Letters to the Editor should be typed double-spaced (including references) with conventional margins. The overall length is limited to five manuscript pages, including not more than one figure or one table.

Evaporative Loss from Small Samples

To the Editor:

We read with interest the observations of Burtis (1) regarding possible sources of analytical error from sample evaporation in the "Dimension 380" (Du Pont, Wilmington, DE 19803) selective-access analytical system. In a similar series of experiments, conducted with the Chem-1 analyzer (Technicon Instruments Corp., Tarrytown, NY 10591), a system that uses similar sample cups and anti-evaporation caps, we confirm the findings of Burtis for 0.5- and 2.0-mL sample cups. However, because the Chem-1 has the capability to analyze pediatric samples, requiring <50 μL of serum, we extended our study to include 0.25-mL cups.

The extent of evaporative loss for various volumes of water pipetted into capped and uncapped cups is shown in Table 1. For uncapped samples, even for cups containing the full volume of 0.25 mL, a significant loss occurs after 1 h. Furthermore, for samples <100 μL, even when capped, exposure to the atmosphere for <30 min can result in a bias greater than the total imprecision expected for many assays in the Chem-1 (2).

In our experience, samples of <100 μL are common from pediatric sources, and the capability of the Chem-1 to analyze samples of this amount is useful. At the moment, we circumvent this high rate of evaporative loss by analyzing these samples at the beginning of a run and covering them immediately after sampling, in case they are required for re-run.

This possible analytical error is inherent in any system that uses 0.25-mL sample cups. Given the trend to design analyzers that require less and less sample, we expect that bias due to evaporative loss will become more widespread. The only satisfactory solution seems to be a closed-sample handling system, which would not only alleviate this problem but also contribute significantly to the confinement of hazardous specimens.

References

Stephen P. Harrison
Ian M. Barlow
Dept. of Biochem.
Bradford Royal Infirmary
Duckworth Lane
Bradford, BD9 6RJ, U.K.

Simplified Procedure to Remove Radiolabeled Contaminants after [3H]Norepinephrine Infusions

To the Editor:

The measurement of apparent norepinephrine kinetics by the use of infusions of [3H]norepinephrine as first described by Esler et al. (1) is commonly used as a more reliable index of sympathetic function than a measurement of the norepinephrine concentration in plasma. In this procedure norepinephrine was extracted from plasma with alumina for the measurement of specific activity, based on the assumption that norepinephrine is the major tritiated component. This assumption was verified by reverse isotope-dilution analysis in the original report, but Howes et al. (2) recently suggested that the metabolite 3,4-dihydroxyphenylethyylene glycol (DHPG) could also contribute significantly to the extracted radioactivity. Subsequent investigation found a contaminant in commercial [3H]norepinephrine, which accumulates in plasma during infusions (3, 4). Consequently, more complex procedures were developed for the purification of plasma norepinephrine, such as radiochromatography on HPLC or combine alumina and cation-exchange extraction (5), both of which make the analysis more difficult and expensive.

Recently, Maycock and Frayn (6) described a study of methods for extracting catecholamines from plasma before analysis by HPLC with electrochemical detection. They conclude that washing the alumina with sodium carbonate before acid elution of catecholamines produced an extract comparable with that obtained by combined alumina and cation-exchange extraction. We found this procedure to be suitable in our plasma catecholamin assay and decided to investigate whether it would also suffice to remove radioactive contaminants from norepinephrine extracts.

For the infusion we used an oil stock of [3H]norepinephrine that contained about 7% impurities. We mix 2 mL of plasma samples with 4 mL of 1 g/L EDTA solution and 1 mL of mol/L Tris·HCl buffer (pH 8.6) containing 20 g of EDTA per liter. We then added 100 mg of acid-washed alumina and shook this in disposable 10-mL columns (Econocolumns; BioRad, Richmond, CA) for 15 min. The columns were then placed in a rad and allowed to drain. The retained alumina was washed twice with 1 mL of distilled water. We then washed half the samples with 10 mL of freshly prepared solution of 200 mmol of sodium carbonate, 5 mmol of EDTA, and 5 mmol of sodium metabisulfite per liter and then made a further two washes with water. After removing residual water by applying gentle air pressure, we used 200 μL of perchloric acid to the alumina (0.3 mol/L was used for the carbonate-washed alm...