of activity, the basal H₂ concentrations are usually within the range 0 to 15 μL/L (3, 4) but occasionally are higher (5). This variation is related in part to a circadian rhythm for breath H₂ concentrations, which increases throughout the hours of sleep that persist to the first-morning sample (5). The consequences of this non-zero baseline for breath H₂ in terms of the accuracy of diagnosis has been recently discussed (6, 7). Kolter et al. (6) propose a preparatory procedure in which their subjects ingested a diet low in dietary fiber residue the day before the breath test. Through this maneuver, they were able to decrease the mean breath H₂ concentrations of fasting patients and thereby improve the sensitivity of the interval-collection breath test by increasing the difference between baseline H₂ and post-ingestion concentration of H₂.

Because early-morning H₂ concentrations seem to respond to the amount of fiber of the previous day's diet, and because the post-waking breath H₂ concentrations of preschool children were often high (5), we chose to evaluate the day-to-day variation in this index in 35 adults. The subjects were instructed in how to collect and store their expired air themselves, at home. On three consecutive mornings, the subjects were collected to expire breath upon waking, before arising from the bed. A 5-L rubber anesthesia bag fitted with a low-resistance, one-way Hans-Rudolph valve was kept at the bedside, and subjects collected air by breathing through the valve into the bag (8). The gas sample was transferred to a 60-mL plastic syringe with three-way stopcock. The syringe was then sealed, and the sample was analyzed within 12 h of collection (9) in a gas-chromatographic H₂ analyzer (MicroLyzer Model CM; Quinton Instruments Co., Milwaukee, WI), calibrated with a reference mixture of H₂: 99 μL/L in air (L1 Squirt; Ideal Gas Co., Edison, NJ). The mean of all 105 such values we obtained for "first-morning breath hydrogen" (FMBH₂) concentration was 24.4 (SD 24.7) μL/L. The coefficient of variation (CV) for FMBH₂ samples from individuals whose overall mean baseline concentration was 10 μL/L (n=9) was 74.3% (SD 45.9%); for those with overall all-three-day means of 10 to 19 μL/L (n=10) it was 82.1% (SD 48.6%); and for individuals whose mean exceeded 20 μL/L (n=17) the CV was 47.8% (SD 26.6%). The high--low difference for FMBH₂ ranged from a minimum of 1 μL/L to a maximum of 16 μL/L in the first group; from 3 to 40 μL/L in the middle group; and from 13 to 113 μL/L for the third group. Overall, the average difference between the extremes of FMBH₂ as a percent of an individual's three-day mean was 124% (SD 80%, range 28-300%).

The concentration of H₂ in the breath of adults upon waking, as is also true for children (5), averages considerably higher than that customarily seen in fasting subjects who have been ambulatory for several hours before supplying the baseline sample for a routine clinical H₂ breath test. Although there is considerable intra-individual variation, subjects can be classified as consistently high or low FMBH₂ producers. Because the H₂ breath test can now be done on an outpatient basis with the subjects beginning the procedures at home, it would seem worthwhile to try to minimize the absolute concentrations of H₂ in the basal samples against which the diagnostic increments are to be measured. If one does not resort to the preparatory diets advocated by Kolter et al. (6), at least one should insist on some period of activity after waking before the sample is taken (while the subject is fasting) and the test-meal ingested.

The intra-individual variation in FMBH₂ is not so large as to preclude its use as a biological marker (perhaps of habitual fiber intake) to interindividual and intergroup survey studies. The present data provide estimates of variance upon which the appropriate sample size for valid testing of hypotheses could be based. Similarly, serial monitoring of the individuals with enforced dietary change could be undertaken with the FMBH₂ determinations and use of an appropriate sample number. For such research applications, however, breath samples should be obtained on at least three consecutive days, to improve confidence that the value for FMBH₂ is representative for a given individual.

References

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More on Determining Serum Sulfate

Laser nephelometry of Serum Sulfate

To the Editor:

Pascoe et al. (1) recently described a very interesting procedure for the turbidity of inorganic sulfate in plasma. The procedure is simple and economical, with adequate linearity and good precision.

A similar method has been developed in our laboratory, but with our procedure the amounts of precipitated BaSO₄ are determined by laser nephelometry. Our procedure requires 200 μL of sample and its sensitivity (0.02 mmol of sulfate per liter) is similar to that of radiometric methods with ¹³⁵Ba (2). It is as follows:

Reagents. Trichloroacetic acid (TCA), 1.8 mol/L. Standard solutions: sodium sulfate (Na₂SO₄ · 10 H₂O), 0.40 and 0.80 mmol/L. Precipitant solution: dissolve 5.00 g (24 mmol) of barium chloride and 90 g of polyethylene glycol 6000 (PEG 6000) in 1000 mL of de-ionized water. Transfer 100 mL of this solution to a 250-mL beaker and add, dropwise, 0.1 mL of Na₂SO₄ · 10H₂O, 60 mmol/L, with magnetic stirring. This reagent should be used within 24 h of being prepared. The Ba–PEG reagent and sodium sulfate solutions are stable separately at 4 °C for several weeks.

Instrument. We use a laser nephelometer (Behringwerke AG, Marburg, F.R.G.) with a He–Ne laser light source (632.8 nm) connected with a Hewlett-Packard HP 85 calculator for automatic evaluation of data.
Blood sampling and procedure. Blood samples were centrifuged as promptly as possible, and the serum was separated and frozen until sulfate determination. We treated 200 μL of serum with 100 μL of TCA solution and centrifuged briefly to sediment precipitated protein. An aliquot of the supernate (150–200 μL) was pipetted into sample cups of the laser nephelometer. Three points were used for calibration: the zero point, corresponding to the blank (de-ionized water); a second point, corresponding to the low standard; and a third point, corresponding to the high standard. We diluted 50 μL of blank, standard solutions (treated as samples with TCA), or TCA-protein-free filtrates with 120 μL of water, then added 100 μL of precipitant solution. After exactly 2.5 min we measured the light-scatter signals (in volts). All these operations could be automated by the preprogrammed microcomputer of module III of the laser nephelometer.

Blank readings were subtracted from the total readings and the specific scattering was plotted vs respective standard concentrations. The sample concentrations were read by the computer and the results were expressed directly in millimoles of sulfate per liter. If the concentration of sulfate exceeds 0.95 mmol/L, samples are prediluted fourfold with de-ionized water and the results multiplied by 4.

The instrumental response (in volts) was linear only up to 2.5 min and this time of the "fixed-time" reaction was used in the present method.

The nonspecific turbidity of protein-free filtrates was negligible; in 28 TCA supernates of lipemic or hyperbilirubinemic sera or sera with hemolysis, the mean concentration and relative SD of sulfate were 0.125 ± 0.012 V (r = 0.01 mmol/L). The standard curve was linear to 0.95 mmol of sulfate per liter. At concentrations >0.95 mmol/L, protein-free filtrates should be prediluted in water and results corrected for the dilution factor.

Analytical recovery of sulfate was 96–102%, even at low concentrations. No influence of phosphate on the sulfate determination was noted.

Heparin in concentrations >1.0 int. unit/mL inhibits BaSO₄ precipitation. This inhibition does not preclude the sulfate determination in plasma of patients who are undergoing hemodialysis because the heparin concentration in postdialysis plasma only rarely and briefly exceeds 1.0 int. unit/mL.

Four different samples (standard solutions and protein-free filtrates) were used for determination of within- and between-batch precision: ±5.5% and ±3.9%, respectively.

The mean value for serum sulfate concentration in healthy adult subjects was 324 μmol/L (SD 62 μmol/L; n = 138).

In conclusion: we believe that the laser nephelometric method offers several advantages as a reliable, rapid, sensitive, and accurate procedure for determination of inorganic sulfate in human serum. We have found a good correlation between the turbidimetric procedure of Pascoe et al. (1) and our nephelometric procedure (slope = 1.02, r=0.981, Scorr = 0.021, n=46). Therefore we think that our semiautomated nephelometric procedure may be an interesting and valid alternative to their automated turbidimetric method.

References
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Microassay of Plasma Sulfate

To the Editor:

The recent report by Pascoe et al. (1) describing a new method for inorganic sulfate (SO₄) determination deserves comment. By developing a turbidimetric technique for measuring a stabilized BaSO₄ precipitate in a centrifugal analyzer, they clearly have established a rapid method for serum sulfate determination that alleviates some of the problems associated with manual methods (2).

However, there are deficiencies inherent to all barium-based methods that do not come to light in their report. The first and foremost is the problem of incomplete precipitation from samples containing less than the 400 μmol of SO₄ per liter that they used for their coefficient of variance estimates. Tallgren (3) showed that the coefficient of variance increases exponentially as SO₄ concentrations drop below 300 μmol/L, a value that is close to the mean for sera from normal adults. We had previously arrived at the same conclusion during the development of a microassay based on the 133Ba radioactivity precipitation method of Miller et al. (4). Our solution was to add known amounts of exogenous SO₄ to all samples, establishing that overall variability was significantly reduced by this strategy (5).

A second deficiency that the barium-based assay suffers from is interference by other anions. Pascoe et al. state that phosphate concentrations below 5 mmol/L do not interfere, but we have found that the BaSO₄ precipitation from serum is significantly diminished when phosphate concentrations exceed 2.5 mmol/L (6), which is not unusual in the neonatal period.

To obviate these difficulties, we have turned to an entirely different method of SO₄ determination, which depends on conductivity of the anion after its separation by ion-exchange chromatography (6, 7). This procedure is considerably more sensitive, precise, and specific than the barium-based methods described to date (7–10). Its drawbacks, however, are the time required for analysis (~20 min) and the expense of dedicated instrumentation (Ion Chromatographs, Sunnyvale, CA).

While we take issue with the authors' statement that theirs is the "first method that can measure inorganic sulfate in microscale samples" (cf. ref. 7), we welcome reports of new assays for this important but poorly understood metabolite, especially ones that are well suited for use in the clinical laboratory.

References