More on Sample Protein
Concentrations and Measurements
of Potassium and Sodium in Serum

To the Editor:

Shyr and Young recently published a letter concerning falsely low values for sodium and potassium obtained when using flame photometry including sample dilution (Clin. Chem. 26: 1517, 1980). I have found that falsely low values occur when using the IL-144 auto-
dilutor and aqueous calibrating solutions, mostly because of differences between serum and calibrator viscosity, i.e., the calibrators more easily pass through the IL-144 than the samples do. However, if you predilute the samples and the calibrators manually, there will be no measurable differences. Thus we always adjust the viscosity of the calibrators to the average serum viscosity of 2.0 cp by means of propane-1,2-diol.

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Suspected Impurities in
Imipramine and Desipramine
Standards and Pharmaceutical
Formulations

To the Editor:

A primary consideration in therapeutic drug monitoring is the primary standard used in the analysis. Clinical laboratories usually obtain these standards from sources such as pharmaceutical companies, in formulations, tablets, or injections, and base their drug content on information found on the label. Often, drug standards are obtained from the United States Pharmacopeia Convention, Inc., which distributes United States Pharmacopeial and National Formulary (USP-NF) Reference Standards.

The method of Narasimhachari et al. (1) is used in this laboratory to measure tricyclic antidepressant drugs. It is rapid, both the tertiary amine and its secondary amine metabolite being determined simultaneously, and it is less complicated than the chemical-ioniza-
tion methods in that the electron impact (EI) mode of the gas chromatograph/mass spectrometer (GC/MS) can be used.

When setting up this method in our laboratory, we noticed that our supposed pure imipramine working standard was contaminated with both desipramine and iminodibenzyl. The same was true for imipramine tablets and USP-NF Reference Standards of imipramine. Similarly, desipramine standards were contaminated with imipramine and iminodibenzyl. We then decided to analyze a series of imipr-
amine and desipramine pharmaceutical formulations prescribed to patients, as well as standards obtained from different sources.

Samples of imipramine and desipramine standards (as hydrochlorides) were obtained from the hospital pharmacy from current lots and deuterated (D4) imipramine, desipramine, and imin-
dibenzyl were prepared from them in our own laboratory by standard exchange procedures (2), in 99% isotopi-
cally pure form.

Samples of standard hydrochlorides were dissolved in methanol to contain 2 g of base per liter. We powdered and suspended 25-mg tablets in 10 mL of water and, after disintegration (30 min), diluted the solutions to 100 mL with water.

For GC/MS determinations we used a Hewlett Packard 5985A or a Finnigan 4000 GC/MS data system. We used 0.9 m (2 mm i.d.) nickel column, packed with 3% SP-2250 on Supelcoport (80/100 mesh), with a helium flow of 30 mL/min. Column temperature was 200 °C for 1 min, programmed to 250 °C at 10 °C/ min. All contaminant compounds were identified by obtaining the total mass spectrum in the EI mode. Quantitative determinations were made by the selected ion monitoring technique with use of both electron impact and meth-
ane chemical ionization methods.

An aliquot containing 100 ng of sample from the stock solution was added to 1 mL of water, and 5 µg of D4-desipra-
mine was added as internal standard to imipramine sample, 5 µg of D4-imipra-
mine was added to the desipramine sample. Five microliters of D4-iminodibe-
zyl was added to both imipramine and desipramine samples.

A calibration curve for iminodibenzyl was obtained by using from 10 ng to 100 ng of iminodibenzyl and 100 ng of D4-iminodibenzyl, and monitoring mo-
olecular ions m/e 195 and 199 on the GC/MS system in the EI selected ion monitor mode. Methanolic solution of imipramine and desipramine standards, aqueous methanolic solutions of tablets, or parenteral preparation were directly injected into the GC/MS system.

From the areas under the peaks of ion current for the sample and internal standard, the quantity of iminodibenzyl in the sample was calculated by standard procedure. Iminodibenzyl in plasma from patients being treated with imipramine or desipramine can be simi-
larly determined after alkaline extraction (1) with 1 µg of D4-iminodibenzyl used as internal standard per 2 mL of plasma sample.

We added 5 µg of D4-desipramine as internal standard to solutions of imipramine samples containing 100 µg of imipramine. After extraction at alkaline pH, the trifluoroacetyl derivative was prepared according to the method of Narasimhachari et al. (1). Ions m/e 208 and 212 were monitored for selected ion monitoring determinations in the EI mode.

Methanolic solutions of desipramine containing D4-imipramine as internal standard were injected directly into the GC/MS system and ions m/e 234 and 238 were monitored.

The EI spectra of iminodibenzyl, imipramine, and the trifluoroacetyl derivative of desipramine found as con-
taminants in the sample were identical in all respects with the spectra of au-
thentic standards. Table 1 shows that all working standards, including the sample obtained from the USP-NF, contained contaminants. The concentration of iminodibenzyl in the samples ranged from 0.2 to 2.8%, of desipramine in imipramine samples from 0.5 to 3.2%, and imipramine in desipramine samples from 0.9 to 4.0%. On recrystallization of an impure sample of imipramine, the percentage of contaminating iminodie-
benzyl and desipramine was considerably lessened (Table 1).

Evidently, samples of imipramine and desipramine in pharmaceutical formulations and working standards contain the basic nucleus, iminodibenzyl, as a major contaminant. Further, formulations of imipramine contain desipra-
ine, and vice versa. While there are no specific limits on these impurities in the U.S. Pharmacopeia, there are guidelines concerning the overall purity of the preparation itself. Indeed, the methodology specified by the USP-NF would not detect these impurities in the concentra-
tions found. Our findings have an effect on the interpretation of results. For instance, the small concentrations of iminodibenzyl found in plasma sam-

mulations stored in the refrigerator (4 °C) showed no notable changes in composition after 30 days.

References


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Lactate Dehydrogenase and Creatine Kinase isoenzymes in Human Vitreous Humor

To the Editor:

After death the vitreous humor of the eye is less susceptible to rapid chemical changes than blood. This vitreous fluid, from autopsy material, is used for several different chemical assays to establish possible cause and time of death (1).

In our laboratory the method of Coutselinis et al. (2) was used to obtain 41 specimens of vitreous humor from autopsy material (average: 10–20 h after death). The carefully preserved material (4 °C) was analyzed as quickly as possible for several constituents. Mean protein concentration, determined by the sulfosalicylic turbidimetric method (3), was 350 mg/L. Enzymes were measured in 20-fold concentrated fluid (Amicon System B15, Amicon Corp., Lexington, MA 02173). Lactate dehydrogenase (LD; EC 1.1.1.27) ranged from 50 to 140 U/L (average 101 U/L). Creatine kinase (CK; EC 2.7.3.2) ranged from 135 to 210 U/L (average 172 U/L). Isoenzymes were assayed with a Corning Electrophoresis System (Corning Medical Co., Palo Alto, CA 94304) and evaluated with a Turner Thrombogrammeter. CK isoenzymes showed the following average distribution (hitherto unknown): LD1 22%, LD2 30%, LD3 32%, LD4 11%, LD5 5%. CK isoenzymes showed the following pattern: CK-MM 3.5%, CK-MB 0, CK-BB 96.5% (the fluid consisted almost exclusively of CK-BB fraction).

We also examined cow eye vitreous humor and found some differences: protein was 450 mg/L (average of 30 specimens), and all three CK isoenzymes were present in the fluid.

References


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Effect of Hyperlipidemia on Homogeneous Enzyme Immunosassay for Thyroxine

To the Editor:

Because enzyme activity may be altered by hyperlipidemia (1, 2), we wondered whether results of enzyme immunoassay (EMIT) procedures, which are based on measuring enzyme activities [notably glucose-6-phosphate dehydrogenase (G-6-PDH, EC 1.1.1.49), and malate dehydrogenase (MDH, EC 1.1.1.37)] are affected by hyperlipidemia. We used EMIT kits (all lot no. J02; Syva Corp., Palo Alto, CA 94304) to determine thyroxine (T4) according to the supplier’s instructions.

A portion of each of 28 lipemic samples was centrifuged at 100,000 × g for 15 min in an “Airfuge” (Spinco Division, Beckman Instruments, Inc., Palo Alto, CA 94304) to clear the lipoprotein. Portions of each sample before and after centrifugation were analyzed simultaneously for total cholesterol, triglycerides, and T4. Total cholesterol and triglycerides were determined by continuous-flow analysis (SMAC; Technicon Instruments Corp., Tarrytown, NY 10591); T4 was determined by the EMIT method. Aliquots of the same samples were sent to BioScience Laboratories, Van Nuys, CA 91405, to be assayed for T4 by a radioimmunoassay method.

Twenty hyperlipemic samples were divided into two lots. One lot was centrifuged (100,000 × g, 10 min, room temperature). Equal amounts of MDH (pig heart) were added to 1.0-ML aliquots of the centrifuged and uncentrifuged sera, followed by equal amounts of G-6-PDH (bakers' yeast; Sigma Chemical Co., St. Louis, MO 63178). Each serum sample was assayed for MDH and G-6-PDH activities with use of enzyme kits from Sigma.

In the MDH assay, 2.5 mL of potassium phosphate buffer (0.1 mol/L, pH 7.5) and 0.1 mL of serum were pipetted directly into a vial containing NADH. After mixing, the vial was left at 25 °C for 20 min, 0.1 mL of 1.0 g/L oxalate acid solution was added, and the contents of the vial were mixed by inversion and absorbances were measured at 340 nm at 30-s intervals for 3 min. The rate of decrease in absorbance (ΔA/min) was used to quantitate MDH activity.

In the G-6-PDH assay, 1 mL of reconstituted G-6-PDH reagent (NADP, 8.2 μmol, and maleimide, 66 μmol in 5.5 mL of water) was added to each cuvette, 10 μL of serum sample was added and, after thorough mixing, the cuvette was allowed to stand for 5 to 10 min at room temperature. G-6-PDH substrate solution (1.05 mmol of d-glucose-6-phosphate) was added and the cuvette was placed in a 37 °C heating block for about 5 min, to attain thermal equilibration. The initial absorbance of each cuvette was measured at 340 nm vs a dichromate blank. Exactly 5 min later, the final absorbance was read. The rate of increase in absorbance (ΔA/min) at 340 nm was used to quantitate G-6-PDH activity.

All absorbance measurements were made with a Stasar III Spectrophotometer (Gildorf Instrument Laboratories, Inc., Oberlin, OH 44074).

Initially, a few specimens with triglyceride concentrations of 2.50–7.00 g/L were used in determining T4 by EMIT before and after ultracentrifugation of these sera as described. Although significant decreases in triglycerides resulted, T4 values by EMIT were not altered significantly—nor were T4 values by radioimmunoassay (Table 1).

For samples with higher triglyceride concentrations (≥7.00 g/L) the effect of ultracentrifugation was generally to lower triglyceride values by 45 to 80% and increase EMIT T4 values by 10 to 120%; one serum showed an increase in T4 of 870% (Table 2). The increase in EMIT T4 values exceeded 50% in 11 of 15 samples with an original triglyceride value >7.00 g/L. T4 as determined by RIA in these sera also was unchanged after ultracentrifugation (p <0.001).

After ultracentrifugation of these lipemic specimens, thyroxine values by EMIT significantly increased, and are then comparable to those obtained by RIA. The change in serum thyroxine result of ultracentrifugation was unrelated to concentrations of triglycerides or cholesterol in serum or to changes in triglyceride or cholesterol concentrations as the result of ultracentrifugation.

The effect of hyperlipemia on the T4 test by EMIT as observed here and as