Addition of 3-Deazaadenosine to Vacutainer Tubes Stabilizes Whole-Blood Homocysteine for At Least 6 Hours at Ambient Temperature

To the Editor:

Increased plasma homocysteine is now well established as a risk factor for cardiac, cerebral, and peripheral vascular disease (1). Despite this, the test is not widely available. This is partly because of practical difficulties in sample collection. The homocysteine concentration increases in whole blood by up to 10% per hour unless samples are kept on ice and separated within 60 min.

The introduction of containers with 3-deazaadenosine (3-dad; DS30 Homocysteine Blood Collection Tubes; Drew Scientific Ltd.), described in a recent report (2) in the Journal, may solve the problem, but they are not in widespread use. Furthermore, we feel that stabilization that requires both special tubes and refrigeration of samples has limited practical value, as transportation (which is unrefrigerated) to the laboratory usually requires 2–6 h, at least in the UK, making the samples unsuitable for homocysteine analysis. This problem can be overcome by preparing tubes that stabilize homocysteine by the simple expedient of injecting 3-dad (3), an inhibitor of S-adenosylhomocysteine hydrolase, into standard Vacutainer Tubes. Samples sent to the laboratory in these tubes can then be separated and the plasma sent to a specialist center for homocysteine analysis. Homocysteine in plasma is stable for several days at room temperature.

We injected 40 µL (10 mmol/L) of 3-dad (Sigma Chemical Company) into 4.0-mL dipotassium EDTA Vacutainers (Becton Dickinson Vacutainer Systems) with an insulin syringe and fine needle. This procedure does not break the vacuum. Control tubes were prepared with 40 µL of saline (150 mmol/L). Blood from 10 healthy volunteers was collected in the modified Vacutainers (two saline and two 3-dad tubes from each volunteer). Saline Vacutainer samples were immediately placed on ice and separated within 60 min or incubated at room temperature for 6 h before separation. 3-dad Vacutainer samples were placed on ice and separated after 6 h or incubated at room temperature for 6 h before separation. Total plasma homocysteine was measured after derivatization with ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBDF) and separation of thiols by isocratic reversed-phase HPLC (4).

Plasma homocysteine concentrations in samples collected into saline and left to stand at room temperature for 6 h showed a mean (SD) increase of 3.2 (0.9) µmol/L compared with samples separated immediately (P < 0.01, Mann–Whitney). In contrast, samples collected into 3-dad showed no increase in plasma homocysteine concentration over 6 h whether the samples were left at room temperature [−0.4 (0.3) µmol/L; P = 0.76] or incubated on ice for 6 h [−0.3 (0.9) µmol/L; P = 0.90].

The process described is a simple and practical means of preparing tubes for homocysteine estimation that prevents the marked in vitro increase seen in unseparated blood. It requires no special equipment, and tubes can be prepared as and when required. It should be noted that blood collected in this way is unsuitable for analysis by assays that convert homocysteine to S-adenosylhomocysteine (5).

References