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 sulfosalicylate 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. LD isoenzymes showed the following average distribution (highestero 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 Immunoassay 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 (potably 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 x g for 15 min in an “Airfuge” (Spinco Division, Beckman Instruments, Inc., Palo Alto, CA 94304) to clear the lipids, and 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 x 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 kept at 25°C for 20 min, 0.1 mL of 1.0 g/L oxalacetic acid solution was added, and the contents of the vials were mixed by inversion and absorptions were measured at 340 nm at 30-s intervals for 3 min. The rate of decrease in absorbance (ΔA/min) was used to quantify 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 Staar III Spectrophotometer (Gildrop 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 hyperlipidemia on the T4 test by EMIT as observed here and as