ments suggest that at physiological concentrations, only urate contributes to the artificially high pre-dialysis values. When a standard serum sample was increased in concentration (in vitro) from 11.6 to 54 mmol/L in urea, from 97 to 1130 mmol/L in creatinine, and from 0.31 to 1.38 mmol/L in urate, values for apparent SFA were unaffected by urea or creatinine changes, but rose 13% in the presence of the additional urate. However, in vivo, there was no direct correlation between the extents of urate and SFA reduction, suggesting that other interfering compounds also contribute to the discrepancy. Among nondialyzable compounds suspected are carbamylated proteins produced by cyanate (5).

We conclude that urate and other compounds artificially increase SFA values in renal-disease patients. During hemodialysis, apparent SFA decreased in 75% of patients tested, sometimes by >20%. Both these facts should be taken into account when the SFA test is used to monitor glycemic control.

References


The RA-1000 random access analyzer (Technicon Instruments Corp., Tarrytown, NY 10591) dispenses reagent and sample by use of stepper motor-driven syringes and Teflon probes. The probe assembly has no sensing reagent to detect resistance to motion, so probes are damaged when the motors drive the probe into (e.g.) misaligned reagent containers or sample tray covers inadvertently placed in their path. The damaged probe must be replaced at considerable expense (e.g. AU$343 for a sample probe, AU$269 for a reagent probe) although in most cases only the Teflon tubing in the probe is damaged. We report here a simple method for reconstructing RA-1000 probes by replacing the Teflon tubing. This procedure, when used with the probe-cleaning procedure reported elsewhere (1), results in probes functionally equivalent to those commercially available.

First remove the damaged sample probe tubing by pulling from above. Stretch a length of thin-wall Teflon tubing (size AWG24, thin wall FT24 natural; Atlantic Tubing Co., Paterson, NJ) until it is thin enough to fit down the Teflon-coated metal casing of the probe, then cut it at its thinnest point with a sharp scalpel blade and thread it down the probe casing from the top of the probe body so that the stretched tubing protrudes from the probe tip. Gently pull the tubing through the casing until unstretched tubing appears at the probe tip. To allow for some end-to-end shrinkage of the tubing, do not cut the probe tubing to length immediately, but leave it untrimmed until the probe is needed; at that time trim it with a sharp scalpel blade, leaving ~1 mm of tubing protruding from the probe casing. Adjust the probe length by releasing the retaining screw that holds the casing and move the casing until the probe tip sits just off the bottom of a reaction cuvette when the probe body is resting on top of the cuvette.

To rethread the reagent probe with thin-wall Teflon tubing (size AWG19, thin wall FT19 natural; Atlantic Tubing Co.), remove the damaged tubing by pulling from below, and stretch and rethread the new tubing through the probe body from below until a firm fit is obtained. Trim the top of the probe flush with the top of the probe body. Adjust the length of the probe so it is just off the bottom of the reaction cuvette when the probe body is resting on the top of the cuvette, then trim it with a sharp scalpel blade. To bore the necessary air-inlet hole through one wall of the tubing, insert a 0.55-mm drill bit along the metal nipple in place on the probe body and turn the bit manually. Remove any burrs on the tubing by inserting a 0.85 mm drill bit into the bore of the tubing and gently turning it manually.

It is essential that the newly made probe be thoroughly cleaned of all residues so that the inert perfluorohydrocarbon (Technicon's "Random Access Fluid," or "Fluorinert" PC-43 (3M Australia)) will form a continuous coating on the inner and outer surfaces of the probe. The ultrasonic cleaning procedure (1) is effective in this regard. Because this method of repair is only attempted when the probe body itself has not been damaged, and because the tubing used matches the manufacturer's product in chemical nature, diameter, and length, we expected (and found) the repaired probes to be functionally equivalent to the original probes (documentation available on request).

References


Lippi et al. (1) proposed a new chemical procedure for separation of high-density-lipoprotein cholesterol (HDL-Chol) from serum, and demonstrated (2) the effectiveness of their procedure for precipitating LDL and VLDL with negligible co-precipitation of HDL, either in normal or in hypertriglyceridemic sera (up to 25 mmol of serum triglycerides per liter). These results were confirmed by Baars et al. (3). HDL can be separated by use of a common diluter (0.1 mL of serum and 1.0 mL of precipitation mixture) and HDL-Chol is measured in (e.g.) the Technicon "RA-1000" (4). The 11-fold dilution of samples requires that the reagents for assay of HDL-Chol in supernates be very sensitive and that the analyzer perform well.

We report results obtained with the "Chem I" system random-access analyzer (Technicon Corp., Tarrytown, NY) for determining HDL-Chol in supernates after precipitation of LDL and VLDL by the method of Lippi et al. (2). Table 1
summarizes the linearity and within-run imprecision of HDL-Chol determinations in diluted control serum and in pooled supernates at different concentrations of analyte. Between-run imprecision was evaluated from 22 determinations done on the preceding samples. The CV are <2.0%. The comparison between 40 cholesterol determinations in the Chem I and in the RA-1000 show differences that were insignificant by Student's paired t-test and yielded the following regression equation: \( y = 1.0065x + 0.0738 \) mmol/L \((r = 0.9956)\).

Thus, the "Chem I" performs well for estimating HDL-Chol in very low concentrations. HDL-Chol can be determined rapidly in randomly sequenced samples or in "batch," with no change in the instrument setting usually seen in determination of serum cholesterol. The results of course must be multiplied by the dilution factor (here, 11).

References

Radioenzymatic Assay for Simultaneous Estimation of Dobutamine and Endogenous Catecholamines, James F. Padbury, Alma M. Martinez, John K. Ludlow, Christopher W. Evans, and Siang L. Thi0 (UCLA School of Med., Dept. of Pediatrics, Harbor-UCLA Med. Center, Torrance, CA 90509)

Dobutamine is a useful inotropic agent with a unique pharmacological profile compared with alternative agents (1, 2). There are few pharmacokinetic data describing its use, because of the poor sensitivity of previous assays for it in plasma. Moreover, dobutamine pharmacokinetics and dynamics may differ in different clinical situations (3).

Here we describe a modification of a radioenzymatic assay for dobutamine in plasma, which also permits simultaneous measurement of catecholamines in plasma.

Dobutamine (DBX) and 3-O-methyldobutamine (3-DBX) were kindly provided by Eli Lilly Pharmaceuticals, Indianapolis, IN 46285. We used, with several modifications, an enzymatic method involving catechol-O-methyl transferase (COMT, EC 2.1.1.6) purified from adult rat liver (4). Norepinephrine (NE), epinephrine (E), and dopamine (DA) standards were prepared in catecholamine-free plasma in concentrations up to 1 ng/mL. Dobutamine was added to a maximum of 100 ng/mL. Before thin-layer chromatography of the reaction products, a 25-µg aliquot of 3-DBX was added along with standard markers. To improve separation of the 3-0-DBX from the solvent front, we pre-ran the plates in equim Graberform/methanol and dried them, then applied the samples and developed them in chloroform/ethanol/ethylamine (80/25/7.5, by vol).

For assay of NE and E, the spots corresponding to their tritiated methyl derivatives were peridilated before the radioactivity was counted. For assay of dopamine and dobutamine, the spots corresponding to 3-methoxytyramine and 3-0-DBX were eluted into 50 mmol/L ammonium hydroxide, back-extracted into toluene and isomyl alcohol, and mixed with scintillation fluid before the radioactivity of all samples was counted in a liquid scintillation spectrometer (efficiency 35%). The 3-O-methyl derivatives of endogenous catecholamines and dobutamine were all well resolved. Rf values for each were: normetanephrine 0.44, metanephrine 0.53, 3-methoxytyramine 0.75, and 3-0-DBX 0.81. There was a dose-dependent, linear incorporation of the tritiated methyl label into each substrate \( r = 0.99, P < 0.001 \). Inter-assay and intra-assay CVs were 10.5% and 8.7%, respectively. The methylation index and the sensitivity of the assay were as follows:

Methylation index* = 6.7 ± 0.2 9.2 ± 0.2 15.5 ± 0.3 8.5 ± 0.06 Sensitivity, pg/mL: 100–125 20–40 20–40 100–200

*Count/min per picogram of added standard base. Defined as 1.5 times the blank value.

Analytical recovery of dobutamine from plasma of volunteers anticoagulated with either EDTA (4 mmol/L) or ethylenebis(oxymethylene)tetraacetic acid (4 mmol/L) and reduced glutathione (3 mmol/L) was 102–110%, vs 87% in heparin. The interference of DBX, 100 ng/mL, with the actual value of each plasma catecholamine was as follows: norepinephrine 0.3 ± 0.2%, epinephrine 0.3 ± 0.2%, dopamine 0.8 ± 0.4%. Isoproterenol or alpha-methyltyrosine added at a final concentration of 1 ng/mL caused 1.1 ± 0.3% and 0.2 ± 0.2% interference, respectively, with DBX. Plasma DBX concentrations measured in critically ill newborn infants receiving 2.5–7.5 µg per kilogram body weight per minute ranged between 20 and 100 ng/mL and showed first-order clearance kinetics.

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References