Human sulfate kinetics

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Human sulfate kinetics. Am J Physiol Regul Integr Comp Physiol 289: R1372–R1380, 2005. First published July 28, 2005; doi:10.1152/ajpregu.00325.2005.—Electrospray tandem mass spectrometry was used to determine steady-state serum and urinary inorganic sulfate and sulfate ester kinetic profiles of nine normal men after intravenous injection of the stable isotope sodium $[^{14}S]$ sulfate. Sulfate ester appearance was traced by eliminating inorganic sulfate from samples, followed by hydrolysis of sulfate esters to inorganic sulfate for analysis. Whole body inorganic sulfate turnover in steady state was calculated using standard tracer techniques. Rate of appearance and disappearance of inorganic sulfate was 841 ± 49 μmol/h. Average urinary inorganic sulfate excretion was 609 ± 41 μmol/h, and the whole body sulfate rate (total rate of disappearance minus rate of urinary excretion) was 232 ± 36 μmol/h. Tracer-labeled sulfate esters appeared in serum and urine within 1 h of tracer injection. The kinetics of inorganic sulfate and sulfate esters were linked by means of a compartmental model. The appearance and excretion of sulfate esters accounted for ~50% of the total sulfate rate. These results indicate that human whole body sulfation accounts for ~27% of inorganic sulfate turnover and that extracellular inorganic sulfate is an important pool for intracellular sulfation. A substantial fraction of newly synthesized sulfate esters promptly enters the extracellular space for excretion in the urine.

inorganic sulfate; stable isotopes; sulfate esters; sulfation

Understanding of the basic features of sulfate metabolism has increased greatly in recent years (6, 8, 29, 47). Sulfate is the substrate in biosynthetic reactions after its activation to the universal sulfate donor 3′-phosphoadenosine-5′-phosphosulfate (PAPS) (6, 18). Sulfotransferases transfer a sulfonate group from PAPS to acceptors to form sulfate esters (6, 18, 47). Human PAPS synthetase is highly active under optimum conditions (55), and inorganic sulfate availability controls the rate of PAPS formation and, hence, in vivo sulfation rates (6, 23). Glycosaminoglycans and galactosaminoglycans (GAGs) are sulfated in the Golgi network (24), whereas O- and N-sulfation of catecholamines, iodothyronines, cholesterol, bile acids, steroids, and some vitamins (vitamin D and aspirin) takes place in the cytosol (6, 18, 47). Also sulfated in the cytosol, especially by hepatocytes, are certain chemicals and drugs such as benzene, after its hydroxylation to phenol (52), and the analgesic drug acetaminophen (N-acetyl-4-aminophenol) (6, 18, 47).

The inorganic sulfate space is considered a valid indicator of the extracellular fluid (ECF) volume (2, 13, 40, 53). However, all cells have the capacity to transport inorganic sulfate (29). Animal and human studies show that the administration of drugs metabolized by sulfation promptly reduces circulating inorganic sulfate concentrations (16, 34). An early human tracer study involving intravenous injection of $[^{35}S]$ sulfate found that radioactivity disappeared from the circulation faster than it was excreted in the urine (44). More recently, we measured human inorganic sulfate turnover by means of primed continuous oral administration of the stable isotope sodium $[^{14}S]$ sulfate and found that it exceeded the urinary excretion rate (12). Because sulfate is almost entirely eliminated from the body in the urine (2, 12, 17, 40, 44), these observations imply that inorganic sulfate is taken up by the cells at a biochemically significant rate for use in intracellular sulfation reactions. To explore this possibility in detail, we carried out a steady-state tracer kinetic study using $[^{34}S]$ sulfate to model steady-state inorganic sulfate turnover of normal fasting humans and the associated appearance of the tracer in sulfate esters.

MATERIALS AND METHODS

Subjects and protocols. Nine healthy nonsmoking men using no medications and with normal blood chemistries were admitted to the Clinical Research Unit of the Jewish General Hospital of Montreal after a 12-h fast. Written consent was obtained to participate in the study, which was approved by the hospital’s Research Ethics Committee. All experiments commenced at 0830.

Aseptically prepared, pyrogen-free sodium $[^{14}S]$ sulfate (99.7% $^{34}S$; Isolife USA, San Francisco CA; 3.0 mg/ml of 154 mmol/l NaCl) was injected in a dose of 2.78 ± 0.29 μmol/kg within 60 s into an antecubital vein flushed with 77 mmol/l NaCl. The dose was verified by weighing the syringe before and after tracer injection. Blood samples were drawn before tracer administration and at 2, 4, 6, 8, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, 120, 150, 180, 210, 240, 300, 360, 420, 480, and 540 min after tracer injection from an arterialized vein of the opposite arm kept patent by constant infusion of 77 mmol/l NaCl (50 ml/h). Urine was collected hourly over the 9-h study. No food was consumed.

Analytical methods. Clotted arterialized venous blood was centrifuged at 1,400 g for 30 min at room temperature; serum and urine samples were stored at −30°C until analysis. All sulfate measurements in urine and serum were obtained with electrospray tandem mass spectrometry using a Quattro II triple quadropole (Micromass, Manchester, UK) configured for negative ion analysis. All water used was type 1 or ultrapure water (resistivity 18.2 MΩ/cm), purified by treatment with Milli-RO Plus and Milli-Q UF systems (Millipore, Bedford MA). Urine and serum inorganic sulfate tracee concentrations and tracer enrichments were measured as previously described (4, 12, 49). Enrichment was measured as the tracer-to-tracee ratio (TTR), which is the conceptual equivalent of specific radioactivity for a radioactive tracer (5).

Urine sulfate esters were isolated using the method of Lundquist et al. (26), in which inorganic sulfate is eliminated by precipitation with
barium chloride and the inorganic sulfate released upon acid hydrolysis is measured. Three milliliters of a solution of 0.977 g BaCl₂·2H₂O and 4.1 ml of concentrated HCl in 100 ml of water were mixed with 1.5 ml of urine and kept at room temperature for 30 min with occasional mixing. The mixture was centrifuged at 1,400 g for 10 min at 20°C, and 1.5 ml of the supernatant was applied to a 0.5-ml column of strong cation exchange resin (Dowex 50W-X8, 100–200 mesh hydrogen form; Bio-Rad Laboratories, Richmond, CA) previously rinsed with 10 ml of 1 mol/l NaOH, 10 ml of water, and 10 ml of 100 mmol/l HCl. The resin was loaded with 2.5 ml of water, and the eluate, now free of barium ions, was collected in screw-cap glass tubes. The sealed tubes were heated in a water bath at 100°C for 30 min to hydrolyze the sulfate esters. To 1 ml of this solution was added 0.1 ml of water (for the enrichment measurement) or 0.1 ml of 600 μmol/l sodium [34S]sulfate (for the tracee concentration measurement) and 5 ml of methanol, and the mixture passed through an OnGuard-Ag cartridge (Dionex no. 39637; Oakville, ON, Canada) previously rinsed with 25 ml of water. This step removed chloride and phosphate ions, which otherwise interfered with the analysis (4). The first 1.5 ml of filtrate were discarded, and the rest was analyzed for tracer enrichment. Tracee concentrations were measured with the aid of an areas ratio standard curve created by adding 0.06 μmol of [34S]sulfate internal standard to tubes containing concentrations of sodium [32S]sulfate ranging from 0 to 150 μmol/l. Recovery of inorganic sulfate from indoxyl sulfate added to urine was 103%.

For serum sulfate ester measurements, 2 ml of serum were mixed with 10 ml of methanol, incubated on ice for 10 min to precipitate proteins, and then centrifuged at 1,400 g for 20 min at 4°C. The supernatant was removed and dried under a stream of nitrogen gas at 50°C. The dried residue was reconstituted in 1.5 ml of water and subjected to ultrasonication. To this was added 0.5 ml of the barium chloride-hydrochloric acid solution described above, and the sample was incubated at room temperature for 1 h with occasional mixing. The barium sulfate precipitate was removed by centrifugation, and 1.5 ml of the supernatant was passed through a 0.25-ml Dowex 50W-X8 column, as described above. The acidic eluate was collected along with a 1-ml methanol wash. The sample was sealed and incubated in boiling water for 30 min, after which 1 ml of the sample was mixed with 1 ml of water for the enrichment measurement or 0.1 ml of internal standard (600 μmol/l sodium [34S]sulfate) for the concentration measurement, followed by 3 ml of methanol. The resulting solution was passed through an OnGuard-Ag cartridge before analysis. In this case, the areas ratio curve used [32S]sulfate concentrations ranging from 0 to 50 μmol/l. Recovery of the sulfate in indoxyl sulfate added to serum was 99.3%.

In all cases, samples [32S]sulfate concentrations were determined using the appropriate standard curve after subtracting the contribution at the [34S]sulfate mass due to the tracer, as determined from a matched sample to which no internal standard had been added. [34S]sulfate concentrations were calculated as the product of TTR and [32S]sulfate concentration.

Urine and serum inorganic sulfate concentrations are approximately six times higher than sulfate ester concentrations, so it was important to verify that what was measured as sulfate ester enrichment was not partly due to incompletely extracted inorganic sulfate. To investigate this possibility, we added sodium [34S]sulfate to urine to an enrichment of 5%, the highest enrichment that occurred in study samples; no tracer was detectable in samples prepared as for sulfate ester analysis. On the other hand, when the tracer was added to serum to an enrichment of 10% (a value somewhat higher than the highest enrichment that occurred in the study), there was an apparent 1.4% sulfate ester enrichment. This result indicated there was <100% inorganic sulfate removal from serum by barium chloride. The contamination increased the apparent serum total sulfate ester concentration by not more than 2%, which is quantitatively trivial, but it was sufficient to artificially indicate non-zero sulfate ester tracer enrichments at time points when inorganic sulfate enrichments were very high. To correct for this, we treated one aliquot of a serum sample in the usual way with barium chloride followed by acid hydrolysis, yielding a mixture of inorganic sulfate derived from esters and the small amount of residual inorganic sulfate that had escaped binding to the barium. A second aliquot was treated with barium but was not hydrolyzed, yielding a sample that contained only the unremoved inorganic sulfate plus a small amount of inorganic sulfate produced by sulfate ester hydrolysis in the acidic milieu even at room temperature. The difference between the inorganic sulfate contents in these two aliquots represents only inorganic sulfate derived from esters. The effect of applying this correction was determined at different time points. Enrichments were unaffected by the correction procedure at the 30-min time point, by which time serum inorganic sulfate enrichments had decreased greatly. With the use of corrected values, serum sulfate ester enrichment at the 2- and 10-min time points (when inorganic sulfate enrichment was maximum) was zero. Therefore, in the reported data, serum sulfate ester enrichments at the 30-min time point and earlier were determined using the correction method.

Conventionally determined water, bromide, and inorganic sulfate spaces. Body weight was recorded, and total body water was measured using bioimpedance analysis (BIA-101A; RJL Systems, Mt. Clemens, MI) just before and immediately after completion of the 9-h study (22). The corrected bromide space was measured as previously described (13). The inorganic sulfate space was determined by plotting the natural logarithms of the [34S]sulfate enrichments against time from hours 4 to 9 and extrapolating back to time 0. The time 0 TTR thus obtained was multiplied by the time 0 [32S]sulfate concentration to obtain the theoretical serum tracer concentration if instantaneously distributed. Division of the tracer dose by this concentration is a measure of the total inorganic sulfate distribution space.

Statistical methods. Comparisons were made between selected average values using Student’s paired t-test, and correlations were made using the Pearson product moment correlation test (GraphPad Prism, version 4.00; GraphPad Software, San Diego, CA). The results are expressed as means ± SE.

MODEL

Glossary

ECF Extracellular fluid space
S Inorganic sulfate tracer mass
S* Inorganic sulfate tracer concentration
S0 Dose of inorganic sulfate tracer injected
S1 Inorganic sulfate tracer mass in the first compartment
S1* Inorganic sulfate tracer mass in the first compartment
S2 Inorganic sulfate tracer mass in the second compartment
S2* Inorganic sulfate tracer mass in the second compartment
S+ Total inorganic sulfate tracer mass
S+S2 Inorganic sulfate tracer mass
E Sulfate ester tracer mass
E* Sulfate ester tracer concentration
C* Tracer inorganic sulfate concentration in serum
C2% Tracer inorganic sulfate concentration in serum
C E Serum sulfate ester concentration
V Volume of distribution of inorganic sulfate
V1 Volume of distribution of the accessible inorganic sulfate compartment
V E Volume of distribution of the accessible sulfate ester compartment
kij Rate constant governing transfer of tracer and tracee from compartment j to compartment i
TTR Tracer-to-tracee ratio
A number of assumptions were made to develop a model for human whole body inorganic sulfate and sulfate ester metabolism. Inorganic sulfate is formed in the body through the oxidation of the amino acids methionine and cysteine. Inorganic sulfate derived from desulfation reactions also contributes. Under steady-state fasting conditions, inorganic sulfate’s rate of entry \( (R_d) \) into the ECF is due to its release from cells. Its rate of disappearance \( (R_d) \) from the ECF is the sum of its elimination from the body in the urine and its reuptake into tissues, because nonurinary routes of elimination from the body are quantitatively unimportant \( (2, 12, 17, 40, 44) \). Inorganic sulfate taken up by the tissues is assumed to be destined solely for sulfation. This assumption is biologically plausible, because there is no other way sulfate is metabolized or stored. Inorganic sulfate \( R_d \) minus urinary excretion rate is the whole body sulfate rate. We assumed that the only significant route of sulfate ester elimination from the ECF is urinary excretion with negligible reuptake by the cells, because, except for GAGs, sulfation is generally understood as conferring water solubility to permit urinary or biliary excretion of metabolites, biliary excretion being negligible compared with urinary excretion.

Noncompartmental calculations of inorganic sulfate model. After injection of the bolus of sodium \( [\text{34S}] \) sulfate, blood samples were collected at intervals for 9 h. Serum tracer concentrations were fitted using a simplex algorithm with the least-squares criterion to the sum of two real, negative exponential functions, given by the function \( C(t) \) shown below as Eq. 16. The need for a third exponential function was obviated by appeal to the Akaike information criterion. Some of the required quantities were calculated without reference to a compartmental model. The average steady-state serum inorganic sulfate concentration is termed \( C_1 \), and the dose of tracer injected is \( S_0 \). Inorganic sulfate \( R_a \) was obtained from linear systems analysis \( (45, 48) \) as

\[
R_a = S_0 \frac{C_1}{\int_0^\infty C_1^\prime dt} \tag{1}
\]

The total mass of inorganic sulfate in the system, \( S_{TOT} \), was estimated according to the Meier-Zierler principle \( (32, 39) \) from the following equation:

\[
S_{TOT} = R_a \cdot \bar{t} \tag{2}
\]

where \( \bar{t} \) is the mean residence time of a sulfate molecule in the system, given by

\[
\bar{t} = \int_0^\infty t C_1^\prime dt/\int_0^\infty C_1^\prime dt \tag{3}
\]

The inorganic sulfate space was then estimated from

\[
V_S = S_{TOT}/C_S \tag{4}
\]

Subsequent calculations required a compartmental model. The model, illustrated in Fig. 1, was developed in two parts that were later linked. The first part governed inorganic sulfate \( (S) \) kinetics and the second sulfate ester \( (E) \) kinetics. Solution of the equations defining the S model provided information about the distribution of inorganic sulfate within its compartmental structure and permitted calculation of \( R_a \).

**Compartmental model for inorganic sulfate.** Inorganic sulfate kinetics were represented by means of a two-compartment model of the extracellular space. Input to and output from the system are confined to compartment 1, which is termed the compartment of access. Compartment 1 communicates with compartment 2, which represents a more static or inert part of the ECF. The compartments communicate by means of the rate constants \( k_{21} \) and \( k_{12} \). Compartment 1 receives the input, \( R_a \); its output is governed by the conjoint rate constant \( (k_{01} + k_{31}) \). These two rate constants are described later; the first governs urinary excretion, and the second governs sulfation.

The kinetics of inorganic sulfate tracee were assumed to be linear and described by the following differential equations:

\[
dS_1/dt = R_a - [(k_{01} + k_{31})S_1 + k_{12}S_2] \tag{5}
\]

\[
dS_2/dt = k_{21}S_1 - k_{12}S_2 \tag{6}
\]

where \( S_1 \) and \( S_2 \) are the inorganic sulfate masses in their...

Fig. 1. Linear compartment model for inorganic sulfate and sulfate ester kinetics. Compartments 1 and 2 represent the extracellular fluid (ECF) through which inorganic sulfate is distributed. The rate constants \( k_{31} \) and \( k_{21} \) govern the exchange of tracer \( [\text{34S}] \) sulfate and tracee \( [\text{32S}] \) sulfate throughout the ECF. The arrow at top left represents the rate of appearance \( (R_a) \) of tracee and injected tracer. Irreversible loss of inorganic sulfate is governed by 2 rate constants, \( k_{01} \) and \( k_{31} \); the first leads inorganic sulfate directly to the urine, and the second leads into the cells where sulfate ester formation takes place. A fraction \( (F) \) of the sulfate esters formed in the cells reappears as sulfate esters in compartment 3 with rate constant \( F \cdot k_{31} \). The rate constant \( k_{33} \), depicted leaving compartment 3, has 2 components: \( k_{330} \), which governs sulfate ester elimination in the urine, and a component leading to an unidentified 4th compartment (not shown) as a first approximation (see Fig. 5).
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respective compartments. Under steady-state conditions, the time derivatives may be set equal to 0, giving

\[ R_d = [(k_{01} + k_{13}) + k_{21}]S_1 - k_{12}S_2 \]  
(7)

\[ k_{21}S_1 - k_{12}S_2 = 0 \]  
(8)

\[ S_{TOT} \]  

was defined as

\[ S_{TOT} = S_1 + S_2 \]  
(9)

Eqs. 7, 8, and 9 were solved for \( S_1 \) and \( R_d \):

\[ S_1 = k_{12}S_{TOT}/(k_{12} + k_{21}) \]  
(10)

where \( S_{TOT} \) is known from Eq. 2, \( S_2 \) is known immediately from Eq. 9, and

\[ R_d = [k_{01} + k_{31}]S_1 \]  
(11)

Analogous to Eqs. 5 and 6, which define tracee kinetics, are the following equations for the tracer of the kinetics:

\[ dS^*_1/dt = S^*_0(i) - [(k_{01} + k_{31}) + k_{21}]S^*_1 + k_{12}S^*_2 \]  
(12)

\[ dS^*_2/dt = k_{21}S^*_1 - k_{12}S^*_2 \]  
(13)

where the input rate of tracer is represented by \( S^*_0(i) \), the delta function of weight \( S^*_0 \). The solution to these differential equations is of the form

\[ S^*_1(t) = B_1e^{-\beta_1t} + B_2e^{-\beta_2t} \]  
(14)

\[ S^*_2(t) = -B_1e^{-\beta_1t} + B_2e^{-\beta_2t} \]  
(15)

where \( S^*_1 \) and \( S^*_2 \) are the tracer inorganic sulfate masses in the two compartments and \( \beta_1 \) and \( \beta_2 \) are real, with \( \beta_1 > \beta_2 > 0 \). The problem is to evaluate the rate constants \( k_{ij} \) numerically. The measured data were serum \([34]^\circ\) sulfate concentrations that were fitted to the sum of two exponential functions:

\[ C_2^*(t) = A_1e^{-\gamma_1t} + A_2e^{-\gamma_2t} \]  
(16)

\( \beta_1, \beta_2, \gamma_1, \) and \( \gamma_2 \) were then known numerically. The equations defining the model are in terms of mass rather than concentration. By assuming the tracee inorganic sulfate concentration is constant throughout the ECF, one can obtain \( S^*_1 \) (a mass) as the product of \( C_2^* \) and the volume of its compartment of access, \( V_S(S_1/S_{TOT}) \). \( B_1 \) was then identified in units of mass with \( A_1V_S(S_1/S_{TOT}) \) and \( B_2 \) with \( A_2V_S(S_2/S_{TOT}) \); \( \gamma_1 \) and \( \gamma_2 \) were identified with \( \beta_1 \) and \( \beta_2 \), respectively. In this way the right-hand side of Eq. 14 was evaluated explicitly. The unknown quantities \( k_{ij} \) could now be expressed in terms of the known quantities \( B_1, B_2, \beta_1, \) and \( \beta_2 \). The solutions are as follows (39):

\[ k_{12} = (\beta_1B_2 + \beta_2B_1)/(B_1 + B_2) \]  
(17)

\[ k_{21} = B_1B_2(\beta_1 - \beta_2)^2/(B_1 + B_2)(\beta_1B_2 + \beta_2B_1) \]  
(18)

\[ (k_{01} + k_{31}) = \beta_1\beta_2(B_1 + B_2)(\beta_1B_2 + \beta_2B_1) \]  
(19)

Compartmental model for sulfate esters. Inorganic sulfate leaves compartment 1 for excretion in the urine and uptake by the cells for sulfation reactions. The rate constant governing excretion into urine, \( k_{01} \), is measured as the average urinary excretion rate of inorganic sulfate divided by \( S_1 \). Subtracting \( k_{01} \) from the joint rate constant \( (k_{01} + k_{31}) \) leaves the value of the rate constant for sulfation, \( k_{31} \). The sulfation rate is \( k_{31}S_1 \). Only a fraction of the products of intracellular sulfation enters the ECF for excretion in the urine. \( F \) is defined as the fraction of the total sulfation rate accounted for by the release of sulfate esters into the ECF for urinary excretion. According to this definition, \( Fk_{31}S_1 \) is the rate of sulfate ester formation in steady state, as shown in Fig. 1.

Sulfate ester kinetics were represented by a model consisting of only a single compartment. The appearance of \( E^* \), the mass of labeled sulfate ester in the compartment of access, was handled by adapting the pharmacokinetic model described by Gibaldi and Perrier (11) for metabolite formation from a drug. The differential equation is

\[ dE^*/dt = Fk_{31}S_1^* - k_EE^* \]  
(20)

The first term on the right-hand side is the rate of entry of sulfate ester mass, and the second term is its rate of removal from the compartment of access. The use of a single compartment model for sulfate ester kinetics might at first seem inadequate. To test this, we developed a two-compartment model for ester kinetics comparable to the one for inorganic sulfate (see APPENDIX). This exercise showed that the values for the rate constant governing reentry of sulfate ester tracer into the compartment of access are extremely small, effectively reducing the two-compartment model to the single-compartment model given by Eq. 20; we therefore continued to use the simpler model.

After substituting \( S_1^* \) from Eq. 14 into Eq. 20, the differential equation for \( E^* \) was solved as an explicit function of time. However, the experimental data used for parameter estimation were sulfate ester enrichments, represented by \( E^*/E \), where \( E \) is the constant mass of sulfate esters in the compartment of access. \( E \) is equal to the product of the serum sulfate ester concentration and the volume of distribution of its compartment of access, \( V_E \). Two parameters were thus to be evaluated from the data: the distribution volume \( V_E \), and the rate constant \( k_E \). In the equation that follows, these parameters are termed \( P_1 \) and \( P_2 \), respectively, and the concentration of sulfate ester in its compartment of access is termed \( C_E \). The final sulfate ester model equation, fitted by the computer to measured sulfate ester enrichment data, was

\[ E^*(t)/E = [k_{31}F(C_EP_1)][(B_1/P_2 - \beta_1)e^{-\beta_1t} - e^{-\beta_2t}] + [B_1/(P_2 - \beta_2)[e^{-\beta_1t} - e^{-\beta_2t}]] \]  
(21)

\( E^*/E \) was measured for each subject over the time course of the experiment. Equation 21 was fitted to the data by a simplex algorithm using an unweighted least-squares criterion, thereby providing numerical values for \( P_1 \) and \( P_2 \), representing \( V_E \) and \( k_E \), respectively.

Noncompartmental derivation of sulfate ester model. In the sulfate ester model defined by Eq. 20, \( S_1^* \) is the mass of \( S^* \) in compartment 1 of the inorganic sulfate model. The three terms \( F, k_{31} \), and \( S_1^* \) may be rewritten as

\[ F = \text{sulfate ester excretion rate/sulfation rate} \]

\[ k_{31} = \text{sulfation rate}/(C_SV_S) \]

where \( C_S \) is the steady-state serum inorganic sulfate concentration and \( V_S \) is the volume of the compartment of access, and

\[ S_1^* = C_S^*V_S \]

where \( C_S^* \) is the serum tracer inorganic sulfate concentration (see Eq. 16).
Substituting these expressions into Eq. 20 and simplifying yields
\[
\frac{dE^*}{dt} = \left( \text{sulfate ester excretion rate} / C_S \right) C_3^* - k_3 E^*
\]
(22)

Because Eq. 22 requires no information or assumptions about \( V_S \) (which cancels out), this formulation is independent of the compartmental inorganic sulfate model. The solution is the same as for Eq. 21, except for the replacements indicated and replacement of the coefficients and rate constants in Eq. 21 with \( A_i \) and \( y_i \) as in Eq. 16. This equation was also fitted to the data as described for Eq. 21.

Alternative calculation of \( F \). \( F \) is the fraction of whole body sulfation directed into urinary excretion of sulfate esters. The sulfation rate, calculated as \( R_d \) minus the urinary inorganic sulfate excretion rate, so \( F \) depends on the hourly inorganic sulfate excretion rate. As reported in RESULTS, this rate decreased over the course of the study, so the value used for hourly excretion was averaged over the entire 9 h. To test the accuracy of calculating \( F \) this way, we used a second method that consists of a static enumeration of the fraction of all \( S^* \) that had entered the cells and reemerged into the ECF as \( E^* \) at that the end of the study. The numerator is the total amount of \( E^* \) excreted in the urine over 9 h plus the amount of \( E^* \) calculated to be present in the ECF at the 9-h time point. The denominator is the amount of \( S^* \) that disappeared into the cells over that time period, calculated as the dose of \( S^* \) injected minus the amount of \( S^* \) recovered in the urine minus the amount of \( S^* \) remaining in the inorganic sulfate space at the 9 h time point.

\[
F = \left( E^*_{\text{urine}} + E^*_{\text{in ECF at 9 h}} \right) / \left( S^*_{\text{dose}} - S^*_{\text{urine}} - S^*_{\text{in ECF at 9 h}} \right)
\]
(23)

RESULTS

The study participants were in normal health and used no medication. Specifically, none of them had consumed acetaminophen within the preceding week. Values are expressed as means \( \pm \) SE; their age was \( 31 \pm 5 \) yr; weight, \( 74 \pm 3 \) kg; body mass index, \( 24 \pm 1 \) kg/m\(^2\); and surface area \( 1.91 \pm 0.04 \) m\(^2\). Total body water was \( 58 \pm 1 \% \) body weight. The corrected bromide space was \( 15.8 \pm 0.4 \) liters (213 \pm 6 ml/kg). The serum creatinine concentration was 81.4 \( \mu \)mol/l, and the glomerular filtration rate (27) was 106 \pm 4 ml/min \(-1\) \times 0.73 m\(^2\). During the study, average total fluid administration from intravenous and drinking water was 402 \pm 41 ml/h; the average urine flow rate was 396 \pm 59 ml/h.

The inorganic sulfate space, determined by log-linear extrapolation to time 0 of the falling portion of the serum tracer enrichment-time curve, was \( 16.1 \pm 0.9 \) liters. As determined using the Meier-Zierler principle, the inorganic sulfate space was \( 15.5 \pm 0.7 \) liters. Figure 2 shows the time course of serum inorganic sulfate and sulfate ester concentrations and their hourly urinary excretion rates. Serum concentrations and the hourly excretion of sulfate esters were constant, but hourly urinary inorganic sulfate excretion decreased by 38% over the 9-h experiment. Figure 3 shows the time course of serum and urinary tracer enrichments in inorganic sulfate and inorganic sulfate esters. Table 1 displays the inorganic sulfate and sulfate ester model parameters. The sulfation rate, calculated as \( R_d \) minus the average urinary inorganic sulfate excretion rate, was \( 232 \pm 36 \) \mu mol/h, representing \( 27.3 \pm 3.9 \% \) of total inorganic sulfate turnover. Inorganic sulfate \( R_d \) calculated by compartmental analysis was precisely equal to \( R_c \) calculated using the area-under-the-curve approach. Figure 4 illustrates the individual serum sulfate ester enrichments and the approximation of...
the function described in Eq. 21 to these data. Use of the noncompartmentally derived formulation of sulfate ester model (Eq. 22) gave virtually identical numerical results, thereby demonstrating its mathematical equivalence.

Calculated as the average urinary sulfate ester excretion rate divided by the sulfation rate, F was 0.496 ± 0.122; calculated according to Eq. 23, F was 0.554 ± 0.117 (not significantly different). The individual F values derived using the two calculation methods were highly correlated ($r = 0.82; P = 0.007$; slope = 0.86, not significantly different from the line of identity).

**DISCUSSION**

In this study, stable isotope methodology and tracer modeling were used to elucidate important characteristics of human whole body sulfate metabolism. As measured in normal men in the postabsorptive state, inorganic sulfate turnover exceeded its urinary excretion rate, implying that a considerable fraction of the inorganic sulfate released by cells is simultaneously taken up by them. The situation is reminiscent of whole body amino acid metabolism, in which amino acids are simultaneously released into the circulation after intracellular proteolysis and taken up by the cells for use in protein synthesis (3, 54).

The average inorganic sulfate $R_d$ was 841 ± 49 $\mu$mol/h, and urinary inorganic sulfate excretion, averaged over the 9-h study, was 609 ± 41 $\mu$mol/h. Inorganic sulfate loss from the body by nonurinary routes is quantitatively unimportant (2, 12, 17, 40, 44), so the difference between $R_d$ and the average rate of tracee sulfate excretion, 232 ± 36 $\mu$mol/h, is the rate at which inorganic sulfate is taken up by the tissues. In keeping with current interpretations of whole body amino acid turnover models, inorganic sulfate uptake into the tissues may be equated with the whole body sulfation rate. (As with the protein synthesis rates determined from whole body amino acid

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<td>Parameter</td>
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<tr>
<td>Serum inorganic sulfate concentration</td>
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<td>Urinary inorganic sulfate excretion</td>
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<td>$k_{12}$</td>
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<td>Urinary sulfate ester excretion</td>
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<tr>
<td>$V_E$</td>
</tr>
<tr>
<td>$k_E$</td>
</tr>
<tr>
<td>$k_{03}$</td>
</tr>
</tbody>
</table>

Serum concentrations are the average values over the 9-h experiment. Excretion rates are the averages of the 9 hourly urine collections. See Glossary for definitions.

Fig. 4. Serum sulfate ester tracer enrichment (TTR, y-axis) and the corresponding model-derived function (hours, x-axis) for each of the 9 subjects.
turnover models, the sulfation rate indicated in this study may be an underestimation, because the model will not detect intracellular sulfate recycling.) A second important finding in this study was that \( \approx 50\% \) of the sulfation rate of healthy men appears to be directed toward the formation of sulfate esters that are excreted in the urine.

The present findings confirm and place a quantitative measure on previous indications that the ECF is an important reservoir for the inorganic sulfate used in sulfation reactions. The analgesic drug acetaminophen is largely metabolized by sulfation, and therapeutic doses of it reduce serum inorganic sulfate concentrations (14, 16, 20, 23, 34). In rats, radioactive harmol sulfate appears in the circulation after coadministration of harmol and \([^{35}S] \) sulfate (35), and the rate of drug sulfation has been reported to depend on the plasma inorganic sulfate concentration (21). It is noteworthy in this regard that serum inorganic sulfate concentrations are physiologically regulated (33). Such regulation may represent a mechanism for maintaining a reservoir of inorganic sulfate in the ECF to draw on for intracellular sulfation reactions.

According to steady-state tracer theory, \( R_d \) can be calculated by compartmental analysis and \( R_s \) by using the area under the curve (45, 48). We carried out both calculations and obtained identical results, demonstrating their mathematical equivalence. We also showed it is possible to derive a sulfate ester model independent of any compartmental assumptions about inorganic sulfate kinetics (see Eq. 22). The two-compartment model describing inorganic sulfate kinetics remains very useful for visualizing the distribution of sulfate within the ECF and its disposition by sulfation and urinary excretion. In principle, any of three different two-compartment models could have been used, depending on the routes of inorganic sulfate exit from the ECF: from the first compartment only, from the second compartment only, or from both compartments. However, only the first of these alternatives was adequate to describe the resulting sulfate ester kinetics, because only this model accommodated the very rapid increase in serum sulfate ester tracer after injection of the inorganic sulfate tracer.

The inorganic sulfate space, \( V_S \), whether measured using the Meier-Zierler principle as in the tracer model (15.5 ± 0.7 liters) or in the conventional way by log-linear extrapolation to time 0 of the falling portion of the serum tracer enrichment-time curve (16.1 ± 0.9 liters), is generally similar to the corrected bromide space (15.8 ± 0.4 liters) and the common rule-of-thumb approximation that the ECF accounts for 20% of normal body weight (14.9 ± 0.5 liters). How can one accommodate the evidence that inorganic sulfate is confined to the ECF with the present evidence that it is taken up by the tissues at a considerable rate? We suggest that the apparent restriction of the inorganic sulfate space to the ECF and the fact that tracer-labeled sulfate esters appear very promptly in the circulation after tracer sulfation injection indicate that the body’s intracellular inorganic sulfate pool is either very small or, as suggested by Cole and Scrivier (7), sequestered, and is hence unable to readily exchange with inorganic sulfate in the ECF.

The predictive equation depicting tracer sulfate ester enrichment over time contains two unknown parameters that were optimized by least-squares fit to the experimental data, yielding two experimental quantities: \( V_E \), the volume of distribution of sulfate ester in its compartment of access, and \( k_{E} \), the rate constant governing both transfer of tracer sulfate ester within its compartment of access and its excretion in the urine. Knowledge of \( V_E \) allowed the calculation of \( k_{03} \), the rate constant governing urinary sulfate ester excretion, as the sulfate ester excretion rate divided by the mass of E in its compartment of access; that is, as urinary sulfate ester excretion divided by the product of \( V_E \) and the sulfate ester concentration. The resulting value (0.297 ± 0.041 h\(^{-1}\)) was similar to the rate constant for inorganic sulfate excretion (0.283 ± 0.022 h\(^{-1}\)).

\( V_E \), the volume of the compartment of access for sulfate ester, was 7.17 ± 0.90 liters, and hence similar to \( V_{S1} \), the volume of the comparable compartment of the inorganic sulfate model (7.95 ± 0.43 liters). This similarity in volumes is compatible with the assumption made at the outset that sulfate ester and inorganic sulfate both distribute in the ECF and that after their synthesis and release into the ECF, sulfate esters are quantitatively excreted in the urine (see Eq. 23).

In the two-compartment sulfate ester model described in the Appendix, the volume of compartment 3 was 6.82 ± 0.89 liters, similar to \( V_E \) in the simpler one-compartment model given by Eq. 20. Also, the rate constant governing return of sulfate esters to this compartment from compartment 4 was very small. As a consequence, the two-compartment model effectively reduced to the one-compartment model. This model fitted a complex function to limited data, but it is of considerable interest that the value of the rate constant it indicated governing transfer of sulfate ester from compartment 3 into compartment 4 was more than 20 times greater than the rate constant in the reverse direction (see Appendix). If taken at face value, this implies that the mass of trace sulfate esters in compartment 4 is more than 20 times that in compartment 3 (see Eq. 8), something that could only occur if some sulfate esters distribute somewhere outside the ECF. If this analysis is
correct, the premise of Eq. 23 that all the E* in the body can be localized to the ECF is not strictly correct, and the resulting values might require adjustment. This unexpected possibility cannot be further explored with the present data.

Despite more than 100 years of research (10), the sulfate esters described here remain unidentified. Human urinary sulfate ester excretion varies (36), but it usually makes up a relatively constant 9–15% fraction of total sulfate excretion (10, 12, 30, 37), or ~2 mmol/day for adults consuming a normal diet and not living in regions where the groundwater is contaminated by inorganic sulfate. In pathological states of small intestinal bacterial overgrowth, certain bacteria metabolize tryptophan to indole and methylindole, which are hydroxylated to indoxyl and skatoxyl, then sulfated in the liver to indoxyl sulfate (indican) and skatoxyl sulfate, and excreted in the urine. Tyrosine may similarly be converted to phenol and indoxyl sulfate (indican) and skatoxyl sulfate, and excreted in pathological states (10, 12, 30, 37), or relatively constant 9–15% fraction of total sulfate excretion (36), but it usually makes up a relatively constant 9–15% fraction of total sulfate excretion. The fate of sulfate ester excretion varies (36), but it usually makes up a relatively constant 9–15% fraction of total sulfate excretion.

Esters described here remain unidentified. Human urinary sulfate is quantitatively trivial (18, 28, 43). GAG excretion cannot be further explored with the present data.

Biosynthetic reactions in the body is interesting from the implications for physiology and medicine. The evidence that human whole body sulfation can be easily and noninvasively detected and assessed congenital and acquired disease states in which sulfation is inhibited.

APPENDIX

Two-Compartment Model for Sulfate Ester Kinetics

The model is shown in Fig. 5. It differs from the model in Fig. 1 by the addition of compartment 4, which communicates with compartment 3 in precisely the same way that compartment 2 connects with compartment 1. The rate constants k43 and k34 govern transfer from compartment 3 to compartment 4 and from compartment 4 to compartment 3, respectively. Compartment 4 has no other inputs or exits. Sulfate ester input to compartment 3 and exit into the urine remain as shown in Fig. 1. The equations that define this model are

\[ \frac{dE^*_3}{dt} = F k_{34} S^*_1 - (k_{43} + k_{03}) E^*_3 + k_{34} E^*_4 \]

where \( E^*_1 \) and \( E^*_2 \) are the masses of sulfate ester tracer in compartments 3 and 4, respectively, and \( S^*_1 \) is the input provided from the inorganic sulfate system (Eq. 14). \( F, k_{34}, \) and \( k_{03} \) are known from the inorganic sulfate model. The solution for \( E^*_3(t) \) is of the form

\[ E^*_3(t) = A_1 e^{-k_{13} t} + A_2 e^{-k_{24} t} + A_3 e^{-k_{33} t} + A_4 e^{-k_{44} t} \]

where \( A_1 \) and \( A_2 \) are known functions of the initial conditions and the rate constants \( k_{34} \) and \( k_{34} \). When this solution was curve fitted to the measured sulfate ester tracer enrichment data, three parameters were estimated from the data:

\[ k_{34} = 0.661 \pm 0.150 \, \text{h}^{-1}, \]

\[ k_{03} = 0.031 \pm 0.015 \, \text{h}^{-1}, \]

and the volume of compartment 3 is

\[ V_3 = 6.64 \pm 0.80 \, \text{liters}. \]

In this model, \( k_{34} + k_{03} = 0.986 \pm 0.200 \, \text{h}^{-1} \) is comparable to \( V_3 = 0.814 \pm 0.135 \, \text{h}^{-1} \) in the one-compartment sulfate ester model defined by Eq. 20. The robustness, and hence reliability, of the fitted parameters was far less than with the one-compartment model, because three rather than two parameters were now being estimated from noisy data. This exercise nevertheless showed that \( k_{34} \), the rate constant for reentry of sulfate ester tracer into the compartment of access, was so small that the two-compartment model was effectively reduced to the one-compartment model.

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