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Renal Clearance of Inorganic Sulfate in Rats: Effect of Acetaminophen-Induced Depletion of Endogenous Sulfate

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Abstract □ Certain drugs that are eliminated partly by conjugation with sulfate cause appreciable depletion of endogenous inorganic sulfate in animals and humans. The limited availability of endogenous inorganic sulfate contributes to, or is responsible for, dose-dependent elimination kinetics and can cause pronounced time-dependent changes in the disposition of drugs that are metabolized to sulfate conjugates. A comprehensive characterization of the pharmacokinetics of such drugs requires, therefore, an interdigitation with the kinetics of endogenous inorganic sulfate. The latter is cleared from the body primarily by renal excretion. The purpose of this investigation was to determine the relationship between the renal clearance and the serum concentration of inorganic sulfate, particularly in the subnormal concentration range during acetaminophen-induced sulfate depletion. Administration of acetaminophen, 150 mg/kg iv, to adult male rats reduced their serum inorganic sulfate concentration from $\simeq$1 m*M* to <0.1 m*M* without affecting the renal clearance of creatinine. The renal clearance ratio, sulfate–creatinine, decreased from between 0.2 and 0.3 at $\simeq$1 m*M* serum sulfate to $\simeq$ 0.05 at <0.1 m*M* serum sulfate concentration. When serum sulfate concentration was increased to $\simeq$1.5 m*M* by iv infusion of sodium sulfate, the sulfate–creatinine renal clearance ratio increased abruptly to $\simeq$1 without affecting creatinine renal clearance. Acetaminophen had no effect on the high renal clearance of inorganic sulfate during hypersulfatemia. The pronounced serum concentration dependence of sulfate renal clearance facilitates inorganic sulfate homeostasis. A survey of the literature indicates that the relatively low endogenous serum sulfate concentration ($\simeq$0.3–0.4 m*M*) in humans (compared with dogs, rabbits, and rats) is due primarily to low formation rate rather than high renal clearance of inorganic sulfate.

Keyphrases □ Inorganic sulfate—renal clearance in rats, effect of acetaminophen-induced depletion of endogenous sulfate □ Renal clearance—inorganic sulfate in rats, effect of acetaminophen-induced depletion of endogenous sulfate □ Acetaminophen—renal clearance of inorganic sulfate in rats, effect of induced depletion of endogenous sulfate

Conjugation with sulfate is an important biotransformation pathway for certain phenolic drugs and for many endogenous compounds including certain steroids and biogenic amines (1). Administration of therapeutic or pharmacologic doses of acetaminophen or salicylamide results in depletion of endogenous sulfate, as reflected by decreased serum concentrations and urinary excretion of inorganic sulfate in humans and animals (2–6). This depletion of inorganic sulfate, and the consequent decrease in the availability of activated sulfate (3′-phosphoadenosine-5′-phosphosulfate) as a cosubstrate for sulfate conjugation, can cause or contribute to pronounced dose dependency in the pharmacokinetics of drugs that are eliminated by conjugation with sulfate and can also cause the pharmacokinetics of such drugs to exhibit marked time-dependency (7, 8). A comprehensive characterization of the pharmacokinetics of these drugs requires, therefore, mathematical models that include a description of the kinetics of endogenous sulfate formation and elimination.

Endogenous sulfate is derived primarily from oxidation of sulfur-containing amino acids and, partly, from inorganic sulfate contained in the diet (9–11). Thus, previous researchers (9) were able to demonstrate a strong correlation between the urinary excretion of sulfate and the dietary intake of methionine and cystine in human subjects. The urinary excretion of sulfate by children with kwashiorkor is only about one-third of normal due to their protein-deficient diet and the resultant reduced intake of sulfur-containing amino acids (10). Inorganic sulfate is eliminated from the body predominantly by urinary excretion as such (12). In nonmedicated humans on a normal diet and with normal renal function, only ~7–20% of total sulfate is excreted in bound form, *i.e.*, as endogenous sulfate conjugates (3,13). The ratio of bound to free sulfate excretion by normal rats is similar to (14) or even lower than in humans (3). Reduced or absent renal function is associated with pronounced retention of inorganic sulfate; there is a strong, positive correlation between the serum concentrations of creatinine and inorganic sulfate in hu-

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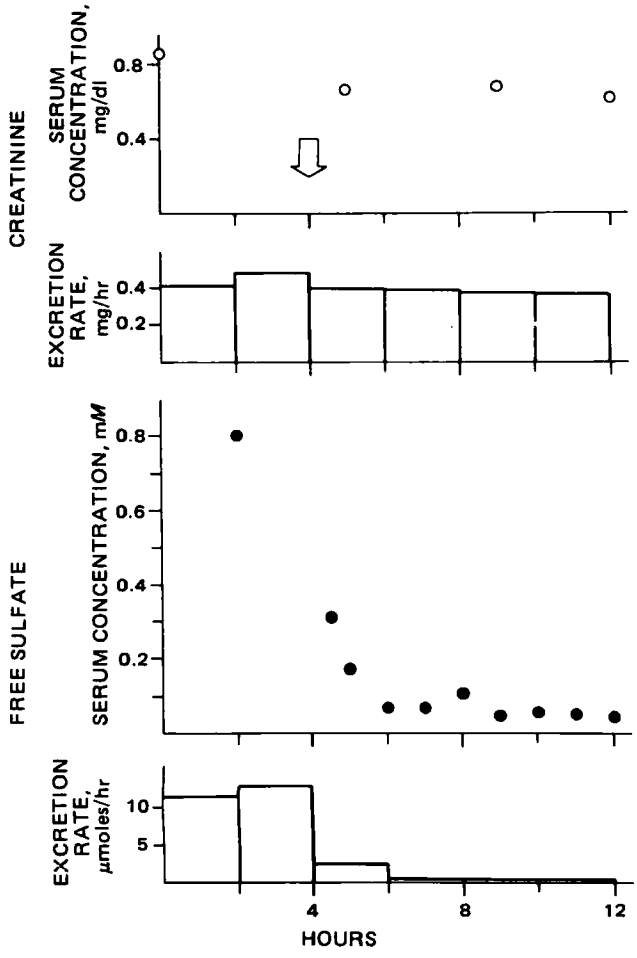

Figure 1—*Effect of an iv injection of acetaminophen (time of injection shown by arrow), 150 mg/kg, on creatinine concentration in serum and urinary excretion rate, and on inorganic sulfate concentration in serum and urinary excretion rate in a rat. This figure shows the experimental design used to determine the renal clearance and renal clearance ratio of inorganic sulfate as a function of sulfate concentration in serum in the normal and subnormal serum concentration range.*

mans (15) and rats (8) with renal dysfunction. Thus, renal clearance is the major determinant of inorganic sulfate elimination kinetics.

Studies on healthy humans and animals (dogs, rabbits, and rats) have shown that the renal clearance of inorganic sulfate is ~10–35% of glomerular filtration rate (GFR) under normal physiological conditions and increases to a rate approximately equal to GFR when serum sulfate concentrations are increased substantially by administration of sodium sulfate (15–20). This renal clearance–serum concentration profile is consistent with glomerular filtration of inorganic sulfate and partial renal tubular reabsorption by a capacity-limited process (19, 20). However, more recent studies have revealed also the presence of a tubular secretory process for sulfate ion in mammals, but it is quite clear that net reabsorption is predominant (21,22). To date, *in vivo* investigations of sulfate renal clearance have been performed under conditions in which serum sulfate concentrations were in the physiological range or elevated by administration of inorganic sulfate. For considerations of the role of endogenous sulfate levels in the elimination of drugs that are subject to sulfate conjugation, the renal clearance of inorganic sulfate at subnormal serum sulfate concentrations

Table I—Urinary Excretion Rate of Inorganic Sulfate, Acetaminophen Sulfate, and Total Conjugated Sulfate Before and After Injection of Acetaminophen [a]

Time Period, hr	Urinary Excretion Rate[b], μmoles/hr/kg		
	Inorganic Sulfate	Acetaminophen Sulfate	Total Conjugated Sulfate
0–4	23.2 ± 4.1	0	1.1 ± 1.9
4–8	5.6 ± 0.5	43.5 ± 8.8	46.9 ± 8.3
8–12	0.4 ± 0.2	28.4 ± 1.2	31.6 ± 2.1

[a] Acetaminophen, 150 mg/kg iv, at 4 hr. [b] Mean ± *SD*, $n = 3$.

is more relevant. This investigation has been designed particularly to determine the concentration dependence of inorganic sulfate renal clearance during sulfate depletion, *i.e.*, in the subnormal serum sulfate concentration range.

EXPERIMENTAL

Adult, male Sprague-Dawley rats (330–380 g) had a cannula implanted in the right jugular vein (23) and another in the urinary bladder, under light ether anesthesia 1 day before the experiment. The animals were kept unrestricted in individual plastic metabolism cages. Food and water were withdrawn in the morning and withheld for the duration of the experiment, which was started in the morning.

The general experimental design is illustrated in Fig. 1. Urine was collected at 2-hr intervals, usually for 12 hr. Each time, the bladder was

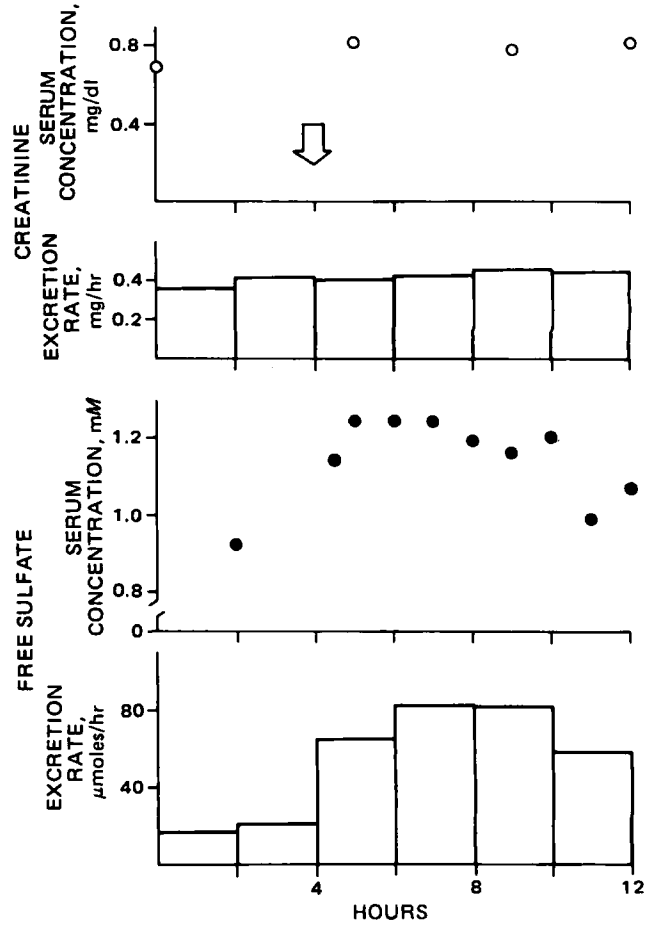

Figure 2—*Effect of an 8-hr infusion of sodium sulfate (start of infusion shown by arrow), 9.6 mg/hr, on creatinine concentration in serum and urinary excretion rate, and on inorganic sulfate concentration in serum and urinary excretion rate in a rat. The figure shows the experimental design used to determine the renal clearance and renal clearance ratio of inorganic sulfate as a function of sulfate concentration in serum in the normal and supernormal serum concentration range.*

Table II—Effect of Acetaminophen or Sodium Sulfate on Serum Concentrations and Renal Clearance of Creatinine in Rats[a]

Time, hr	Acetaminophen[b]		Sodium Sulfate[c]	
	Creatinine Concentration in Serum, mg/100 ml	Creatinine Renal Clearance, ml/min/kg	Creatinine Concentration in Serum, mg/100 ml	Creatinine Renal Clearance, ml/min/kg
0	0.67 ± 0.22	3.14 ± 1.22	0.55 ± 0.08	3.41 ± 0.84
5	0.59 ± 0.13	3.49 ± 1.75	0.59 ± 0.13	3.19 ± 0.85
7	0.63 ± 0.15	3.29 ± 1.35	0.59 ± 0.17	3.48 ± 1.26
12	0.59 ± 0.22	2.96 ± 1.22	0.64 ± 0.13	3.40 ± 1.28

[a] Results are expressed as mean ± *SD*, $n=11$ (for acetaminophen) or $n=6$ (for sodium sulfate). [b] 150 mg/kg iv at 4 hr. [c] 9.6 mg/hr was infused for 8 hr starting at the 4th hour of the experiment.

washed with three 1-ml portions of normal saline solution and these were combined with the collected urine. Blood samples were obtained at frequent intervals, as shown in Fig. 1. After a 4-hr control period for collection of two 2-hr urine samples and two blood samples (one for creatinine and the other for inorganic sulfate determination), the animals received an injection of acetaminophen, 150 mg/kg iv, through the cannula. The drug was administered as a 2.5% solution in 40% propylene glycol–normal saline. Blood samples (0.35 or 0.60 ml) were collected in plastic syringes, and serum was separated by centrifugation. To ensure that mainstream blood was obtained, the saline solution (without heparin) in the cannula and a small volume of blood were aspirated into another syringe before sample collection and then reinjected. One milliliter of normal saline solution was also injected after each blood withdrawal, to replace blood volume, fill the cannula, and stimulate urine flow.

Another group of rats had an additional cannula implanted in the left carotid artery for sodium sulfate infusion. These animals received 9.6 mg/hr/rat, as an isotonic solution containing 0.8% sodium sulfate and 0.45% sodium chloride in water. The solution was infused at a rate of 1.2 ml/hr from the 4th to the 12th hour of the experiment. The urine and blood collection schedule for this experiment is shown in Fig. 3. Some rats received both acetaminophen and sodium sulfate, the former as an injection at 4 hr and the latter as an infusion from the 8th to the 12th hour.

The concentration of inorganic sulfate in serum or urine was determined by the turbidimetric method of Berglund and Sörbo (24) as modified by Krijgsheld *et al.* (25). The method was scaled down for serum assays to a serum volume of 0.15 ml. The concentration of bound sulfate in urine was determined as the difference in sulfate concentrations before and after acid hydrolysis (diluted urine–10 *N* hydrochloric acid, 10:1, v/v, heated for 2 hr at 100°).

Creatinine concentrations in serum and urine were determined with a commercial kit[1], which is based on a modification of a previous assay procedure (26).

Acetaminophen sulfate in urine was assayed by high-performance liquid chromatography (HPLC) based on a previous method (27,28).

Renal clearances of inorganic sulfate and creatinine in the control periods were determined as excretion rate divided by serum concentration, and were normalized for body weight. During periods of changing serum sulfate concentrations, (after acetaminophen injection and during sodium sulfate infusion), the renal clearance of inorganic sulfate was calculated as the amount excreted in the urine divided by the area under the serum concentration–time curve during the urine collection interval. Some of the results are reported as clearance ratio, *i.e.*, the renal clearance of sulfate divided by the renal clearance of creatinine.

RESULTS

Control renal clearance determinations were made on 32 animals. The serum sulfate concentration was 0.89 ± 0.08 m*M*, urinary excretion of inorganic sulfate averaged 0.55 ± 0.26 μmole/min/kg, and renal clearance of inorganic sulfate was 0.64 ± 0.24 ml/min/kg. Serum creatinine concentrations were 0.72 ± 0.24 mg/dl, and creatinine clearance was 3.0 ± 1.2 ml/min/kg. The renal clearance ratio of inorganic sulfate was 0.24 ± 0.14. All of these results are mean values ±*SD*.

Injection of acetaminophen, 150 mg/kg, produced a rapid, pronounced, and persistent decline of serum concentrations and urinary excretion of inorganic sulfate (Fig. 1). A separate study showed that under these conditions, acetaminophen concentrations in plasma average ~150 μg/ml initially and decline with an apparent half-life of ~1 hr (7).

The urinary excretion of sulfate before acetaminophen administration consisted mostly of inorganic (free) sulfate; <10% of total sulfate was of the bound form (*i.e.*, sulfate conjugated with endogenous substances). The pronounced reduction of inorganic sulfate excretion after acetaminophen administration was accompanied by a very large increase in the excretion rate of bound (conjugated) sulfate, mostly in the form of acetaminophen sulfate (Table I).

Acetaminophen had no apparent effect on the serum concentration and urinary excretion rate of creatinine and, therefore, on the renal clearance of endogenous creatinine (Fig. 1 and Table II). This lack of effect became apparent in preliminary experiments and justified a reduction of serum creatinine determinations (which required an extra 0.25-ml of blood per sample) to four during 12 hr, to minimize blood volume depletion. Sulfate renal clearance ratios were, therefore, determined on the basis of an average creatinine clearance for each animal, calculated by dividing the 12-hr creatinine excretion rate by the average of the four successive serum creatinine concentrations.

Control experiments were performed to determine the possible effects of normal saline solution and propylene glycol on the renal clearances of creatinine and inorganic sulfate in otherwise unmedicated rats. Seven rats were studied during a 4-hr control period and during 4 hr of normal saline infusion at a rate of 1.2 ml/hr. The ratios, control–treatment, of individual creatinine and sulfate clearance values were 1.00 ± 0.18 (*SD*) and 0.93 ± 0.15, respectively. The effect of propylene glycol was determined in an experiment identical to that described in Fig. 1 except for the omission of acetaminophen. The injection solution consisted of 40% propylene glycol in normal saline, 6 ml/kg. Again, there was no effect on the renal clearance of creatinine or sulfate; the control–treatment ratios were 0.92 ± 0.15 for creatinine and 1.02 ± 0.18 for sulfate renal clearance.

Infusion of sodium sulfate caused a significant increase of serum concentrations and urinary excretion rates of inorganic sulfate, without any apparent effect on the serum concentrations and renal clearance of creatinine (Fig. 2 and Table II).

Depletion of endogenous inorganic sulfate by acetaminophen and supplementation by sodium sulfate administration permitted determination of the relationship between serum concentration and renal clearance of sulfate over a wide concentration range (Fig. 3). To rule out a possible direct effect of acetaminophen on sulfate clearance (*i.e.*, an

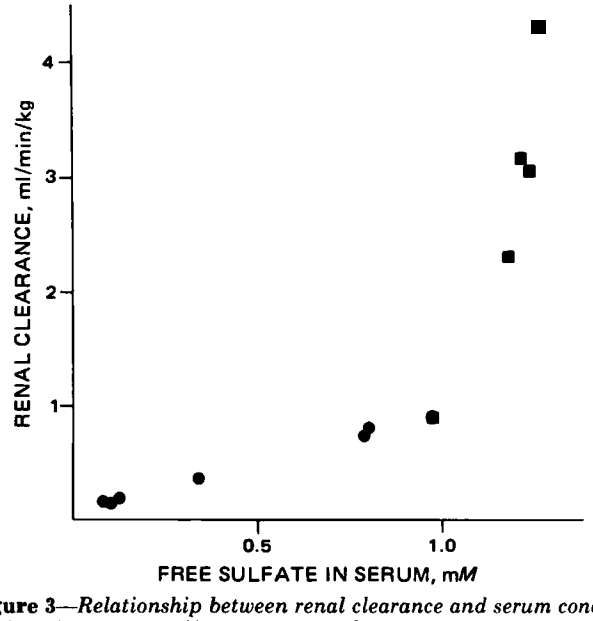

Figure 3—*Relationship between renal clearance and serum concentration of inorganic sulfate in two rats. One animal received acetaminophen (●); the other received sodium sulfate (■).*

[1] Creatinine Kit, No. 555-A, Sigma Chemical Co., St. Louis, Mo.

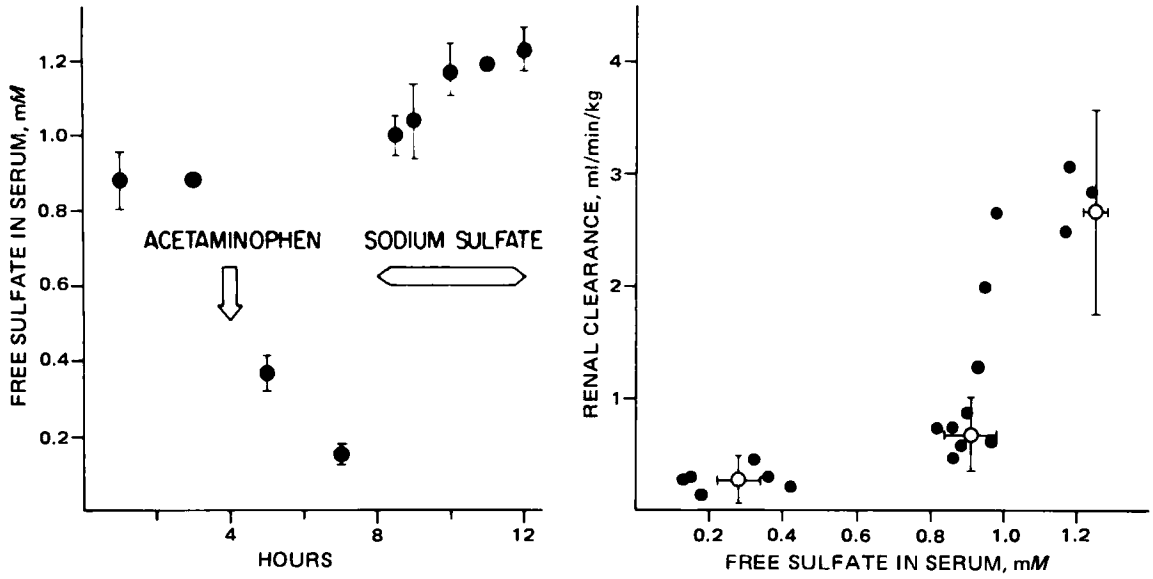

Figure 4—*Effect of acetaminophen on the renal clearance of inorganic sulfate at subnormal and supernormal serum sulfate concentrations in rats. Left panel: Inorganic sulfate concentrations (mean ±SD) in serum of 3 rats during a 4-hr control period and after intravenous injection of acetaminophen at 4 hr followed by a 4-hr infusion of sodium sulfate, 9.6 mg/hr, from 8 to 12 hr. Right panel: Renal clearance of inorganic sulfate as a function of sulfate concentration in serum, derived from the experiment shown in the left panel (●). Also shown (○) are results obtained, at selected concentration ranges, from animals who received either only acetaminophen or only sodium sulfate, or neither (mean ±SD).*

effect unrelated to the acetaminophen-induced diminished serum sulfate concentrations), sulfate depletion following acetaminophen administration was reversed by infusion of sodium sulfate. The renal clearance of inorganic sulfate at elevated serum sulfate concentrations was unaffected by acetaminophen (Fig. 4).

A summary of all renal clearance data obtained over a wide range (<0.1–1.5 m*M*) of serum sulfate concentrations is presented in Fig. 5. Excluded from that figure are data from two rats with some evidence of renal dysfunction. To facilitate the presentation of the combined results of experiments on 15 rats, the data were normalized with respect to creatinine clearance by being expressed as clearance ratios. The clearance ratio of inorganic sulfate is very low (<0.1 on the average) at serum sulfate concentrations <0.1 m*M* and increases to ≃1 when serum sulfate concentration is ~1.5 m*M*. There is a very steep increase of the clearance ratio when serum sulfate concentration slightly exceeds 1 m*M*, *i.e.*, the usual physiological level of inorganic sulfate in rats. In the serum concentration range from <0.1 to 1 m*M* (*i.e.*, during hyposulfatemia in rats), the renal clearance of sulfate also increases with increasing serum concentration, albeit not as steeply as between serum concentrations from 1 to 1.5 m*M*. The serum concentration dependence of sulfate renal clearance in the subnormal concentration range was established by the following objective criteria, applied to the group of animals that received acetaminophen: (*a*) comparison of renal clearance values at serum sulfate concentrations <0.25 m*M* to those at >0.5 m*M* indicated the latter to be significantly higher ($p < 0.001$ by paired t test), and (*b*) analysis of data for each animal separately showed a strong positive correlation between renal clearance and serum concentration of sulfate for every rat in the study ($r > 0.85$, $p < 0.05$).

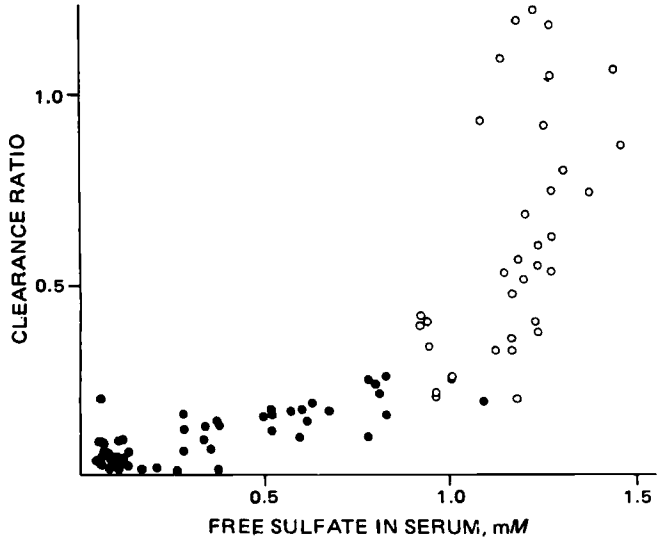

Figure 5—*Renal clearance ratio of inorganic sulfate (sulfate clearance–creatinine clearance) as a function of sulfate concentration in serum of nine rats before and after acetaminophen injection (●) and six rats before and during sodium sulfate infusion (○).*

DISCUSSION

Baseline data obtained during the control periods in this investigation are in excellent agreement with literature values. This includes serum inorganic sulfate concentration (11, 19, 29), urinary excretion rate of inorganic sulfate (3, 11), serum creatinine concentration (30), and renal clearance of creatinine (31) in rats. Previous researchers (14) observed a circadian rhythm with respect to serum concentration and urinary excretion rate of inorganic sulfate in rats; serum sulfate concentration decreased rapidly from ≃0.9 to ≃0.6 m*M* in the evening while sulfate excretion increased. Apparently, this phenomenon does not occur consistently, since the same investigators reported essentially constant serum sulfate concentrations in rats during a 24-hr period in another study (11). Experiments in this investigation were always started in the morning and lasted 12 hr; serum concentrations and urinary excretion of inorganic sulfate remained essentially constant during this time in unmedicated rats.

It appears that the renal tubular reabsorption of sulfate is coupled to active transport of sodium through a sodium–sulfate cotransport system (32, 33). Under certain conditions, administration of sodium chloride causes a transient decrease of serum sulfate concentrations in rats (5, 25). The use of sodium chloride in this investigation to adjust the tonicity of the sodium sulfate infusion solution and, as normal saline solution, to replace blood volume and stimulate urine flow, had no apparent effect on the serum concentration and urinary excretion of endogenous inorganic sulfate. Propylene glycol, used as a solvent for acetaminophen, also had no effect on endogenous sulfate serum concentration and urinary excretion under the experimental conditions.

Under normal physiological conditions, the renal clearance of sulfate is considerably below GFR even though sulfate in plasma is completely ultrafiltrable (34). Thus, inorganic sulfate undergoes considerable renal tubular reabsorption. This process is saturable, causing sulfate renal clearance to increase with increasing serum sulfate concentration. This concentration dependence is strikingly apparent in the rat when serum sulfate concentrations are increased by infusion of sodium sulfate (Figs. 3 and 5). More problematical has been the search for the existence of a

Table III—Interspecies Comparison of Free Sulfate Kinetics

	Humans[a]	Dog[b]	Rabbit[c]	Rat[d]
Reported or assumed body weight, kg	70	12	2.5	0.35
Serum-free sulfate concentration, m*M*	0.44	1.40	1.21	0.89
Renal clearance of creatinine, ml/min/kg	1.7	3.4	4.3	3.0
Renal clearance of sulfate, ml/min/kg	0.54	0.35	0.57	0.64
Renal clearance ratio, sulfate/creatinine	0.33	0.10	0.13	0.24
Urinary excretion rate of free sulfate, μmoles/min/kg	0.24	0.48	0.76	0.55

[a] Reference 13. [b] Reference 38. [c] Reference 15. [d] This study.

renal tubular secretory process for sulfate. Studies in several species, including humans and dogs, have failed to demonstrate that renal clearance of sulfate can exceed GFR (18, 20, 35). While sulfate renal clearance ratios in the present investigation have occasionally exceeded unity at high serum sulfate concentrations (Fig. 5), these values have all been close to unity. Clearance ratio determinations are based on four measurements (sulfate and creatinine in serum and urine) and are therefore subject to some error. The results of our renal clearance experiments at elevated serum sulfate concentrations provide no definitive evidence of renal secretion of sulfate and are consistent with the experiences of other investigators who have not been able to demonstrate conclusively, by conventional clearance determinations in mammals, that inorganic sulfate is subject to renal tubular secretion (22). However, a renal secretory process for sulfate has been found by tubular microperfusion techniques but appears to be rudimentary (21, 22). In any event, the focus of this investigation has been on the concentration dependence of sulfate in the subnormal serum concentration range where the secretory process is overshadowed by the reabsorption process.

The use of a drug to deplete endogeneous sulfate has permitted, apparently for the first time, the determination of sulfate renal clearance at subnormal serum sulfate concentrations. This information is important for the comprehensive characterization of the pharmacokinetics of drugs that are subject to sulfate conjugation and whose biotransformation is associated with a depletion of endogenous sulfate. It was found that the renal clearance of sulfate decreases continuously with decreasing serum sulfate concentrations. When endogenous sulfate is severely depleted, there occurs almost complete reabsorption of sulfate from the glomerular filtrate. Thus, the serum concentration dependence of renal sulfate clearance in rats facilitates sulfate homeostasis by retaining sulfate when this ion is depleted and by excreting it rapidly when serum concentrations are elevated above normal. These conclusions apply also to humans, as is evident from the results of an ongoing investigation[2].

There are very large species differences in serum sulfate concentrations; the goat and rooster have concentrations $\simeq$2.5 m*M*, the rat and mouse $\simeq$1 m*M*, and the monkey and humans are at the lower extreme with concentrations $\simeq$0.3 m*M* (14). The two most likely reasons for these species differences are variations in the formation rate (or dietary intake) of sulfate and in the renal sulfate clearance. Volume of distribution differences are much less likely; sulfate ion is not protein bound and distributes almost exclusively in extracellular space (36, 37). A survey of the literature shows that, based on body weight, the renal clearance of sulfate in humans under physiological conditions is similar to that of rats, rabbits, and dogs (Table III). The low serum sulfate concentration in humans under these conditions suggests that their sulfate formation rate is relatively low, perhaps due to lower dietary intake of precursors. This conclusion is supported by the fact that the urinary excretion rate of free sulfate (which is a good approximation of the formation rate since tracer doses of sulfate-S^{35} are excreted largely as such), is much less in humans than in the other three species (Table III).

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