distinct advantages in terms of participant acceptability and logistical considerations in purely clinical or clinical/epidemiologic environments. A recent observation (6) that concentrations of reduced homocysteine peak 2 h after an oral methionine load may provide an additional rationale for use of this abbreviated protocol.

Potential determinants of postmethionine-load homocysteinemia include B-vitamin status, in particular $B_6$ (7); heterozygosity for CS deficiency (1); and, perhaps, thermolability of methylenetetrahydrofolate reductase (8). The 2-h methionine-loading protocol should facilitate further study of these and other possible determinants of postmethionine-load homocysteinemia.

In conclusion, we have demonstrated that a 2-h postmethionine-loading total plasma homocysteine determination can accurately identify the same individuals who would be considered hyperhomocysteinemic on the basis of their 4-h postmethionine-load total plasma homocysteine concentration.

Supported in part by General Clinical Research Center Grant M01 RR 00054, NIH Grant DK 02120 (R.R.), USDA Contracts 53-3K06-10, and an Arthritis Foundation Clinical Science Grant (R.R.).

References

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Reagent-induced Variation in Specificity of Serum Phenylalanine Assays

To the Editor:

Serum Phe is measured in our laboratory with an automated centrifugal analyzer (Cobas-FARA; Roche Diagnostics Systems, Branchburg, NJ) (1). The method is based on the phenylalanine dehydrogenase (PDH)-catalyzed oxidative deamination of Phe to phenylpyruvate with concomitant consumption of NAD+. In the course of evaluating this method, we identified significant differences in activity and specificity in preparations of PDH obtained from Calbiochem (La Jolla, CA), Biocatalysts (Ponypridd, UK), and Sigma (St. Louis, MO).

The assay calls for 0.35 U of PDH per incubation. We analyzed serum specimens for Phe by using 0.35 U (based on labeled activity) of each of the three PDH preparations and compared the results with values measured by a fluorometric method based on the reaction with copper/ninhydrin reagent (2). PDH from Sigma or Biocatalysts yielded greater apparent Phe concentrations than that from Calbiochem (Fig. 1). The discrepancies were evident as increased y-intercepts: Calbiochem = 1.01 fluorometric + 6 μmol/L; Biocatalysts = 0.99 fluorometric + 79 μmol/L; and Sigma = 1.04 fluorometric + 54 μmol/L (Fig. 1, top three panels).

A potential source of this constant positive bias is cation exchange of amino acids in serum other than Phe. A previous study of PDH specificity had found modest activity with several amino acids (3). To test this hypothesis, we supplemented serum with various pure amino acids and analyzed the resulting solutions with the Sigma PDH preparation. No interference was seen with His (2000 μmol/L), Glu (4000 μmol/L), Trp (600 μmol/L), Ile (1500 μmol/L), Val (4000 μmol/L), or Met (1500 μmol/L), concentrations greatly in excess of the normal concentrations of these amino acids. This preliminary experiment did, however, suggest an increase in apparent Phe concentration when Tyr or Leu was present. To further characterize this effect, we analyzed with each of the three enzyme preparations a pooled serum containing 745 μmol/L Phe, to which increasing concentrations of Tyr or Leu were added. The apparent Phe increased systematically in those assays done with PDH from Biocatalysts (0.36 μmol/L increase per 1 μmol/L Leu; 0.30 μmol/L increase per 1 μmol/L Tyr) and Sigma (0.24 μmol/L increase per 1 μmol/L Leu; 0.06 μmol/L increase per 1 μmol/L Tyr), but to a lesser extent in the assays with Calbiochem PDH (0.02 μmol/L increase per 1 μmol/L Leu; 0.02 μmol/L increase per 1 μmol/L Tyr).

Examination of the time course of reactions showed that 0.35 U of labeled activity of each of the three preparations were not equivalent, despite identical definition of activity units and stated assay conditions that were very similar (all used 30°C and pH 10.5–10.7) for all three manufacturers. When the amounts of Sigma or Biocatalysts PDH added were adjusted so that 90% of the substrate in a specimen containing 1211 μmol/L Phe was consumed in 3 min, determinations made with these lower amounts of labeled PDH activity (0.12 U of Sigma PDH, 0.18 U of Biocatalysts PDH) were in better agreement with the comparison method results (Biocatalysts = 1.01 fluorometric + 42 μmol/L; Sigma = 1.02 fluorometric + 18 μmol/L) (Fig. 1, lower two panels). Use of lesser amounts of Sigma or Biocatalysts PDH also decreased the apparent Phe contributed by Tyr or Leu in experiments in which sera with in-
increasing concentrations of these amino acids were analyzed (data not shown).

These results suggest that Tyr and Leu in serum are measured as Phe to various degrees by the PDH preparations examined here. The increased utilization of Tyr and Leu by the Sigma and Biocatalysts preparations of PDH accounts for the positive biases obtained with these PDH sources. The Calbiochem and Biocatalysts PDH are prepared from Rhodococcus, whereas Sigma PDH is isolated from Sporosarcina ureae. A previous report found substantial reaction of other amino acids with PDH from S. ureae (3), which may explain the reaction with Tyr and Leu by the Sigma PDH preparation seen in our studies. The differences in utilization of Tyr and Leu between the Biocatalysts and Calbiochem PDH preparations might reflect different degrees of purity, such that contaminating activity in the Biocatalysts preparation oxidizes amino acids other than Phe. Of the three sources, only Biocatalysts makes a claim in regard to the specificity of the PDH, stating that reaction with Tyr is <0.5% of that with Phe. Even this low extent of reaction with Tyr can result in substantial interference in end-point clinical assays, where the excess PDH activity added to assure timely, complete reaction also enhances the opportunity of contaminating activity to catalyze side reactions. We urge further effort on the part of manufacturers of PDH to remove contaminating activities or utilize bacterial sources of PDH with minimal cross-reactivity with other amino acids. Until preparations become available that are aimed at (and assessed for) clinical application, users must evaluate independently the actual activity and specificity of PDH preparations they wish to employ.

References

Saliva and Serum Neopterin Concentrations Not Significantly Correlated in HIV-1 Infection

To the Editor:
The pteridine neopterin, derived from guanosine triphosphate (GTP), is released by macrophages after stimulation by interferon-y (1), which is itself secreted by stimulated T lymphocytes. Serum neopterin concentrations increase as HIV disease progresses, probably due to continual activation by antigenic challenge, and thus form a prognostic marker for progression to AIDS (2). Neopterin concentration in other body fluids such as bronchoalveolar lavage fluid is an indicator of macrophage activation (3) and cell-mediated immunity (4).

Neopterin has also been detected in human saliva (5); in one study the concentration was increased in the saliva of patients with HIV-1 infection (6). It was not known if this increase was due to local production or was a reflection of the concentrations of neopterin in the serum.

The aim of our study was to investigate whether any correlation exists between concentrations of neopterin in serum and in saliva. Other clinical details such as whether the patient smoked and whether oral candida was present were also recorded.

We determined the neopterin concentration in saliva by RIA (Henning-Berlin, Berlin, Germany). Saliva excretion was stimulated by chewing for 1 min on an inert plastic film and the sample collected into a sterile container. Participants in the study were 9 adults with no known risk factors for HIV infection or known to be HIV antibody-negative (control subjects), and 31 HIV-positive subjects, 19 with AIDS and 6 with oral candida. Ethical approval for the project was obtained.