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### The Microchromatographic Estimation of Fetal Hemoglobin Levels in Hereditary Persistence of Fetal Hemoglobin

To the Editor:

Abraham et al. (1) measured fetal hemoglobin by a macrochromatographic method in the presence of Hb A. Later (2), this group determined fetal hemoglobin by a microchromatographic method in the absence of Hb A in sickle-cell syndromes, including the condition of Hb S-hereditary persistence of fetal hemoglobin (HPFH). We report the application of microchromatography to measurement of fetal hemoglobin in two other patients with different combinations of HPFH—with and without Hb A. To our knowledge, this is the first report of microchromatographic quantification of Hb F in the presence of Hb A.

Our first patient was an asymptomatic black man whose hemolysate had an Hb CFA pattern by both cellulose acetate and citrate agar electrophoresis (Helena Laboratories, Beaumont, TX 77704). The mean cell volume and Hb were 86 fL and 160 g/L, respectively. The hemoglobin was solubility negative (Dade Division, American Hospital Supply Corp., Miami, FL 33152). The proportion of Hb C and Hb A<sub>2</sub> was 38% (3). The Kleihauer smear (4) had an even Hb F pattern (Bio-Dynamics/bmc, Indianapolis, IN 46250). The fetal hemoglobin by alkali denaturation (F<sub>AD</sub>) was 32.5% and 24.1% by the methods of Singer et al. (5) and Betke et al. (6), respectively. The Hb F by radial immunodiffusion (7, Helena Labs.) was 36% at a 10-fold sample dilution. The Hb F by densitometry (8) was 40%. Microchromatographically, the Hb F was 35.0% and 35.6% on two occasions (2). Concentrates of fast hemoglobin eluates from cation-exchange microchromatography showed a major band with Hb F mobility and minor band with Hb A mobility representing Hb A<sub>1</sub> on cellulose acetate electrophoresis (9).

Our second patient was an asymptomatic Southeast Asian man with a mean cell volume of 61 fL and an Hb of 115 g/L. The Kleihauer smear (4) had an even distribution of Hb F. The cellulose acetate electropherogram had an Hb CF pattern; the citrate agar electropherogram had an Hb AF pattern. Fetal hemoglobin was 37.5% by densitometry (8) and 27.4% by microchromatography (2).

We believe our first patient has a combination of Hb C and a rare type of HPFH with associated beta-chain production (10, 11), whereas our second patient has the Hb E-HPFH condition. We conclude that our data show clinically adequate agreement between the microchromatographic and other methods of Hb F measurement. Both of the above HPFH syndromes are uncommon and, to our knowledge, microchromatographic Hb F determination has not been applied to them before. Finally, the results for our first patient demonstrate the feasibility of microchromatographic Hb F measurement in the presence of a substantial percentage of Hb A.

We thank Dr. P. F. Milner for the gift of blood from our second patient.

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### Day-to-Day Variation in Breath Hydrogen Concentration on Awakening

To the Editor:

Measurement of hydrogen in the breath has become an important diagnostic test for evaluating intra-intestinal carbohydrate metabolism (1). Most commonly, the procedure involves collection of breath samples before and at intervals after an oral dose of a carbohydrate substrate of interest, such as lactose or sucrose. The criterion for incomplete absorption that is most often applied is the increment in breath H<sub>2</sub> concentration above fasting values; an increase exceeding 20  $\mu$ L/L represents a positive test result for malabsorption (2). Because most subjects arrive for their tests after several hours

of activity, the basal H<sub>2</sub> 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 H<sub>2</sub> concentration, with increases throughout the hours of sleep that persist to the first-morning sample (5). The consequences of this non-zero baseline for breath H<sub>2</sub> 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 H<sub>2</sub> concentrations of fasting patients and thereby improve the sensitivity of the interval-collection breath test by increasing the difference between baseline H<sub>2</sub> and post-ingestion concentration of H<sub>2</sub>.

Because early-morning H<sub>2</sub> concentrations seem to respond to the amount of fiber of the previous day's diet, and because the post-waking breath H<sub>2</sub> 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 to collect expired air 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 H<sub>2</sub> analyzer (MicroLyzer Model CM; Quintron Instruments Co., Milwaukee, WI), calibrated with a reference mixture of H<sub>2</sub>: 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" (FMBH<sub>2</sub>) concentration was 24.4 (SD 24.7) μL/L. The coefficient of variation (CV) for FMBH<sub>2</sub> samples from individuals whose overall mean baseline concentration was 10 μL/L (n=9) was 74.3% (SD 45.9%); for those with overall 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 26.0%). The high-low difference for FMBH<sub>2</sub> 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 FMBH<sub>2</sub> as a percent of an individual's three-day mean was 124% (SD 80%,

range 28–300%).

The concentration of H<sub>2</sub> 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 H<sub>2</sub> breath test. Although there is considerable intra-individual variation, subjects can be classified as consistently high or low FMBH<sub>2</sub> producers. Because the H<sub>2</sub> 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 H<sub>2</sub> 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 FMBH<sub>2</sub> is not so large as to preclude its use as a biological marker (perhaps of habitual fiber intake) to *inter*individual and *inter*group 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 changes in diet could be undertaken with the FMBH<sub>2</sub> 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 FMBH<sub>2</sub> is representative for a given individual.

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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 turbidimetry 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 BaSO<sub>4</sub> 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 <sup>133</sup>Ba (2). It is as follows:

**Reagents.** Trichloroacetic acid (TCA), 1.8 mol/L. Standard solutions: sodium sulfate (Na<sub>2</sub>SO<sub>4</sub> · 10 H<sub>2</sub>O), 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 deionized water. Transfer 100 mL of this solution to a 250-mL beaker and add, dropwise, 0.1 mL of Na<sub>2</sub>SO<sub>4</sub> · 10H<sub>2</sub>O, 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.