinine clearance ratio and acetaminophen sulfate serum concentration for concentrations <0.06 mM (this excludes a total of three sets of data from two subjects), but the correlation was not statistically significant when all data were included. There was no apparent correlation between normalized renal clearance and serum concentration in the cases of acetaminophen and acetaminophen glucuronide, respectively (Table II). Also, there was no statistically significant correlation between the normalized renal clearance of either conjugate and the serum concentration of acetaminophen.

The absolute renal clearances of acetaminophen glucuronide and acetaminophen sulfate for individual subjects (except one outlier) were strongly correlated (Fig. 2). A statistically significant correlation was found also with respect to normalized clearance values for the individual subjects ($r = 0.876$, $p < 0.001$), again excluding the one outlier. The latter individual yielded, on two occasions, significantly lower renal clearance values for acetaminophen sulfate than any of the other subjects in the study, but renal clearance values for acetaminophen glucuronide (Fig. 2) and the serum-free fraction of acetaminophen sulfate (≃0.4) for this subject were in the normal range.

Neither acetaminophen nor acetaminophen glucuronide was significantly protein bound in the serum of subjects who had taken acetaminophen or in human albumin solution and blank serum to which these compounds had been added *in vitro* (Table III). On the other hand, acetaminophen sulfate was

[5] Union Carbide Corp., New York, N.Y.
[6] By Dr. R. E. Galinsky.
[7] Dr. Leonard Weintraub of Bristol-Myers Products, Hillside, N.Y.
[8] Gelman Sepratek Electrophoresis System.
[9] This range is based on individual determinations while the ranges in Table I are averages of two determinations per subject.
[10] Individual urine and serum concentration data are available on request.

Table II—Concentration Dependence of the Renal Clearance and Clearance Ratio of Acetaminophen, Acetaminophen Glucuronide, and Acetaminophen Sulfate [a]

	Range of Serum Conc., mM	Range of Clearance, mL/min/1.73 m²	Range of Clearance Ratio[c]	Correlation Coefficient	
				Clearance	Clearance Ratio
Acetaminophen	0.070–0.200	3.26–11.3	0.030–0.127	0.095	0.229
Acetaminophen glucuronide	0.052–0.134	71.7–132	0.530–1.169	−0.104	−0.086
Acetaminophen sulfate[b]	0.018–0.083	115–233	0.889–2.12	−0.718[d]	−0.145

[a] Ranges and correlation analyses based on 15 individual sets of values from eight subjects (all but one subject participated in two studies). One mM of acetaminophen glucuronide and acetaminophen sulfate is equal to 327 mg/L and 231 mg/L, respectively. [b] One outlier (see footnote c in Table I) was excluded. [c] Relative to creatinine. [d] $p < 0.01$.

appreciably (>50%) protein bound in all of these fluids. These results, obtained by equilibrium dialysis, were confirmed by ultrafiltration experiments. For example, the serum free fraction value (mean ±*SD*, $n = 3$ or 4) of acetaminophen sulfate was 0.460 ± 0.021 by equilibrium dialysis and 0.425 ± 0.021 by ultrafiltration. Preliminary ultrafiltration experiments with protein-free aqueous solutions of acetaminophen and its two conjugates yielded average "free fraction" values consistently near unity (0.988–1.07), indicative of negligible binding to the cellophane membrane. Freezing and thawing of serum (one or two times) had no apparent effect on the protein binding of acetaminophen and its conjugates.

The albumin binding of acetaminophen was independent of drug concentration over a wide range, but increased with increasing albumin concentration (Fig. 3). On the other hand, the albumin binding of acetaminophen sulfate decreased with increasing concentration of the conjugate and increased with increasing albumin concentration (Fig. 4). The protein binding of acetaminophen glucuronide was very low (<10%) and not significantly affected by its concentration or by the concentration of albumin[11].

DISCUSSION

Acetaminophen, in nontoxic doses, has no apparent effect on renal creatinine clearance in humans (17) and on glomerular filtration rate and other indices of renal function in experimental animals (18–20). Consistent with previous reports (7, 8), the renal clearance of unmetabolized acetaminophen is quite low despite its negligible serum protein binding and is positively correlated with urine flow rate (7). It is also independent of acetaminophen concentration in the serum, at least under the conditions of this investigation. These observations confirm studies in dogs which have shown that acetaminophen undergoes glomerular filtration and renal tubular reabsorption by simple diffusion (4). The mean renal clearance of acetaminophen obtained in this investigation, 6.32 ± 1.93 mL/min/1.73 m², is somewhat lower than that reported by Prescott *et al.* (8) for 12 normal subjects (11.9 ± 4.9 mL/min/67 kg average body weight). This may be due to different urine flow rates in the two studies. In general, small differences in the average renal clearances of acetaminophen and its major metabolites, observed in this investigation and in that by Prescott *et al.* (8), may be due to the fact that the data reported here are based on a single serum concentration determination at the midpoint of a 2-h urine collection period while Prescott *et al.* employed the usually preferable area-under-the-curve method. However, the latter method would not reveal concentration dependence of renal clearance if the determinations are based on the total area under the plasma concentration–time curve and total urinary excretion. The conditions of the present study (*i.e.*, a constant absolute dose given to subjects of widely differing body weight) facilitated assessment of the possible serum concentration dependence of the renal clearances.

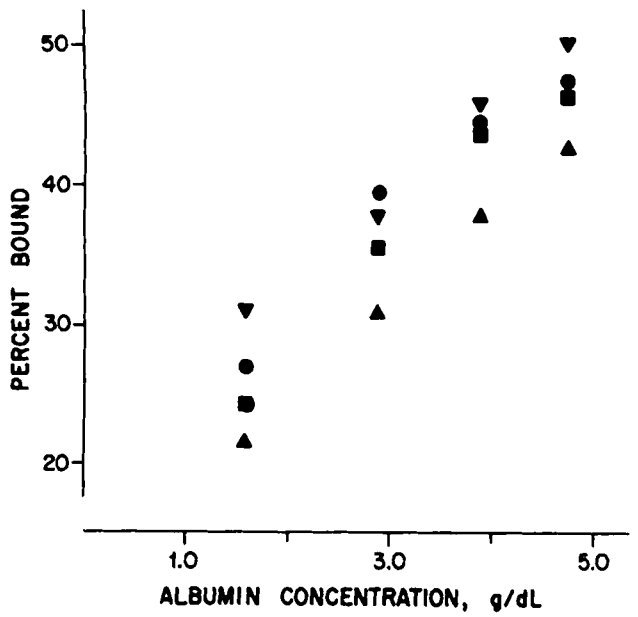

Figure 4—*Protein binding of acetaminophen sulfate in human albumin solutions (1.57, 2.88, 3.88, and 4.79 g/dL), determined by equilibrium dialysis, at acetaminophen sulfate concentrations of 1.22 ± 0.040 mM (mean ± SD) (▲), 0.564 ± 0.038 mM (■), 0.252 ± 0.021 mM (●), and 0.107 ± 0.007 mM (▼).*

[11] Unpublished results.

Renal clearance values for the acetaminophen glucuronide and sulfate conjugates determined following administration of acetaminophen, rather than of the conjugates themselves, must be considered, in principle, as estimates of apparent renal clearances. This is because studies on isolated perfused rat kidneys (18–20) and isolated rat kidney cells (21) have shown that renal tissues form acetaminophen glucuronide and sulfate conjugates, albeit at very low rates. These conjugates do not appear in the perfusate of isolated kidneys, but are immediately excreted in the urine. It is likely that the renal contribution to acetaminophen conjugation is also quantitatively negligible in humans since the elimination of acetaminophen by biotransformation is not impaired in anephric patients (22).

Previous studies by Duggin and Mudge in dogs (4) and by Lin and Levy in rats (6) have shown that the renal clearance of acetaminophen sulfate is substantially higher than glomerular filtration rate at low serum concentrations of the conjugate and that the renal clearance decreases with increasing serum concentration. The present study in humans also yielded renal clearance ratios, relative to creatinine, appreciably higher than unity. This demonstrates that acetaminophen sulfate is subject to renal tubular secretion. While, in theory, similar results may be obtained if the conjugate was excreted only by glomerular filtration and formed at a high rate in the renal tissues, that possibility is unlikely in view of the evidence reviewed in the preceding paragraph. Moreover, there was no relationship between the normalized apparent renal clearance of acetaminophen sulfate and the serum concentration of acetaminophen. A relationship would be expected had there been appreciable renal synthesis of the conjugate. The renal clearance value obtained in this study (161 ± 30.6 mL/min/1.73 m²) is in good agreement with that of Prescott *et al.* (166 ● 29 mL/min) in 12 healthy volunteers, with an average body weight of 67 kg, who received a single dose of acetaminophen, 20 mg/kg (8). More direct comparison is not possible since Prescott *et al.* did not report individual or normalized average values or glomerular filtration rates.

The apparent renal clearance of acetaminophen glucuronide determined in this study, 100 ± 13.6 mL/min/1.73 m², is also in reasonable agreement

Table III—Serum Protein Binding of Acetaminophen, Acetaminophen Glucuronide, and Acetaminophen Sulfate [a]

	Free Fraction		
	Acetaminophen	Acetaminophen Glucuronide	Acetaminophen Sulfate
Serum after administration of acetaminophen	0.819 ± 0.018	0.923 ± 0.009	0.460 ± 0.021
Human albumin, 5%	0.818 ± 0.013	0.928 ± 0.012	0.469 ± 0.019
Blank serum	0.780 ± 0.020	0.916 ± 0.019	0.391 ± 0.016
	0.844 ± 0.017[b]	0.954 ± 0.019[b]	0.387 ± 0.015[b]

[a] Results expressed as mean ±*SD* ($n = 3$). Serum was dialyzed for 6–8 h at 37°C against isotonic phosphate buffer, pH 7.4. The approximate concentrations of acetaminophen, acetaminophen glucuronide, and acetaminophen sulfate at equilibrium were 0.09, 0.06, and 0.05 mM, respectively, in serum from medicated subjects, and 0.2, 0.1, and 0.05 mM in albumin solution and blank serum. [b] Determined by 6-h dialysis against Tris buffer instead of phosphate buffer.

with that of Prescott *et al.* (8), 131 ± 22 mL/min, but did not, on the average, exceed creatinine clearance. However, there were four individual renal clearance ratio values (of two subjects) above unity: the highest of these was 1.17. Thus, the results of the present study do not provide strong evidence for renal tubular secretion of acetaminophen glucuronide, perhaps because of the opposing effects of tubular secretion and reabsorption. Duggin and Mudge (4) found that acetaminophen glucuronide is subject to both processes in the dog.

This investigation revealed a striking positive correlation between the renal clearances of acetaminophen glucuronide and acetaminophen sulfate in normal human subjects. Such correlation could reflect the existence of a common renal tubular secretory (transport) process for the two conjugates. Alternatively, the secretion rate of both conjugates could be blood flow rate dependent, either in terms of overall renal blood flow or with respect to the blood perfusion of the secretory region of the kidneys. We favor the second of these alternatives because of evidence suggesting separate renal transport processes for the two conjugates. First, probenecid has been found to inhibit the net renal tubular secretion of acetaminophen sulfate, but not that of the glucuronide, in dogs (4). Second, the present investigation identified one apparently healthy male subject, with normal creatinine, inorganic sulfate (17), and acetaminophen glucuronide renal clearances, but markedly subnormal renal clearance of acetaminophen sulfate during two experiments ~8 months apart. Such selectivity suggests a dissociation of the secretory processes for acetaminophen glucuronide and sulfate or, alternatively, the existence of separate specialized reabsorption processes together with a common or separate process(es) for renal tubular secretion of the two conjugates.

The serum protein binding of acetaminophen and acetaminophen glucuronide is very limited, so that correction of the renal clearance values of these compounds for protein binding will have only a quantitatively small effect. On the other hand, acetaminophen sulfate was found to be appreciably bound to serum proteins, so that its renal clearance referenced to the unbound metabolite would be more than double the value reported in Table III. While the limited protein binding of acetaminophen and acetominophen glucuronide is consistent with the results of previous studies (4, 22, 23), one of these studies had also indicated that acetaminophen sulfate binding to serum proteins is similarly negligible. Consequently, the protein binding of acetaminophen sulfate was examined in considerable detail in this investigation. Appreciable (>50%) binding was found by equilibrium dialysis and ultrafiltration, with good agreement between the results of the two methods. These binding experiments were performed on serum obtained from acetaminophen-treated subjects, blank serum with acetaminophen and its two major conjugates added *in vitro*, and human albumin solution with added acetaminophen and conjugates. All of these fluids yielded similar free fraction estimates. Moreover, protein binding was also determined in serum from rats pretreated with acetaminophen, with the following average results (percent binding, concentration): acetaminophen 23%, 0.55 mM; acetaminophen glucuronide 5%, 0.15 mM; acetaminophen sulfate 65%, 0.28 mM. Several studies by other investigators have shown that various organic sulfate esters are extensively bound to plasma proteins (24–26).

Acetaminophen is almost entirely metabolized to the glucuronide and sulfate conjugates under ordinary conditions, and these conjugates are excreted in the urine. Renal failure results, therefore, in substantial accumulation of these metabolites in plasma (22). The present study provides information relevant to the elimination of these metabolites by human subjects with normal renal function under conditions yielding serum concentrations of acetaminophen and its glucuronide and sulfate conjugates in the usual therapeutic range.

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