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Circadian rhythm of serum sulfate levels in man and acetaminophen pharmacokinetics

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Summary. The circadian variation of serum inorganic sulfate levels was studied in healthy volunteers. The effect of subchronic acetaminophen administration (650 mg q.i.d. for 4 days) on serum inorganic sulfate levels was investigated and the possible role of fluctuating serum inorganic sulfate levels on the pharmacokinetics of acetaminophen was evaluated.

During a 24 h cycle, serum inorganic sulfate levels were lowest in the morning (11.00 h) and typically increased in the afternoon to reach a maximum in the early evening (19.00 h). Average 24 h serum concentrations were 360 μM and the difference between minimum and maximum levels was on average 25.8%. Subchronic administration of acetaminophen (650 mg q.i.d. for 4 days) significantly reduced serum inorganic sulfate levels to a 24 h average of 253 μM. The circadian rhythm, however, was not affected and the difference between minimum (12.00 h) and maximum (18.50 h) serum concentrations was 31.3%. Subchronic acetaminophen administration lead to a significant decrease in the renal excretion (−51%) and renal clearance (−33%) of inorganic sulfate. No significant differences were found in the disposition kinetics of acetaminophen and its glucuronide and sulfate conjugates during two consecutive dosing intervals (08.00–14.00 h, 14.00–20.00 h) on Day 4 of the acetaminophen regimen.

Key words: Acetaminophen, Serum inorganic sulfate; Circadian rhythm, Chronopharmacokinetics

Sulfate conjugation is an important biotransformation pathway for many endogenous compounds and xenobiotics, particularly those having a phenolic -OH group. The metabolic reaction involves activation of inorganic sulfate by a series of ATP catalyzed reactions which produce adenosine-3′-phosphate-5′-sulfatophosphate (PAPS). The sulfoconjugation process is considered to be influenced by the supply of inorganic sulfate, the concentration of PAPS and the activity of sulfotransferase enzymes. In several animal species, the plasma pool of inorganic sulfate seems to be in rapid equilibrium with the metabolically active sulfate pool in the body [Herbai 1980; Mulder and Scholtens 1978; Waschek et al. 1986].

Inorganic sulfate is acquired by the body both by gastrointestinal absorption of the ion from dietary sources, and by oxidation of the amino acids, cysteine and methionine. Inorganic sulfate is primarily eliminated by glomerular filtration. The clearance pattern of the sulfate anion is consistent with partial renal tubular reabsorption by a capacity-limited process [Lin and Levy 1983; Morris and Levy 1983 b].

The levels and excretion of several inorganic ions, including sulfate, vary during the day [Wever 1975; Ferguson and Botchway 1980]. A circadian variation in serum concentrations of inorganic sulfate has been reported in rats [Krijgsheld et al. 1980] and man [Meier and Schmidt-Kessen 1978] with slightly elevated levels occurring during the day for rats and at night for man.

The purpose of this study was to investigate the circadian variation of inorganic serum sulfate levels in man and to determine (1) if the rhythm is affected by subchronic administration of a substrate which undergoes sulfate conjugation, and (2) if the rhythm, in turn, affects the elimination kinetics of such a substrate. The substrate chosen was acetaminophen, a commonly used analgesic which is mainly metabolized to sulfate and glucuronide conjugates. Studies in laboratory animals and man have shown pronounced depletion of serum (and body stores of) inorganic sulfate following administration of drugs subject to sulfoconjugation, such as acetaminophen and salicylamide [Greiling and Schuler 1963; Lin and Levy 1981; Hendrix-Treacy et al. 1986]. Also, lower levels of inorganic sulfate have been associated with reduced sulfoconjugation of the drug [Levy and Yamada 1971].

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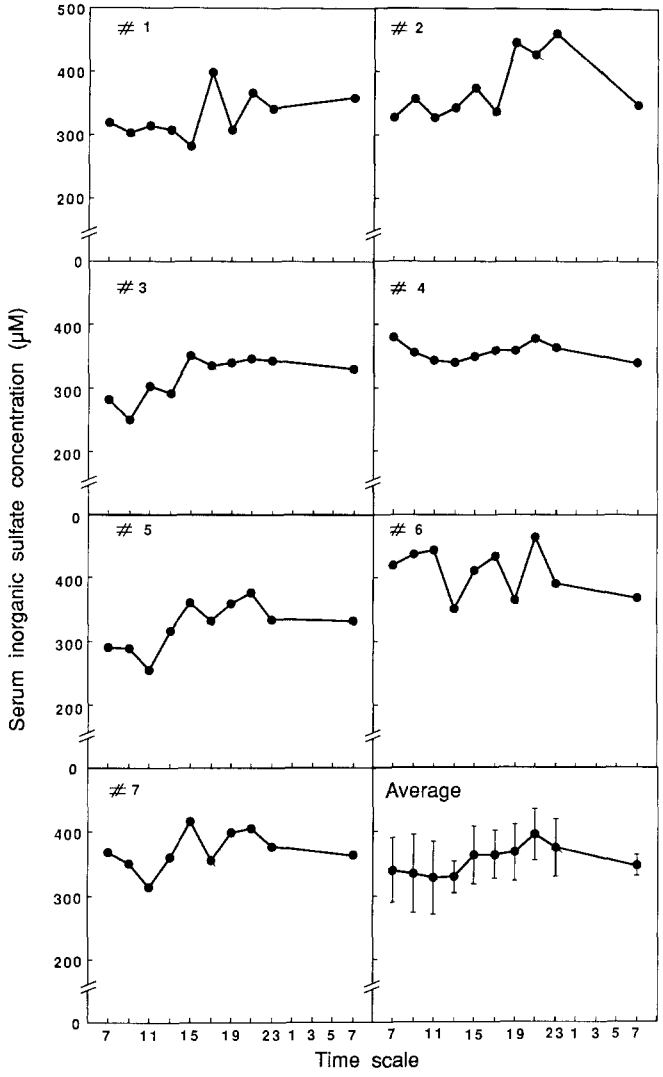

Fig. 1. Individual and mean (SD) serum inorganic sulfate concentrations in the 7 volunteers who participated in Phase I (24 h control period) of the study

Materials and methods

Subjects

Seven male subjects, between 20 and 28 y old (weight: 79.6 (6.0) kg) volunteered to participate in the study, which was approved by the University Human Ethics Committee. They were all in good health as judged by appropriate biochemical tests and physical examination and had no evidence of hematologic, renal or hepatic dysfunction. All were non-smokers and none had been taking any medication for at least one week prior to and during the study. Alcohol was not permitted 2 days before and throughout both study periods.

Study protocol

All 7 volunteers completed Phase I of the study: a 24 h period during which blood samples were collected at 07.00, 09.00, 11.00, 13.00, 15.00, 17.00, 19.00, 21.00, 23.00, and 07.00 h the next morning. Blood samples were collected in red-stoppered Venoject tubes, permitted to clot at room temperature and serum separated by centrifugation (2.000 × g). Urine was collected from 0–4, 4–8, 8–12, 12–16, and 16–24 h (0 h representing 07.00 h). The serum and urine samples were immediately frozen and stored at −20 °C pending analysis. Each volunteer consumed the same foods and drinks at the following times: breakfast at 07.30 h, lunch at 12.00 h, and dinner at 18.00 h. Each volunteer also ate 2 snacks, one at 15.00 h and the other at 21.00 h.

Six of the 7 volunteers also participated in Phase II of the study. During this phase each volunteer was instructed to ingest 650 mg acetaminophen (Tylenol® tablets) 4 times daily, at 08.00, 14.00, 20.00 and 02.00 h. On the fourth day of this acetaminophen regimen, blood and urine samples were collected during 2 consecutive dosing intervals; i.e., from 08.00–14.00 h and from 14.00–20.00 h. Blood samples were collected during each of these 2 dosing intervals at 0 (just before ingestion of the 650 mg acetaminophen tablet), 0.5, 1, 1.5, 2, 3, 4, and 6 h following acetaminophen administration. Urine was collected during the 6 h of each dosing interval. Serum and urine samples were stored at −20 °C pending analysis. The same meals and drinks, as described above, were provided to each volunteer 0.5 h earlier than in Phase I of the study.

Inorganic sulfate assay

Serum samples were deproteinized by membrane filtration using an MPS-1 Micropartition System (Amicon Corporation, Danvers, MA). Before membranes were used, sulfate was removed from the membrane by filtering HPLC grade water (Fisher Scientific Ltd., Fairlawn, NJ) through the apparatus. Ultrafiltration of the undiluted serum sample (0.5 ml) was achieved by centrifuging the device at room temperature for 17 min at 2.000 × g in a centrifuge with a fixed angle rotor (IEC HN S11 centrifuge, International Equipment Company, Needham Heights, MA). The ultrafiltrate was diluted (8 fold) with HPLC grade water and an aliquot (50 μl) injected onto the HPLC column. Urine samples were diluted (80 to 100 fold) with HPLC grade water and a 50 μl aliquot injected onto the column. Each plasma and urine sample was analyzed in duplicate.

The HPLC system consisted of a Rheodyne 7125 injector (Rheodyne Inc., Cotati, CA), an LKB pump model 2150 (LKB, Broma, Sweden), an anion exchange column (Anion IC-Pak, Waters Associates, Waters-Millipore, Milford, MA) protected by a Waters IC-Pak Anion guard column, and an ion conductivity detector (Waters-Millipore, model 430). The mobile phase (4 mM potassium hydrogen phthalate pH 4.5 aqueous solution) was pumped through the column at a flow rate of 1.0 ml · min^{-1}. The retention time of the sulfate ion peak was 6.8 min. The only other peak appearing in the chromatograms was a system peak with a retention time of 10.5 min.

Standard solutions (25 to 250 μM), made by adding known amounts of inorganic sulfate (anhydrous sodium sulfate, Fisher Scientific Company Ltd.) to HPLC grade water, were directly injected onto the HPLC system. Standard curves (sulfate peak height

Table 1. Circadian variation of serum inorganic sulfate levels (mean ± S.D.) in healthy men ($n = 7$), and effect of multiple dose acetaminophen administration ($n = 6$)

Parameter	Phase I 07.00–07.00	Phase I[b] 07.00–19.00	Phase II 08.00–20.00
C_{AVE} (μM)	360 (29.2)	352 (35.4)	253 (18.9)[a]
C_{max} (μM)	408 (42.7)	404 (39.0)	309 (38.7)[a]
C_{min} (μM)	302 (40.2)	320 (34.9)	211 (16.6)[a]
ΔC (%)	25.8 (6.3)	20.5 (8.5)	31.3 (5.1)
t_{max} (h)	19.00 h (3.3)	16.00 h (3.0)	18.50 h (2.7)
t_{min} (h)	11.00 h (2.6)	11.40 h (1.0)	12.00 h (2.4)

[a] value significantly different ($P < 0.05$, T test) from the value obtained during Phase I of the study.

[b] to evaluate the effect of acetaminophen administration on serum sulfate concentrations, a comparison is made between the 12-h control (Phase I) period (i.e. 07.00–19.00 h) coinciding most closely to the two consecutive dosing intervals (i.e. 08.00–20.00 h) during Phase II in the 6 volunteers who participated in both study phases

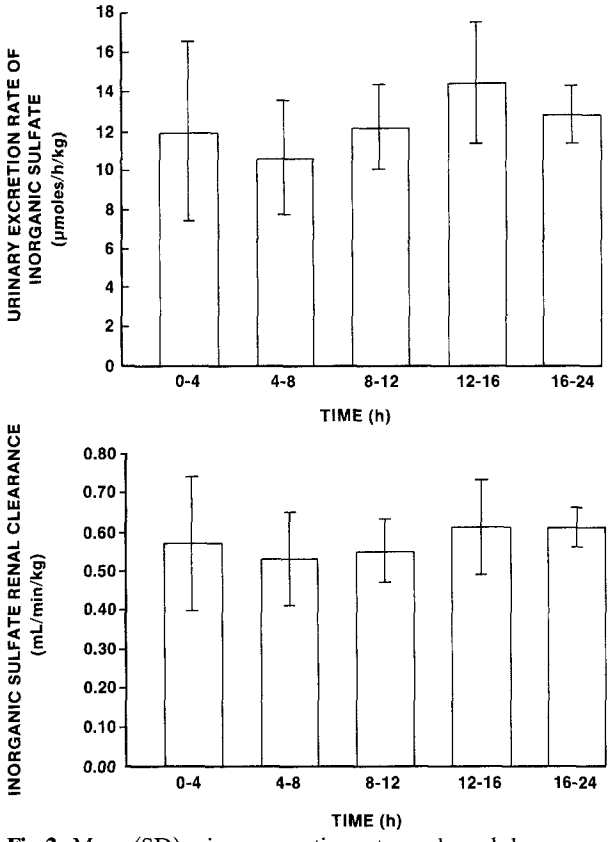

Fig. 2. Mean (SD) urinary excretion rates and renal clearances of inorganic sulfate in 7 volunteers during a 24 h period (Phase I)

vs sulfate concentration) were linear and had negligible y-intercepts ($r > 0.99$). The coefficient of variation (C.V.) for the slopes of the calibration curves ($n = 22$) was 4.8%. Between-run C.V. for inorganic sulfate over the range of sulfate concentrations studied (25 and 250 μM) was less than 6%. Quality control standards (100 μM) were injected at the end of each day of analysis and gave the following concentrations: 102.3 (2.8) μM, and 100.8 (2.6) μM (mean (SD); $n = 11$) for serum and urine, respectively. Details of the assay will be published elsewhere.

Acetaminophen and conjugates assay

A 250 μl aliquot of serum was transferred to a Reacti-vial (Pierce Chemical Company, Rockford, IL) containing 5 μg of the internal standard (IS), 3-acetamidophenol (Sigma Chemical Company, St. Louis, MO). Acetonitrile (375 μl) (HPLC grade, BDH Chemicals, Toronto, Ontario) was added, the contents of the vial mixed (Vibrax VXR Mixer, Ika-Werk, Staufen, Germany) and then centrifuged at 2000 × g (4 °C, 10 min) (Accuspin-FR Centrifuge, Beckman Instruments Inc., Palo Alto, CA). An aliquot of the clear supernatant was transferred to a clean tube and evaporated in a waterbath (40 °C) under a stream of N_2 (Meyer N-Evap Analytical Evaporator, Organomation Associates Inc., Northborough, MA). The residue was dissolved in 100 μl HPLC grade water and an aliquot injected onto the HPLC system. Urine samples were centrifuged at room temperature, an aliquot (10 to 20 μl) transferred to a clean tube containing 20 μg 3-acetamidophenol (IS) and diluted with HPLC grade water to a final volume of 2.0 ml. An aliquot of this diluted urine was injected onto the HPLC system. Standards for calibration curves were prepared by adding known amounts of acetaminophen, its glucuronide and sulfate conjugates to blank serum and urine.

The HPLC system for the analysis of acetaminophen and its conjugates in serum consisted of a Rheodyne 7125 injector, an LKB pump model 2150, a guard column, an Altex Utrashere ODS column (250 × 4 mm, 5 μ particle size, Beckman Instruments, Berkley, CA), a Waters Programmable Wavelength Detector Model 490, and a chart recorder. The mobile phase consisted of 0.015 M K_2HPO_4 (adjusted to pH 2.65 with 0.9 M H_3PO_4) and acetonitrile in a ratio of 93:7 (v/v). The flow rate was 1.2 ml · min^{-1} and the absorbance of the eluate was monitored at 249 nm. For the assay of urine samples, the mobile phase was modified: 0.015 M K_2HPO_4 (adjusted to pH 3.0): acetonitrile (95:5, v/v). Using these conditions, baseline resolution was obtained for all compounds of interest with retention times no longer than 14 min for the serum assay, and no longer than 20 min for the urine assay.

Calibration curves for acetaminophen and the two conjugates were always linear over the ranges studied: 0.5–20.0 μg · ml^{-1} (serum) and 0.125–4.0 μg · ml^{-1} (urine) for acetaminophen, 4.0–30.0 μg · ml^{-1} (serum) and 2.0–30.0 μg · ml^{-1} (urine) for the two conjugates. The coefficient of variation for the three compounds in serum and urine over these concentration ranges were smaller than 5%.

Data analysis

The maximum (C_{max}) and minimum (C_{min}) serum sulfate concentrations and the times at which they occurred (t_{max} and t_{min}) were determined for each subject. The individual fluctuation in serum sulfate levels (ΔC) was calculated as follows:

$$\Delta C = \frac{C_{max} - C_{min}}{C_{max}} 100$$

The average serum sulfate concentration (C_{AVE}) was determined by dividing the area under the serum sulfate concentration (AUC, trapezoidal rule) during a time interval by the time interval. Renal clearance of inorganic sulfate was calculated by dividing the urinary excretion rate of inorganic sulfate by the midpoint serum sulfate concentration.

Pharmacokinetic parameters of acetaminophen and its conjugates were estimated using standard pharmacokinetic techniques. The partial metabolic clearances of the sulfate and glucuronide conjugate were estimated using the following equations (Houston 1982):

$$CL_{AP \to S} = CL/f \cdot f_{eS}$$
$$CL_{AP \to G} = CL/f \cdot f_{eG}$$

where f_{eS} and f_{eG} represent the fractions of the acetaminophen dose excreted in urine during one dosing interval as the sulfate or glucuronide conjugate respectively. Renal clearance of acetaminophen and its conjugates was estimated as the amount of a compound excreted in urine during one dosing interval divided by the AUC of that compound during that dosing interval.

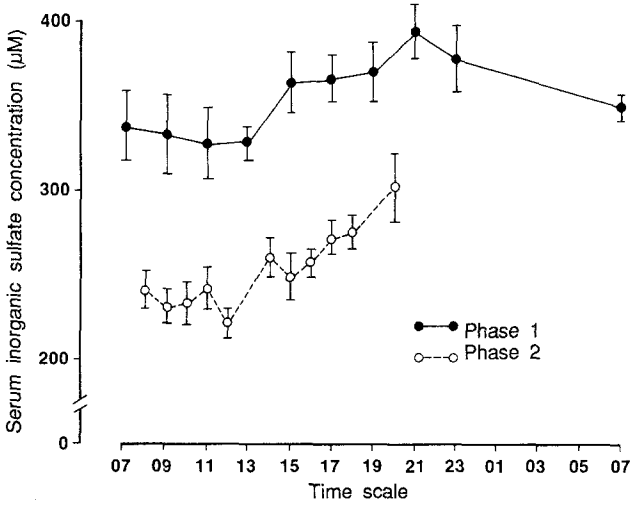

Fig. 3. Mean (SEM) serum inorganic sulfate concentrations in six volunteers who participated in both study phases

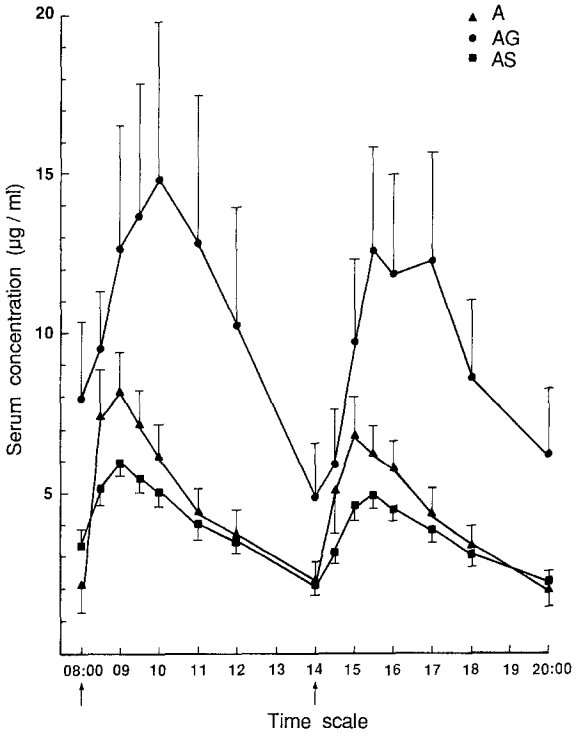

Fig. 4. Mean (SD) serum concentration-time profiles of acetaminophen (▲—▲), acetaminophen glucuronide (●—●), and acetaminophen sulfate (■—■) during two consecutive dosing intervals on Day 4 of Phase II

Statistical comparison of data was by paired Student t-test. A P-value of 0.05 or less was considered significant. All results in the text and tables are expressed as mean (standard deviation) unless otherwise indicated.

Results

In all 7 volunteers, serum sulfate levels were lowest in the morning or early afternoon and reached maximum levels in the evening (Table 1, Fig. 1). Pronounced interindividual variability in the fluctuations of serum sulfate levels was found (Fig. 1). The time at which the serum sulfate levels peaked was on average 19.00 h (range: 15.00–23.00 h) (Fig. 1). Minimum levels occurred on average at 11.00 h (range: 07.00–15.00 h). C_{max} and C_{min} values were 408 (29.2) µM and 302 (40.2) µM respectively and were significantly different ($P < 0.005$). The average difference between maximum and minimum serum sulfate levels was 25.8 (6.3)%. The urinary excretion rate of inorganic sulfate over the entire 24 h period was 12.5 (2.4) $\mu mol \cdot h^{-1} \cdot kg^{-1}$. The renal clearance of inorganic sulfate was 0.58 (0.08) $ml \cdot min^{-1} \cdot kg^{-1}$. Both parameters showed slight variations over the 24 h study period (Fig. 2) which seemed to parallel the variation observed in the serum sulfate levels.

Serum sulfate levels were only followed for a 12 h period (08.00–20.00 h) during Phase II (acetaminophen administration study) and were therefore compared to the 07.00–19.00 h time interval of Phase I (Table 1). Acetaminophen intake resulted in a significant reduction in average serum sulfate levels over the 12 h period studied: from 352 (35.4) µM during the control period (Phase I) to 253 (18.9) µM following acetaminophen intake (Phase II) (Fig. 3). However, the pattern of fluctuations in serum sulfate levels was affected very little by acetaminophen intake (Fig. 3). The pattern was similar to that observed during the control phase. The highest serum sulfate concentration during Phase II was observed at the last sampling time i.e. 20.00 h. It is therefore not certain whether or not maximum levels had been reached at that time. Urinary excretion (12.2 (2.9) $\mu mol \cdot h^{-1} \cdot kg^{-1}$, Phase I; 6.0 (1.9) $\mu mol \cdot min^{-1} \cdot kg^{-1}$, Phase II) and renal clearance of inorganic sulfate (0.58 (0.11) $ml \cdot h^{-1} \cdot kg^{-1}$, Phase I; 0.39 (0.10) $ml \cdot min^{-1} \cdot kg^{-1}$, Phase II) were significantly ($P < 0.05$) less following acetaminophen intake as compared to the control period.

Figure 4 shows the mean plasma concentration-time profiles of acetaminophen and its glucuronide and sulfate conjugates in the 6 volunteers following oral ingestion of a 650 mg acetaminophen tablet during 2 consecutive dosing intervals. With the exception of elimination half-life of acetaminophen, which was slightly but significantly prolonged during interval 2, no significant differences were found in the disposition of acetaminophen and its 2 conjugates between the 2 dosing intervals (Table 2). AUCs of acetaminophen and its sulfate conjugate were similar and approximately 2 to 2.5 times smaller than the AUC of acetaminophen glucuronide (Fig. 4). Urinary recovery of the sum of acetaminophen and its 2 conjugates was 96.4 (15.7)% and 93.4 (12.1)% of the administered dose during dosing interval 1 and 2, respectively. Approximately 66% of the urinary recovery was in the form of the glucuronide conjugate, and approximately 31% as the sulfate conjugate. Renal clearance of both acetaminophen conjugates was much greater than CL_R of unchanged drug.

Table 2. Pharmacokinetics of acetaminophen following multiple dose administration: comparison between two consecutive dosing intervals (mean and (SD), $n = 6$)[b]

Compound	Parameter	Interval 1 (08.00–14.00)	Interval 2 (14.00–20.00)
Acetaminophen	AUC ($\mu g \cdot h \cdot ml^{-1}$)	28.6 (11.9)	25.8 (10.7)
	$t_{1/2}$ (h)	2.1 (0.2)	2.4 (0.1)[a]
	V_z/f ($l \cdot kg^{-1}$)	1.0 (0.4)	1.2 (0.4)
	CL/f ($ml \cdot min^{-1} \cdot kg^{-1}$)	5.3 (1.6)	5.9 (1.8)
	$CL_{AP \to S}$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	1.8 (0.6)	1.8 (0.5)
	$CL_{AP \to G}$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	3.6 (0.4)	4.2 (0.7)
	CL_R ($ml \cdot min^{-1} \cdot kg^{-1}$)	0.15 (0.03)	0.12 (0.02)
	X_U (% of dose)	2.7 (0.9)	2.0 (0.5)
Acetaminophen sulfate	AUC[c] ($\mu g \cdot h \cdot ml^{-1}$)	24.5 (4.6)	21.2 (4.0)
	$t_{1/2}$ (h)	2.4 (0.4)	2.9 (0.3)
	CL_R ($ml \cdot min^{-1} \cdot kg^{-1}$)	2.3 (0.1)	2.8 (0.4)
	X_U (% of dose)	30.9 (10.1)	28.9 (7.50)
Acetaminophen glucuronide	AUC[c] ($\mu g \cdot h \cdot ml^{-1}$)	63.6 (45.7)	56.0 (35.6)
	$t_{1/2}$ (h)	2.3 (1.1)	2.1 (0.6)
	CL_R ($ml \cdot min^{-1} \cdot kg^{-1}$)	4.3 (4.0)	5.1 (3.5)
	X_U (% of dose)	62.8 (7.4)	65.9 (7.5)

[a] value significantly different ($P < 0.05$) form the value obtained during interval 1

[b] all parameters based on urinary recovery are for $n = 5$ because of an incomplete urine collection in one volunteer

[c] based on conjugate concentrations (not acetaminophen equivalents)

Discussion

The concentration of inorganic sulfate in biological fluids has been most commonly measured by colorimetric or turbidimetric methods based on the formation of barium sulfate [Häkkinen and Häkkinen 1959; Berglund and Sörbo 1960; Cole et al. 1979; Krijgsheld et al. 1979; Lundquist et al. 1980]. These methods were generally time-consuming and were often not very reproducible. More recently, suppressed (dual-column) and nonsuppressed ion chromatographic techniques have been developed to measure inorganic sulfate concentrations in urine and serum [Cole and Scriver, 1981; Krijgsheld et al. 1982; Reiter et al. 1987; Morris et al., 1988]. The single-column (nonsuppressed) anion chromatography assay we developed is based on a method described by Morris and Levy (1983 a). Since inorganic sulfate does not bind to plasma proteins [Cole and Landry, 1985], we used ultrafiltration rather than the more common precipitation techniques to remove the proteins from the serum samples. Incomplete removal of proteins from the biological samples will lead to early deterioration of the anion-exchange column [Reiter et al., 1987]. The developed assay to measure inorganic sulfate in serum and urine was very sensitive and highly reproducible.

The serum concentrations, urinary excretion rate and renal clearance of inorganic sulfate in our 7 young volunteers were in good agreement with previously reported values [Becker et al. 1960; Cochetto and Levy, 1981; Morris and Levy, 1983 b]. Our study also confirmed an earlier report by Meier and Schmidt-Kessen (1978) showing a circadian rhythm in serum sulfate levels. Similar to our findings, they reported maximum levels in the early evening (at 18.00 h) and a difference between minimum and maximum concentrations of approximately 15–30%. Meier and Schmidt-Kessen (1978) investigated the effect of protein intake on renal excretion and on serum levels of inorganic sulfate and concluded that the circadian rhythm they observed was very likely the result of circadian variation in dietary sulfate intake. Dietary protein intake has been shown to contribute partly to the variation in serum sulfate levels [Cole and Scriver, 1980] and is one factor affecting sulfate homeostasis in man [Tallgren, 1980]. To minimize intersubject variability in the fluctuations of serum sulfate levels due to differences in diet, our study subjects were all required to consume the same meals and drinks during the study periods.

Acetaminophen has been shown to lower serum inorganic sulfate levels in healthy volunteers [Morris and Levy, 1983 b; Hendrix-Treacy et al., 1986], even following administration of a single therapeutic dose. In the present study 650 mg acetaminophen taken orally four times a day produced a 28% reduction in average daytime serum sulfate levels on the fourth day of drug administration. Decreased serum sulfate levels were associated with a 33% reduction in the renal clearance of inorganic sulfate. This result is consistent with capacity-limited tubular reabsorption of inorganic sulfate in the kidney, a mechanism which leads to preservation of inorganic sulfate body stores in case serum sulfate levels are decreased. The existence of this renal mechanism to regulate serum sulfate levels has been previously demonstrated in rats [Lin and Levy, 1983] and in man [Morris and Levy, 1983 b]. Since the circadian variation in serum sulfate levels was not affected by acetaminophen intake, the experiment provided us with the opportunity to investigate the effect of a circadian rhythm in serum sulfate levels on the elimination kinetics of acetaminophen, in particular its sulfate conjugation.

Several studies have investigated temporal variations in acetaminophen disposition in man [Shively and Vesell 1975; Malan et al. 1985; Kamali et al. 1986]. Shively and Vesell (1975) demonstrated a small (15%) but statistically significant prolongation of the acetaminophen half-life in healthy men when a single dose (975 mg) of the drug was given at 06.00 h as compared to 14.00 h. The volume of distribution was 13% lower following the afternoon dose. No alteration in the urinary recovery of acetaminophen glucuronide was observed between the morning and afternoon dose. Malan et al. (1985) studied the single dose (1 g) pharmacokinetics of acetaminophen in 6 male volunteers following oral administration at 08.00, 14.00 and 20.00 h. No significant differences were found in the plasma pharmacokinetics of acetaminophen between these 3 administration times. Most recently, Kamali et al. (1986) reported that acetaminophen plasma clearance, elimination half-life and distribution volume did not differ when the drug was administered by i.v. infusion (1.5 g) at 08.00 h or 20.00 h. However, following oral administration (1.5 g) significantly more acetaminophen glucuronide was recovered in the 0–4 h urine when the drug was given at 08.00 h as compared to 20.00 h. They thought that the variation in urinary excretion of acetaminophen glucuronide as a function of time of administration was probably related to a variation in absorption. Consistent with these previous reports, our results showed also only minor variations in the pharmacokinetic parameters of acetaminophen and its glucuronide and sulfate conjugates following 08.00 and 14.00 h oral administration of 650 mg of the drug. In our study the volunteers had been taking acetaminophen 2.6 g per day for 3 days prior to the pharmacokinetic studies, which resulted in lower serum inorganic sulfate levels. The moderately lower serum sulfate levels during the morning as compared to the afternoon dosing interval, however, did not significantly affect the extent of sulfation of acetaminophen.

In conclusion, serum sulfate levels showed a circadian rhythm in healthy men, reaching a maximum during the evening. Subchronic acetaminophen administration lowered the serum sulfate levels without abolishing the circadian variation. The pharmacokinetics of acetaminophen (including sulfoconjugation) were not different following oral administration of the drug at 08.00 h and at 14.00 h despite higher serum sulfate levels during the afternoon dosing interval.

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References

Becker EL, Heinemann AO, Igarashi K, Hodler JE, Gershberg H (1960) Renal mechanisms for the excretion of inorganic sulfate in man. J Clin Invest 39: 1909–1913

Berglund F, Sörbo B (1960) Turbidimetric analysis of inorganic sulfate in serum, plasma and urine. Scand J Clin Lab Invest 12: 147–153

Chaudhary A, Szakacs CBN, Herman RJ (1989) Lorazepam disposition in Gilbert's Syndrome: effect of feeding and fasting. Clin Pharmacol Ther 45: 166

Cocchetto DM, Levy G (1981) Absorption of orally administered sodium sulfate in humans. J Pharm Sci 70: 331–333

Cole DEC, Landry DA (1985) Determination of inorganic sulfate in human saliva and sweat by controlled-flow anion chromatography. Normal values in adult humans. J Chromatogr 337: 267–278

Cole DEC, Mohyuddin F, Scriver CR (1979) A micro-assay for analysis of serum sulphate. Anal Biochem 100: 339–342

Cole DEC, Scriver CR (1980) Age-dependent serum sulfate levels in children and adolescents. Clin Chem Acta 107: 135–139

Cole DEC, Scriver CR (1981) Microassay of inorganic sulfate in biological fluids by controlled flow anion chromatography. J Chromatogr 225: 359–367

Critchley JAJH, Clements JA, Prescott LF (1986) Concentration-dependent renal clearance of paracetamol sulphate and glucuronide in man. Br J Clin Pharmacol 21: 98P

Ferguson DB, Botchway CA (1980) Circadian variations in the flow rate and composition of whole saliva stimulated by mastication. Arch Oral Biol 24: 877–881

Greiling H, Schuler B (1963) Zur Wirkungsweise der Salizylsäure und des Salizylamids. Z Rheumaforsch 22: 47–56

Häkkinen IPT and Häkkinen LM (1959) Spectrophotometric determination of sulphate in serum and in urine with barium chloranilate. Scand J Clin Lab Invest 11: 294–297

Hendrix-Treacy S, Wallace SM, Hindmarsh KW, Wyant GM, Danilkewich A (1986) The effect of acetaminophen administration on its disposition and body stores of sulphate. Eur J Clin Pharmacol 30: 273–278

Herbai G (1970) A double isotope method for determination of the miscible inorganic sulfate pool of the mouse applied in in vivo studies of sulfate incorporation into costal cartilage. Acta Physiol Scand 80: 470–491

Houston JB (1982) Drug metabolite kinetics. Pharmacol Ther 15: 521–552

Kamali F, Fry GR, Bell GD (1987) Temporal variations in paracetamol absorption and metabolism in man. Xenobiotica 17: 635–642

Krijgsheld KR, Frankema H, Scholtens E, Zweens J, Mulder GJ (1979) Absorption, serum levels and urinary excretion of inorganic sulfate after oral administration of sodium sulfate in the conscious rat. Biochim Biophys Acta 586: 492–500

Krijgsheld KR, Scholtens E, Mulder GJ (1980) Serum concentration of inorganic sulfate in mammals: species differences and circadian rhythm. Biochem Pharmacol 67: 683–686

Krijgsheld KR, Scholtens E, Mulder GJ (1982) The dependence of the rate of sulfate conjugation on the plasma concentration of inorganic sulfate in the rat *in vivo*. Biochem Pharmacol 31: 3997–4000

Levy G (1986) Sulfate conjugation in drug metabolism: role of inorganic sulfate. Federation Proc 45: 2235–2240

Lin JH, Levy G (1981) Sulfate depletion after acetaminophen administration and replenishment by infusion of sodium sulfate or N-acetylcysteine in rats. Biochem Pharmacol 30: 2723–2725

Lin JH, Levy G (1983) Renal clearance of inorganic sulfate in rats: effect of acetaminophen-induced depletion of endogenous sulfate. J Pharm Sci 72: 213–217

Lundquist P, Martensson J, Sörbo, Öhman S (1980) Turbidimetry of inorganic sulfate, ester sulfate and total sulfur in urine. Clin Chem 26: 1178–1181

Malan J, Moncrieff J, Bosch E (1985) Chronopharmacokinetics of paracetamol in normal subjects. Br J Clin Pharmacol 19: 843–845

Meier MS. Schmidt-Kessen W (1978) Untersuchungen über den Stoffwechsel des anorganischen Sulfates. MMW 120: 357–362

Morris ME, Kwon O, Mansfield IL (1988) Sulfate homeostasis. I. Effects of salicylic acid and its metabolites on inorganic sulfate in rats. J Pharmacol Exp Ther 244: 945–949

Morris ME, Levy G (1983a) Absorption of sulfate from orally administered magnesium sulfate in man. J Toxicol Clin Toxicol 20: 107–114

Morris ME, Levy G (1983b) Serum concentration and renal excretion by normal adults of inorganic sulfate after acetaminophen, ascorbic acid, or sodium sulfate. Clin Pharmacol Ther 33: 529–536

Mulder GJ (1981) Sulfate availability in vivo. In: Mulder GJ (ed). Sulfation of drugs and related compounds. CRC Press, Boca Raton, FL, pp 31–52

Mulder GJ and Scholtens E (1978) The availability of inorganic sulphate in blood for sulphate conjugation of drugs in rat liver in vivo. [^{35}S]-sulphate incorporation into harmol sulphate. Biochem J 172: 247–251

Reiter C, Müller S, Müller T (1987) Improved method for the determination of sulphate in human serum using ion chromatography. J Chromatogr 413: 251–256

Shively CA, Vesell ES (1975) Temporal variations in acetaminophen and phenacetin half-life in man. Clin Pharmacol Ther 18: 413–424

Tallgren LG (1980) Inorganic sulphates in relation to serum thyroxine level and in renal failure. Acta Med Scand [Suppl] 640: 1–100

Waschek JA, Fielding RM, Tozer TN, Effeney DJ, Pond SM (1986) Availability of plasma sulfate for conjugation of salicylamide in dogs. Biochem Pharmacol 35: 544–546

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