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AN EVALUATION OF METHODS TO DECREASE THE AVAILABILITY OF INORGANIC SULPHATE FOR SULPHATE CONJUGATION IN THE RAT *IN VIVO*

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Abstract—The concentration of inorganic sulphate in serum of the rat (about 0.9 mM) could be lowered in the following three different ways. (1) Oral administration of sodium chloride (8 mmol/kg) decreased serum sulphate within 2 hr to 0.5 mM. Eight hours after administration serum sulphate had returned to the control level. (2) Feeding of a low-protein diet (8 per cent casein, without supplements of sulphur-containing amino acids or inorganic sulphate salts) reduced urinary sulphate excretion in 2 days to 10 per cent of control. Concomitantly, serum sulphate was decreased to half the control level. (3) Paracetamol (1.0 or 1.5 mmol/kg, orally), a substrate of sulphation, reduced serum sulphate within 3 hr to 30 per cent of control. Eight hours after administration the sulphate concentration tended to rise again. Fasting initially increased serum sulphate; after 3 days of fasting still considerable amounts of inorganic sulphate were excreted in urine (50–70 per cent of control). Even after 3 days serum sulphate was not yet significantly decreased below control. Lowering of the serum sulphate concentration results in a decreased availability of inorganic sulphate.

Sulphation of a high dose of phenol (266 μmol/kg) was decreased at a serum concentration of sulphate of 0.3 mM, presumably because sulphate was depleted by the high dose of phenol. Feeding the low-protein diet, however, caused no decrease in sulphation at a tracer dose of [^{14}C]-phenol (1.25 μmol/kg), while paracetamol pretreatment did cause a decrease in the fraction of the dose that became sulphated, probably because remaining unconjugated paracetamol competed with phenol for sulphation; the tracer dose of [^{14}C]-phenol did not further deplete sulphate. The findings are discussed in relation to implications for the toxicity of many xenobiotics that are eliminated as sulphate conjugates.

Inorganic sulphate is needed for the sulphation of many endogenous substances such as sulpholipids and glycosaminoglycans; conjugation with sulphate is also an important route of elimination for e.g. catecholamines, bile acids and steroids in mammals. In addition, many exogenous substances are detoxified by sulphation; in some cases, however, sulphate conjugation can give rise to toxic, highly reactive intermediates [1].

Activation of inorganic sulphate to the cosubstrate of sulphation, adenosine 3′-phosphate 5′-sulphatophosphate (PAPS), requires ATP. Sulphate can be supplied in two ways: by absorption of inorganic sulphate from the gut [2] or by oxidation of cysteine [3]. Inorganic sulphate circulating in the blood is directly available for sulphation [4, 5]; the availability of sulphate is considered to be one of the limiting factors for sulphation *in vivo* [6, 7, 8]. In the past several investigators have tried to deplete sulphate *in vivo* by various means in order to evaluate the role of sulphation in toxification and detoxification of various compounds [6, 9–27]. A sulphate depletion was usually concluded, however, from indirect data: if administration of sulphate or sulphate precursors caused an increase in sulphation, sulphate depletion was assumed to have occurred by a certain treatment. Yet, sulphate concentrations in blood were never determined, and the possibility that administration of sulphate (precursor) increased serum sulphate levels above normal, leading to an abnormally high sulphation rate, was not considered in these experiments. Therefore, the analysis of the findings remains ambiguous as has been discussed elsewhere [8]. Because it is important for an evaluation of sulphation as a toxifying and detoxifying pathway to have methods at disposal which deplete sulphate in a defined way, we report here various methods that lead to a decrease in sulphate concentration in serum in the rat. Two methods have been used to investigate the consequences of a decreased concentration of sulphate in serum for the sulphation of phenol.

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MATERIALS AND METHODS

Chemicals. Paracetamol was used, which was micronised (<20 μm) by grinding the material in a jet mill. Methylcellulose was 400 cps (Lamers & Indemans, 's-Hertogenbosch, The Netherlands). Phenol sulphatase (Sigma, St. Louis, MO) was type VI from *Aerobacter aerogenes*.

[^{14}C]-labelled phenol was obtained from New England Nuclear Inc. (Dreieich, West Germany) (specific radioactivity 5.6 mCi/mmol; dissolved in benzene). A stock solution for injection was prepared by extraction of phenol from the benzene layer into aq. 0.9 per cent (v/v)NaCl; this solution was diluted

with a solution of cold phenol to the appropriate concentration of phenol and radioactivity.

The low-protein diet (LP-diet). The low-protein diet (LP-diet) consisted of: Casein (84 per cent protein), 8.0 per cent; glucose (cerelose), 66.6 per cent; corn starch (gelatinized), 10.0 per cent; alpha cellulose, 5.0 per cent; sunflower oil, 5.0 per cent; calcium diphosphate, 2.5 per cent; NaCl, 0.3 per cent; KCl, 1.0 per cent; MgO, 0.2 per cent; choline chloride (50 per cent), 0.4 per cent; standard vitamin and trace element mixture (without inorganic sulphate), 1.0 per cent. This diet, obtained as meal, was squeezed into lumps after addition of 20 per cent tapwater (w/w); the lumps were dried overnight at room temperature, which caused a 50 per cent loss of the water added. Elemental analysis showed the sulphur content of control and LP-diet to be 0.32 and 0.09 per cent respectively, and the nitrogen content 3.74 and 1.11 per cent respectively.

Animals. Male Wistar rats had free access to food and water during the experiments, unless stated otherwise. They were fed a commercial rat diet (RMH-B, Hope Farms, Woerden, The Netherlands). Sodium chloride (8 mmol/kg body wt) and paracetamol (1.0 mmol/kg) were administered to rats of 280–350 g body wt under light ether anaesthesia by stomach tube in a volume of 6 ml of distilled water per kg rat. The higher dose of paracetamol, 1.5 mmol/kg, was given in a suspension of methylcellulose (1 per cent, w/v). Control animals received the same volume of distilled water or methylcellulose suspension. The experiments were started at 9 a.m. (paracetamol) or 10 a.m. (sodium chloride). At different time intervals after administration blood was withdrawn with a syringe from the aorta under ether aneasthesia.

For dietary experiments rats of 160–230 g body weight were housed in metabolism cages to allow separate collection of urine and faeces. They were fed a low-protein diet (LP-diet), containing 8 per cent casein, without addition of sulphur-containing amino acids and inorganic sulphate salts. Control animals received the normal commercial diet. Urine was collected every 24 hr and blood was obtained under ether aneasthesia from the aorta at about 2 p.m.

During fasting, the animals had free access to water. In a first experiment to determine the effect of overnight food deprivation on serum sulphate concentration, blood was obtained from the aorta at 8 a.m., 4 and 11 p.m. at the day after the overnight fasting. To follow concomitantly serum sulphate and urinary excretion of inorganic sulphate, rats of 190–200 g body weight were housed in the metabolism cages during a fast for up to 3 days. Urine was collected every 24 hr and blood was obtained at about 10 a.m. Urinary samples were centrifuged to get rid of coarse contaminating materials. Urines and sera were stored at −30°.

After pretreatment with paracetamol or feeding the LP-diet the conjugation of [^{14}C]-phenol was investigated in rats (about 200 g body wt), housed individually in metabolism cages. Phenol was administered at about 2 p.m., 4 hr after oral administration of paracetamol, or after 3 days on LP-diet. [^{14}C]-phenol (7 μCi/kg) was injected intravenously in the tail vein of conscious rats, at three dose levels (tracer dose, 26.6 and 226 μmol phenol per kg body wt). Just before injection a small sample of blood (about 0.5 ml) was obtained by orbital punction (without anaesthesia) to determine the concentration of inorganic sulphate in serum. Urine was collected during the following 24 hr and analyzed for phenol conjugates as described by Weitering *et al.* [28].

Determination of inorganic sulphate in serum and urine. Inorganic sulphate in serum and urine was determined by the turbidimetric method of Berglund and Sörbo [29], as modified by Krijgsheld *et al.* [2]. When only small serum samples were available 150 μl of serum was supplemented with distilled water to 500 μl. If low concentrations of sulphate could be expected the distilled water contained 0.75 mM sodium sulphate to prevent inaccurate measurements at low barium sulphate turbidity. Since the sulphate concentration in urine is very high, urine was diluted 30 to 50-fold with distilled water. When little or no dilution was required, a high blank background absorption made the assay unreliable. This was overcome by treating the urine samples with activated charcoal. The calibration curve for inorganic sulphate, made with standard solutions in water or diluted urine, was not influenced by charcoal treatment.

Determination of paracetamol and its sulphate conjugate. Paracetamol and paracetamol sulphate concentrations in rat serum were estimated by high-performance liquid chromatography (HPLC). To 0.5 ml serum 3.0 ml methanol with internal standard (4-methoxyphenyl acetic acid, 50 mg/l) was added. After centrifugation of the mixture 25 μl of the clear supernatant was used for HPLC. HPLC was performed with a Waters Associates chromatograph (Model 6000A) that was equipped with an u.v. absorbance detector model 440 (at 254 nm), and a LiChrosorb 10RP18 column (25 cm × 4.6 mm i.d.; Merck, Darmstadt, West Germany) which was combined with a pre-column Co Pell ODS (10 cm × 2.1 mm i.d.; Merck). The mobile phase was sodium phosphate buffer (0.02 M, pH 7.2)/methanol (85:15, v/v; pH 7.4), used at a flow rate of 2.0 ml/min. Retention times for paracetamol, paracetamol sulphate and internal standard were 4.4, 2.3 and 5.6 min respectively. The retention time for paracetamol glucoronide was 1.8 min, therefore no interference with the determination of paracetamol or its sulphate conjugate occurred. A calibration curve for paracetamol was made with blank serum to which known amounts of paracetamol were added. The conversion factor for paracetamol sulphate was determined by hydrolysis of this conjugate with phenol sulphatase and estimation of paracetamol liberated. The detection limit of this method for paracetamol as well as for paracetamol sulphate was 1 μmol/l plasma.

Amino acid analysis in serum. Analysis of amino acid concentrations in sera of LP-diet and control fed rats was performed on a Kontron Liquimat III amino acid analyzer (Kontron AG-Zürich, Switzerland) [30, 31]. Serum samples were deproteinized by addition of an equal volume of sulphosalicylic acid solution (6 per cent, w/v). Nor-leucine was used as internal standard (100 nmol/ml).

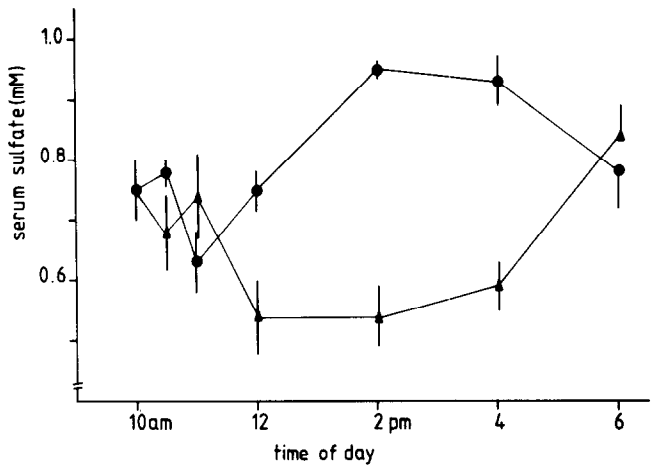

Fig. 1. Effect of oral administration of sodium chloride on the inorganic sulphate concentration in serum. At 10 a.m. 8 mmol/kg body wt was administered by gastric tube (▲). Control rats (●) received the same volume of water (6.0 ml/kg). At different time intervals after administration blood was collected from the aorta under ether anaesthesia. Each point represents the mean (± S.E.M.) of 3 individual rats.

RESULTS

Oral administration of sodium chloride

Two hours after sodium chloride administration the serum sulphate concentration was significantly decreased (Fig. 1) to levels between 0.5 and 0.6 mM ($P<0.005$, Wilcoxon's test). The lowered sulphate concentration remained at this level for about 4 hr, while in control rats serum sulphate increased to about 1.0 mM, in agreement with the circadian rhythm observed previously [32]. The difference between the serum sulphate concentration in control and sodium chloride-treated animals was maximally 0.4 mM. Eight hours after sodium chloride administration control levels were reached again. The extent of the effect of sodium chloride administration was rather variable and the decrease in serum sulphate was often smaller than that shown in Fig. 1 (see Discussion).

Low-protein diet

Rats were fed a low protein diet (LP-diet), containing 8 per cent casein with no supplement of sulphur-containing amino acids and inorganic sulphate salts. During the first 2 days the body wt of the rats decreased by about 3 per cent. In the following days their weights stabilized. When the diet was fed for more than 4 days, the rats kept the same body wt or showed a slight increase. The amount of food consumed (as determined every day by weighing the food) was not significantly different between control and LP-diet fed animals.

Rats fed the LP-diet showed a rapid decrease in urinary excretion of inorganic sulphate (Fig. 2). During the first day sulphate excretion was about half that of control rats, which were fed on the normal diet. After 3–4 days the urinary excretion of sulphate of LP-diet fed animals decreased to about 10 per cent of control. The concomitant sulphate

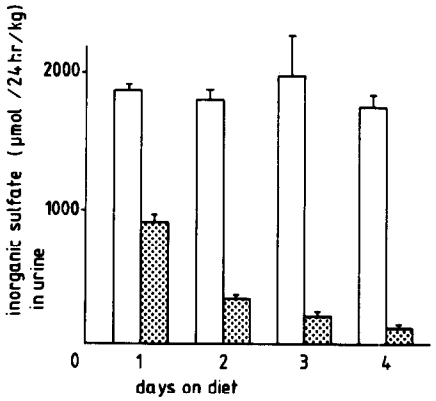

Fig. 2. Urinary excretion of inorganic sulphate after feeding of a low-protein diet. Urine was collected in 24 hr samples. Open bars: sulphate excretion of control rats, which were fed the normal diet (N = 9, 6, 3 and 3 on the respective days). Hatched bars: sulphate excretion of rats fed the low-protein diet (N = 15, 10, 5 and 5 respectively). The S.E.M. has been indicated on top of the bars.

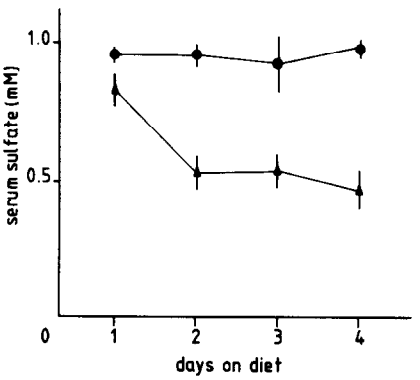

Fig. 3. Effect of feeding a low-protein diet on the concentration of inorganic sulphate in serum. After starting the diet, at each day blood was collected under ether anaesthesia from the aorta at about 2 p.m. Each point represents the mean (±S.E.M.) of 5 (LP-diet) or 3 (normal diet) individual rats; ▲, LP-diet and ●, control diet.

Table 1. Effect of fasting on the inorganic sulphate concentration in serum

Number of days of food deprivation	Time of blood collection	Serum sulphate concentration (mM)* Fasted rats	Fed controls
1†	8 a.m.	1.00 ± 0.06 (4)‡	0.85 ± 0.02 (4)
	4 p.m.	0.90 ± 0.03 (8)§	1.01 ± 0.03 (8)
	11 p.m.	0.81 ± 0.04 (4)‡	0.65 ± 0.04 (4)
2	10 a.m.	0.76 ± 0.01 (3)	0.88/0.84 (2)
3	10 a.m.	0.94 ± 0.003 (3)	0.83 ± 0.06 (3)

* Mean ± S.E.M.; number of rats used in parentheses.
† Deprived of food on the day before, in the afternoon.
‡ Significantly different from control, $P < 0.05$ (Wilcoxon's test).
§ The same at $P < 0.025$.

concentration in serum was determined in these rats (Fig. 3). After 2 days serum sulphate was decreased significantly below control levels, to about 0.5 mM. During days 3 and 4 on the LP-diet the sulphate concentration remained at this level. Amino acid analysis of the sera revealed comparable amino acid patterns for LP-diet and control fed rats. Serum concentrations of methionine and cystine were not significantly changed by the LP-diet. However, the LP-diet caused a large decrease in urea concentration, to about 30 per cent of control.

In some initial experiments the effect of the LP-diet on serum sulphate had been even more pronounced: after 3–4 days the sulphate concentration could reach levels as low as 0.1–0.2 mM. We have no explanation for this variation, but it may have been due to the condition of the LP-diet that showed a tendency to deteriorate rapidly. To prevent this as much as possible we stored the LP-diet at −30° and provided the rats every day with food taken freshly from the freezer.

The effect of fasting on sulphate metabolism. In the first fasting experiment rats were deprived of food during one night and blood was collected on the following day. The normal fed control animals showed a relative high serum sulphate concentration in the afternoon and a low sulphate concentration in the night (Table 1, Fig. 1). In the fasted animals serum sulphate was increased as compared with controls, except at 4 p.m. (Table 1). After a longer period of starvation, as investigated in the second experiment, serum sulphate was not significantly reduced below control level, even after 3 days of fasting (Table 1). The excretion in urine of inorganic sulphate was decreased to about half the amount excreted by fed animals, but remained constant during the 3 days of food deprivation (Fig. 4).

The effect of paracetamol on serum sulphate concentration. Paracetamol is a drug that is eliminated mainly after sulphation and glucuronidation. Oral

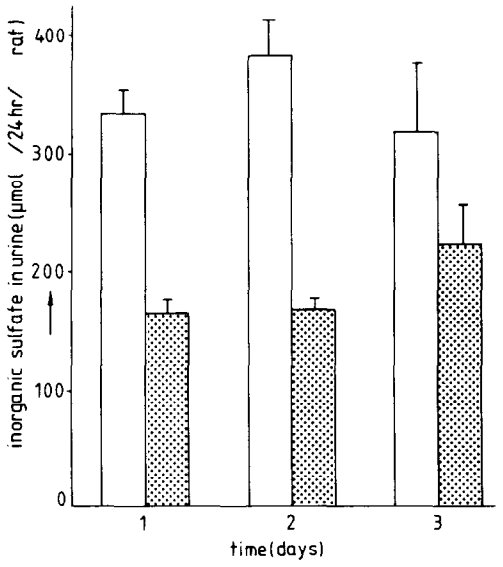

Fig. 4. Urinary excretion of inorganic sulphate by fasted rats. Up to 3 days after the start of food deprivation 24 hr urine samples were collected. Sulphate excretion is expressed in µmol/24 hr per rat (±S.E.M.) for fed (open bars) and fasted (filled bars) rats. Because body weight declined during the experiment we have expressed the urinary excretion per rat; body weight at start was 190–200 g. During the first 2 days $N = 6$, on the third day $N = 3$.

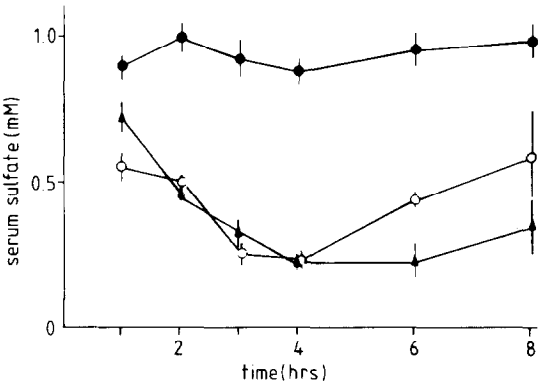

Fig. 5. Effect of oral administration of paracetamol on the inorganic sulphate concentration in serum. At about 9 a.m. 1.0 mmol (○) or 1.5 mmol (▲) paracetamol per kg body weight was administered by gastric tube. Control rats (●) received the same volume of water (6.0 ml/kg). At different time intervals after administration blood was collected from the aorta under ether anaesthesia. Each point represents the mean (±S.E.M.) of 4 individual rats.

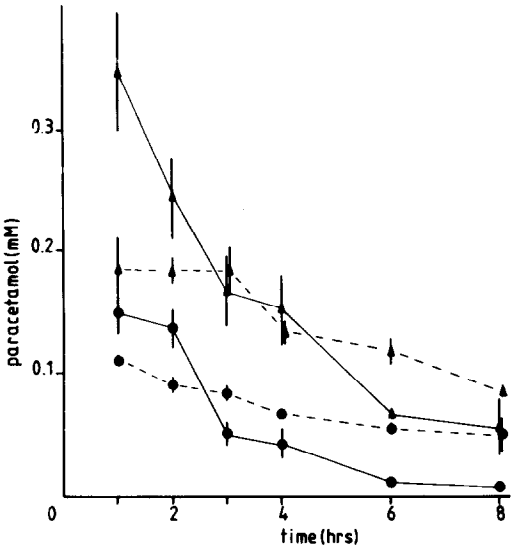

Fig. 6. Concentration of paracetamol and its sulphate conjugate in serum after oral administration of paracetamol to the rat (●, 1.0 mmol/kg and ▲, 1.5 mmol/kg body weight). At different time intervals after administration blood was collected from the aorta under ether anaesthesia. Unconjugated paracetamol (—) and paracetamol sulphate (---) were determined by HPLC. Each point represents the mean (±S.E.M.) of 4 individual rats.

administration of 1.0 or 1.5 mmol of paracetamol per kg reduced the sulphate concentration in serum within 3 hr to about 30 per cent of control (Fig. 5). At these doses of paracetamol serum sulphate tended to rise again 6–8 hr after administration. At that moment unconjugated paracetamol was still present in serum, but only at relatively low concentrations (Fig. 6).

Significant levels of paracetamol sulphate appeared in serum (Fig. 6). During the first 3 hr after oral administration of 1.5 mmol of paracetamol per kg the concentration of the sulphate conjugate in serum seemed in steady state. In the following hours paracetamol sulphate was eliminated with the same half-life at both paracetamol dose levels.

The effect of LP-diet and paracetamol pretreatment on sulphation of phenol. The conjugation of [^{14}C]-phenol was investigated after decreasing serum sulphate from 1.0 to about 0.3 mM by pretreatment with paracetamol (1.0 mmol/kg body wt, orally) or by feeding the LP-diet for 3 days (Table 2). Phenol is conjugated with sulphate or glucuronic acid and, subsequently, almost completely excreted in urine within 24 hr. The sulphation of phenol, administered 4 hr after pretreatment with paracetamol was significantly decreased at each of the 3 doses of phenol used ($P<0.005$). The inhibition of sulphation increased with increasing dose of phenol: from 6 per cent inhibition at the tracer dose to 35 per cent at 266 μmol phenol per kg body wt. Feeding the LP-diet caused a decrease in sulphation at the highest phenol dose of 45 per cent, as compared to controls ($P<0.005$); at the lower doses of phenol no significant changes in sulphation were observed.

DISCUSSION

A decrease in the availability of inorganic sulfate may have important consequences for the elimination and toxicity of various xenobiotics. The capacity of sulphation as a route of elimination will be reduced. A decreased sulphate concentration in the extracellular fluid will reduce the rate of synthesis of adenosine 3′-phosphate 5′-sulphatophosphate (PAPS), the cosubstrate of sulphation, and, consequently, the rate of sulphation since the rate of incorporation of intravenously injected [^{35}S]-labelled sodium sulphate into sulphate conjugates of phenolic compounds indicates a rapid equilibration of sulphate in blood with the intracellular pool used for conjugation [4, 5].

The concentration of inorganic sulphate in serum may be decreased by at least three different mechanisms: (a) an increase in urinary sulphate excretion; (b) a decrease in the supply of sulphate by the various sources of inorganic sulphate; and (c) consumption of the available sulphate by sulphate conjugation of a substrate. The decrease of serum sulphate after oral administration of sodium chloride may be explained by an increased excretion of sulphate by the kidneys, caused by the sodium chloride. Berglund and Lotspeich [33] observed in the dog a markedly decreased T_m of sulphate reabsorption after an intravenous dose of 1.7 mmol NaCl/kg body wt. Possibly the glomerular filtration rate determines for a major part the serum sulphate concentration, which explains the variable results of sodium chloride. A reduction in glomeruler filtration rate by e.g. stress could reduce urinary sulfate excretion and therefore diminish the sodium chloride effect.

Wellers *et al.* [34, 35] found that a decreased supply of sulphate sources in the rat diet reduces the output of inorganic sulphate in urine. In man, omission of sulphur-containing amino acids from the food caused a reduction in sulphate excretion of about 65 per cent on the average [36]. Unfortunately, the concentration of sulphate in blood was not determined in these experiments. Our results in the rat show that a limited supply of sulphur-containing amino acids and inorganic sulphate not only reduced the urinary sulphate excretion within a few days to 10% of control, but also the serum sulphate concentration to at least half of control. The comparable amino acid patterns in sera of LP-diet and control fed rats suggest an efficient utilization of the amino acids in the LP-rats, which is supported by the finding of a decreased ureum concentration in the sera of the rats on LP-diet. Surprisingly, we found no significant decrease in serum sulphate after fasting during 3 days. On the contrary, after one night of fasting the sulphate concentration was significantly increased. One might speculate that this temporary increase is caused by a reduced glomerular filtration rate, resulting from behavioural changes and, therefore, a reduction in the urinary excretion of sulphate. Although the sulphate excretion was decreased during starvation, even on the third day sulphate was still excreted in considerable amounts: 50–70 per cent of control. These results indicate that the situation after fasting is very different from that after feeding a low protein diet. Starving rats catabolize

Table 2. The effect of paracetamol pretreatment or LP-diet on the conjugation of phenol in the rat *in vivo*

(A) Paracetamol pretreatment (1.0 mmol/kg, orally)

Dose of [^{14}C]-phenol (μmol/kg)	Pretreated −/+	Approximate serum sulphate (mM)	Phenyl sulphate	Phenyl glucuronide
			(Per cent of the dose)	
Tracer dose	−	1.0	85.7 ± 1.0	14.3 ± 1.0
	+	0.5	80.8 ± 0.6*	19.2 ± 0.6*
26.6	−	1.0	65.4 ± 1.4	34.6 ± 1.4
	+	0.3	51.0 ± 1.4*	49.0 ± 1.4*
266	−	1.1	46.1 ± 2.2	53.9 ± 2.2
	+	0.3	30.5 ± 2.0*	69.5 ± 2.0*

(B) Low-protein diet (for 3 days)

Dose of [^{14}C]-phenol (μmol/kg)	Pretreated −/+	Approximate serum sulphate (mM)	Phenyl sulphate	Phenyl glucuronide
			(Per cent of the dose)	
Tracer dose	−	1.0	84.2 ± 1.2	15.8 ± 1.2
	+	0.2	83.6 ± 1.5	16.4 ± 1.5
26.6	−	0.9	69.6 ± 1.7	30.4 ± 1.7
	+	0.2	65.0 ± 3.0	35.0 ± 3.0
266	−	1.0	49.5 ± 2.5	50.5 ± 2.5
	+	0.3	30.4 ± 2.9*	69.6 ± 2.9*

At three dose levels ^{14}C-labelled phenol (7 μCi/kg) was injected intravenously in the tail vein of rats housed in metabolism cages. Injection was performed at about 2 p.m., 4 hr after oral administration of paracetamol, 1.0 mmol/kg (A), or after 3 days on LP-diet (B). Just before phenol administration a small sample of blood was obtained by orbital puncture to determine the approximate concentration of sulphate in serum. In the urine collected during the following 24 hr the administered [^{14}C]-radioactivity was completely recovered. The sulphate and glucuronide conjugates were separated by t.l.c. and determined by counting the radioactivity of the [^{14}C]-phenol in the conjugate. Data are expressed as the mean ± S.E.M.; each group consisted of 5 or 6 rats.

* Significantly different from control, $P < 0.005$ (Wilcoxon's test).

body proteins and this will cause a release of sulphur-containing amino acids, that are subsequently converted to inorganic sulphate, instead of being incorporated into newly synthesized proteins. In addition, degradation of highly sulphated body constituents like glycosaminoglycans may release sulphate. Probably, the LP-diet still permits protein synthesis and utilization of cysteine. Fasting experiments in man revealed even an increase in urinary excretion of sulphate [37].

Exhaustion of the endogenous sulphate pool is often assumed to occur after administration of a large dose of a substrate that becomes sulphated [6, 7], see for review [8]. However, a sulphate depletion was always concluded from indirect experiments, such as the increase of the percentage of the dose of a substrate that became sulphated after administration of sulphate or sulphate precursors. Thus, Weitering *et al.* [28] found that intravenous administration of a high dose of phenol (266 μmol/kg) in the rat caused only a small and very temporary decrease in serum sulphate concentration. Paracetamol can be administered in much larger doses than phenol, without acute toxic effects. From the data presented here it is clear that paracetamol can deplete the directly available sulphate pool very strongly.

For many phenolic compounds a relative decrease of sulphation of phenols is observed at increasing dose (see, for instance, Table 2). Since only a small drop in serum sulphate occurred after a high dose of phenol (266 μmol/kg) [28], it is not very likely that the dose-dependent relative decrease of sulphation is caused by sulphate depletion. However, the large decrease in sulphate availability to 30 per cent of control by oral administration of paracetamol or feeding the LP-diet, clearly lowered the capacity of sulphation, as shown by the data presented in Table 2. The directly available amount of sulphate at the low serum sulphate is only approximately 75 μmol/kg body wt, assuming a distribution volume for inorganic sulphate of 25% of body weight as compared to 250 μmol/kg in controls. At the high dose of phenol (266 μmol/kg) this was inadequate for the sulphation of the same fraction of the dose as in control rats, since about 135 μmol/kg is sulphated in controls. But at the two lower doses enough sulphate is available to conjugate the same amount as in control animals with sulfate. Indeed, no significant decrease in sulphation is seen after feeding the LP-diet in these cases. However, after paracetamol pretreatment also at these low dose levels a significant inhibition of sulphation occurred, albeit less pronounced than at high doses. This effect may be explained by the

presence of unconjugated paracetamol in the blood at the moment of injection of phenol that may compete with phenol for the sulphotransferase. At the same time, these data indicate a very efficient use of the available sulphate, since after LP-diet feeding, the fraction of the lower doses of phenol that became sulphated was the same as in control rats, in spite of the low sulphate concentration in serum.

Because sulphation is a major route of elimination for many xenobiotics it is important to know what the consequences are of a reduced sulphate availability [6–27]. Methods to decrease sulphate availability will also be valuable as experimental tool in research on toxification mechanisms by sulphation, as is the case e.g. for the toxicity of *N*-hydroxyacetylamino fluorene [38]. However, the three methods presented here have their limitations, and their usefulness will depend on the aim of the experiment. The decrease of the inorganic sulphate concentration in serum by oral administration of sodium chloride or high doses of substrates for sulphation like paracetamol, is of limited duration. In addition, in the case of paracetamol, which has been used to deplete sulphate by several authors [7, 10, 12, 13, 15, 16, 24–27], still unconjugated drug will be present in the blood, while serum sulphate is already increasing again. The remaining free paracetamol will compete with the drug to be investigated for the available sulphate left. To the contrary, the LP-diet causes a prolonged decrease in serum sulphate but will, no doubt, have more effects. For instance, decreasing the amount of sulphur-containing amino acids in the rat diet induced an increase in UDP-glucuronyltransferase activity [39], that catalyzes the competing conjugation reaction of sulphation-substrates.

The presented methods may be of help to study the effect of a decreased sulphate concentration in the blood and a concomitant decrease in sulphate availability on the rate of sulphation and the extent to which sulphation of a substrate is still possible. Subsequently, it may be possible to evaluate the consequences of a decreased sulphate availability on elimination or toxification of a drug by sulphation.

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