it to be a complex mixture, containing a minimum of 20 components. No information was available to indicate which component was responsible for the observed activity.

The results of dry column separation of the chloroform fraction indicated the presence of at least two active materials (Table I), a higher R_f fraction (Fraction C) possessing *in vitro* KB activity and a more polar fraction (Fraction H) possessing PS activity. While certain additional fractions possessed marginal KB activity, subsequent fractionation efforts were directed at the two most active materials. With biological data available from dry column chromatographic analysis, larger scale gradient-elution column chromatographic procedures could be aimed specifically at the resupply of the active materials.

DISCUSSION

The complex nature of the biologically active *E. cyparissias* chloroform fraction presented a formidable challenge to the dry column chromatographic technique. Although all components of this mixture were not separated completely (some fractions contain more than one component), useful separation was achieved and, on the basis of dry column chromatographic results, subsequent efforts could be directed toward a limited group of materials possessing bioactivity. The value of the dry column technique in the preliminary analysis of a complex active fraction thus was demonstrated. In examples not presented here, pure materials could be isolated and their activity determined directly from the dry column procedure.

In certain investigations involving the fractionation of biologically active plant or fermentation extracts, a previously isolated active material may be presumed to be responsible for the observed activity based on phytochemical or chemotaxonomic information. Much effort may be saved in these instances by employing the dry column chromatographic procedure as the initial fractionation step. If the R_f of the material is known, the corresponding dry column chromatographic band can be obtained and analyzed for activity as well as for the presence of the known compound.

As indicated in Table I, a developing solvent for dry column chromatographic analysis can be utilized that will produce results roughly similar to those obtained from extensive column chromatographic procedures. By employing dry column procedures first, however, careful liquid chromatographic techniques need only be employed to separate components of active fractions, allowing inactive materials to be collected together. Thus, some time, effort, and expense of conventional techniques may be saved.

The described dry column technique represents only minor modification of previously described dry column chromatographic techniques. Although such methods have not been heavily utilized by the natural product chemist, they should become increasingly important in improving the efficiency and speed of fractionation efforts.

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Absorption of Orally Administered Sodium Sulfate in Humans

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Abstract □ Sodium sulfate can be used to enhance the conjugation of phenolic drugs with sulfate and to treat hypercalcemia. It is thought that sulfate ion is absorbed slowly and incompletely from the digestive tract. The purposes of this investigation were to determine the absorption of a large amount of sodium sulfate (18.1 g as the decahydrate, equivalent to 8.0 g of the anhydrous salt) and to compare the bioavailability when this amount is administered orally to normal subjects as a single dose and as four equally divided hourly doses. The 72-hr urinary recovery of free sulfate following single and divided doses was 53.4 ± 15.8 and 61.8 ± 7.8%, respectively (mean ± *SD*, $n=5$, $p > 0.2$). The single dose produced severe diarrhea while the divided doses caused only mild or no diarrhea. Thus, a large amount of sodium sulfate, when administered orally in divided doses over 3 hr, is well tolerated and is absorbed to a significant extent. Orally administered sodium sulfate may be useful for the early treatment of acetaminophen overdose.

Keyphrases □ Sodium sulfate—absorption after oral administration, single and divided doses, bioavailability, humans □ Acetaminophen—use of sodium sulfate for early treatment of toxicity, absorption of orally administered sodium sulfate, single and divided doses □ Bioavailability—sodium sulfate, comparison of single and divided doses, role in treatment of acetaminophen toxicity

Humans and animals have a limited capacity to conjugate phenolic drugs with sulfate (1–5). The limiting factor is the availability of sulfate ion rather than its activation or the transfer of activated sulfate to the acceptor molecule (3, 5). Sulfate ion is acquired by the body partly as such from dietary sources and partly by oxidation of cysteine and methionine (6). The possibility of enhancing the formation of phenolic sulfates by direct administration of inorganic sulfate was first proposed in 1876 and has been demonstrated by several investigators (6, 7). Typically,

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Table I—Urinary Excretion of Free Sulfate by Normal Men after Oral Administration of 18 g of Sodium Sulfate Decahydrate in Single and Divided Doses

Subject	Age, years	Body Weight, kg	Baseline Excretion Rate, mmoles/24 hr[a]	Cumulative Percent of Dose Excreted					
				Single Dose			Divided Dose[b]		
				24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
PM	25	79	22.9 ± 2.9	24.5	27.1	38.8	37.2	49.7	57.2
DC	25	66	16.7 ± 1.2	40.7	62.9	71.5	44.6	52.8	63.2
DS	34	77	24.3 ± 6.5	55.2	65.3	68.9	48.3	60.0	69.3
UW	36	74	25.0 ± 6.4	44.4	49.0	39.4	59.8	60.4	68.4
SR	28	66	13.0 ± 2.1	17.3	43.0	48.5	27.9	42.5	50.8
Mean				36.4	49.5	53.4	43.5[c]	53.1[c]	61.8[c]
SD				15.4	15.6	15.8	12.0	7.5	7.8

[a] Mean ± *SD*, $n = 3$ consecutive days. [b] Four equally divided doses administered at hourly intervals. [c] Not significantly different ($p > 0.1$) from corresponding value after single dose.

sodium sulfate has been administered parenterally but it has also been effective when given orally in small doses (3, 6).

BACKGROUND

It may be desirable under certain circumstances to administer relatively large doses of sodium sulfate orally, provided that it can be ascertained that the sulfate ion is absorbed reasonably well. For example, intravenous administration of >100 g of sodium sulfate decahydrate to adults is considered to be an effective and relatively safe treatment for hypercalcemia (8). Parenteral administration of sodium sulfate enhances the conjugation of large doses of acetaminophen to acetaminophen sulfate in rats and thereby accelerates drug elimination (5). Overdoses of acetaminophen may cause serious and sometimes fatal hepatotoxicity, apparently due to the formation of one or several quantitatively minor but highly reactive metabolites (9). It is reasonable to assume that enhanced formation of acetaminophen sulfate by a parallel and competing pathway will reduce the formation of hepatotoxic metabolite(s) and thereby reduce the toxicity of acetaminophen (10). In fact, parenteral administration of sodium sulfate reduced the acute toxicity of acetaminophen in mice (11).

Oral administration of a tracer dose of sodium [^{35}S]sulfate to normal men resulted in the recovery of ~80% of the radiolabel in the 24-hr urine, compared to a recovery of ~86% following intravenous injection of the same dose (12). However, the intestinal absorption of sulfate ion occurs by active transport, at least in rats, hamsters, and rabbits (13), and, therefore, is subject to saturation. Large doses of sodium sulfate (~15 g of the decahydrate in adults) are used as an osmotic cathartic on the basis that sulfate ion is only slowly and incompletely absorbed (14). No report concerning the actual bioavailability of sodium sulfate administered in such large doses to humans was found.

In view of the potential therapeutic usefulness of systemic sodium sulfate, particularly for reducing or preventing acetaminophen toxicity (10, 11), the absorption of a relatively large amount of sodium sulfate was determined following oral administration to human volunteers as a single large dose and as four equally divided hourly doses.

EXPERIMENTAL

Five healthy men, who had given their written informed consent, participated in the study. They collected all urine for three separate 24-hr periods for determination of baseline free sulfate output. They then received 18.1 g of sodium sulfate USP (the decahydrate, equivalent to 8.00 g of anhydrous sodium sulfate) orally on two occasions, at least 1 week apart. At these times, the subjects fasted overnight and consumed a low fat breakfast in the morning. They then emptied their bladder and ingested the sodium sulfate dissolved in 50 ml of warm water, either as a single dose or in four equally divided hourly doses. They ate lunch, and their subsequent meals and fluid intake were not restricted or controlled. All urine was collected in sterile plastic bags over 0–24, 24–48, and 48–72 hr. The urines were frozen immediately after collection, pending assay.

The concentration of free (inorganic) sulfate was determined by the method of Häkkinen and Häkkinen (15), which involves precipitation of urinary calcium as the oxalate (to avoid assay interference), precipitation of free sulfate as the barium salt by addition of barium chloranilate, and spectrophotometric assay of the liberated chloranilic acid. Total sulfate (*i.e.*, the sum of free and organically bound sulfate) was determined by the same procedure following acid hydrolysis at 80° (15). The procedures were performed in quadruplicate; one reaction mixture was used to prime the filter paper, which then was used to filter the other three. Results of the three assays were averaged (coefficient of variation usually was ≃5%).

RESULTS

The results are summarized in Table I. The baseline individual average excretion rate of inorganic sulfate ranged from 13 to 25 mmoles/24 hr and was relatively constant (coefficient of variation of 7–27%). The individuals with the lowest body weight (DC and SR) exhibited the lowest baseline values. Compared with the amount of sodium sulfate taken by the subjects (56.3 mmoles), the baseline excretion rate of inorganic sulfate was substantial.

The baseline excretion rate of free sulfate was not affected by relatively large changes in urine flow rate, but the baseline excretion rate of total sulfate (and, therefore, of organically bound sulfate) increased essentially linearly with increasing urine flow rate (Fig. 1). This differential effect of the urine flow rate also was observed after sodium sulfate administration.

The cumulative amounts of free sulfate excreted in the urine 24, 48, and 72 hr after sodium sulfate administration (*i.e.*, the sum of endogenous and exogenous free sulfate excretion) were significantly larger than the amounts of free sulfate excreted during the same lengths of time in control experiments ($p < 0.01$ by paired t test). The average urinary recovery of administered sulfate, calculated as the 72-hr excretion of free sulfate minus the baseline excretion, averaged 53.4% from the single dose and 61.8% from the divided doses. There was considerably less interindividual variation in urinary recovery of free sulfate from the divided doses (Table I).

All subjects experienced severe diarrhea after the single dose of sodium sulfate, starting typically after 2 hr and lasting up to 24 hr. The same amount of sodium sulfate taken in four equal hourly doses produced either no diarrhea or mild diarrhea of short duration. No other adverse effects were reported.

DISCUSSION

Sodium [^{35}S]sulfate has been used extensively in tracer doses to estimate extracellular fluid volume in humans (12, 16–18). Typically, plasma sulfur 35-derived radioactivity declines biexponentially after intravenous injection, with a terminal apparent half-life of ~4 hr (17). The apparent volume of distribution is usually ~20% of the body volume (12, 17, 18); the renal clearance is ~35 ml/min/1.73 m^2 (16, 17), *i.e.*, approximately one-third of the glomerular filtration rate. Since sulfate ion is not appreciably bound to plasma proteins, the difference between its renal clearance and the glomerular filtration rate is probably due to renal tubular reabsorption. The renal tubular reabsorption of sulfate is capacity limited (19). However, the urinary excretion of free sulfate is apparently not urine flow rate dependent, at least in the flow rate range encountered in this study (Fig. 1). That is not so with respect to organically bound (ethereal) sulfate; total nonradioactive sulfate in the urine is, therefore, not a suitable index of sulfate absorption.

Urinary recovery of sulfur 35 activity in humans after administration of a tracer dose of sodium [^{35}S]sulfate was ~38% of an oral dose in 5 hr (18), and ~86% in 24 hr (12) and 95% within 5 days (17) after an intravenous dose. It was also reported that 90–100% of a 117-g iv dose of nonradioactive sodium sulfate decahydrate was excreted in the urine within 1 day, but no details were given (8). More than 92% of the radioactivity excreted in the urine within 4 hr after intravenous injection of

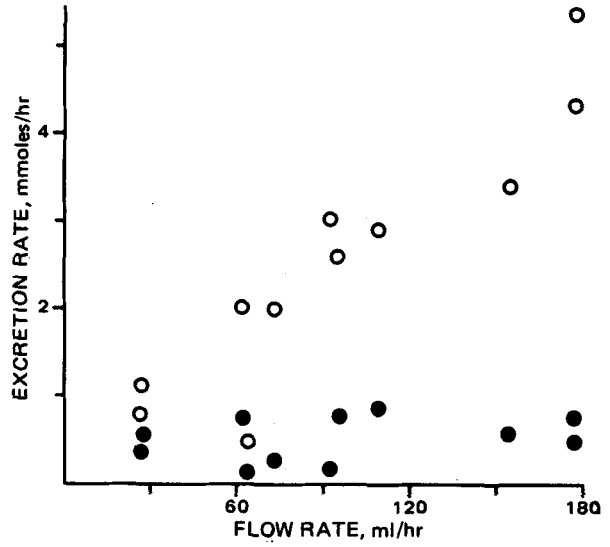

Figure 1—*Relationship between urine flow rate and baseline excretion rate of free (●) and total (○) sulfate for Subject DC. Urine was collected every 2–3 hr for 24 hr.*

sodium [^{35}S]sulfate is free sulfate, *i.e.*, sulfate ion which could be precipitated as benzidine sulfate (16).

The baseline excretion of free sulfate in the urine (*i.e.*, excretion without administration of a sulfate salt) is affected by the diet, particularly by the intake of proteins and green vegetables (6, 20). Most sulfur excretion is in the form of inorganic sulfate (20, 21). The average baseline urinary excretion rate of free sulfate by normal men is ~22 mmoles/day (22). This rate is substantial when compared with the dose of sodium sulfate used in the present study (56.3 mmoles). Ideally, therefore, the drug should have been administered together with ^{35}S-labeled sodium sulfate, but it was considered inadvisable to expose the volunteers to sulfur 35, particularly without first attempting to perform the study without radioactive sulfate. This conservative approach was justified by the results. The individual 72-hr urinary recoveries were reasonably consistent, especially after administration of sodium sulfate in divided doses (Table I). Cumulative recovery increased progressively over 3 days, except for Subject UW following the single dose[1]. Thus, the relatively large amount of drug administered and the relatively consistent baseline excretion rate of free sulfate made it possible to conduct this investigation without radioactive tracer.

Considering its pronounced cathartic effect, the single large dose of sodium sulfate was surprisingly well absorbed. However, the same is true in rats. Oral administration of ~250 mg of sodium [^{35}S]sulfate/kg to adult male rats resulted in the recovery of 73.7% of the administered radioactivity in the urine, compared with a recovery of 76.7% after intraperitoneal injection (7). Another investigator administered oral doses of 0.35, 0.70, 1.41, and 2.0 g/kg to adult male and female rats and recovered 57–74% of the radioactivity in urine over 72 hr, with no apparent relationship between the dose and urinary recovery (23). The same investigator determined that the median effective laxative dose of sodium sulfate in rats was 1.6 g/kg and that a dose of 1.22 g/kg had no laxative effect, while a dose of 2.8 g/kg was 100% effective by his criteria (23). Thus, the relative bioavailability of sodium sulfate in rats was not affected by the degree of catharsis, which also reflects the relative intestinal motility or transit rate. Our results in men were similar: there was no statistically significant difference in the urinary recovery of free sulfate after administration of a large amount of sodium sulfate as a single dose (which produced severe diarrhea) or in divided doses (which produced little or no diarrhea).

In conclusion, oral administration of sodium sulfate is a viable means of introducing large amounts of free sulfate into the systemic circulation. We also found, in an unpublished study, that sodium sulfate is not significantly adsorbed onto activated charcoal. The latter is effective in inhibiting the absorption of acetaminophen (24). Administration of as little as 2 g of acetaminophen to healthy men caused depletion of endogenous sulfate as reflected by reduced urinary excretion of free sulfate and endogenous sulfate conjugates (25), while administration of sodium sulfate enhanced conjugation of acetaminophen with sulfate in humans and animals (3, 5, 25) and reduced the acute toxicity of acetaminophen in animals (11). Consequently, the combined use of sodium sulfate and activated charcoal, both being widely available home remedies, for the initial treatment of acetaminophen overdose deserves clinical investigation. However, pending such investigation, this treatment is not advocated for routine use and should certainly not take the place of presently accepted therapy.

[1] An apparent decrease in the urinary recovery of free sulfate, as observed in Subject UW from 48 to 72 hr (Table I), can occur if the baseline excretion rate determined in the drug-free control period is higher than the excretion rate of endogenous free sulfate after sodium sulfate administration.

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