A Nonenzymic Reaction That Interferes with Measurement of Serum Aspartate Aminotransferase Activity at pH 6

To the Editor:

A low pH is used for one of the rate measurements in the differential kinetic assay (by absorbance at 340 nm) of the isoenzymes of aspartate aminotransferase (AAT; EC 2.6.1.1) (*1*). During early experiments directed towards improving the performance of this procedure we observed that progress records at pH 6 were often severely curved, such that the rate of change of absorbance became progressively slower during the first few minutes. The effect, which varied greatly from one serum sample to another, was sometimes severe enough that one was obliged to wait for several minutes before the rate became stable.

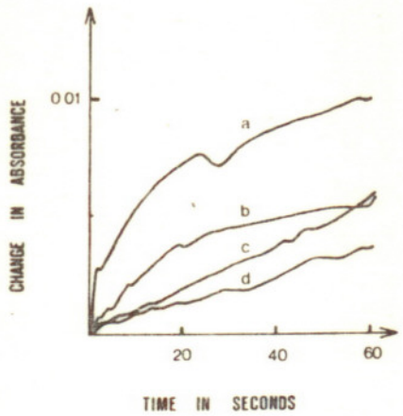

Fig. 1. Change of absorbance at 340 nm

Curves taken directly from recordings made with an LKB 2086 Reaction Rate Analyzer, beginning at the moment of adding the second substrate. The same serum sample was used throughout and was selected as a striking example of the phenomenon. Readings were not all made on the same day. Conditions were: pH 6.0, 37 °C, 33 mmol/L sodium phosphate buffer, L-aspartate (where present) 5 mmol/L, 2-oxoglutarate (where present) 20 mmol/L; malate dehydrogenase 1.0 U, NADH (where present) 0.1 mg, serum 0.2 mL, in a final volume of 1.2 mL. Concentrations quoted are final concentrations. The reaction mixture was incubated at 37 °C for at least 7.5 min before the second substrate was added, then mixed and recordings initiated automatically. (*a*) 2-oxoglutarate added last; (*b*) same as *a*, but NADH omitted; (*c*) L-aspartate added last (NADH present); (*d*) same as *a*, but serum sample dialyzed for 24 h vs distilled water before the test

This interference was traced to a reaction between 2-oxoglutarate (or conceivably a contaminant) and a dialyzable serum component, giving a total absorbance change of as much as 0.01 *A* and showing a half-life of about 20 s (Figure 1). Malate dehydrogenase (EC 1.1.1.37) is not involved, because serum ultrafiltrate shows the effect (not illustrated).

In practice, the interference can be eliminated by pre-incubating the sample with 2-oxoglutarate for a sufficient period (say, 8–10 min) and, accordingly, this should always be done when conducting such measurements at pH 6. It follows that L-aspartate should be the substrate added last, to start the reaction—the opposite of common practice with assays of total AAT at pH 7.4–8.0. In principle, correct results can be obtained by adding 2-oxoglutarate last and waiting several minutes for a stable rate, but this is time-wasting and diminishes confidence in the procedure—and it is then difficult to arrange automation.

No such effect was observed at pH 7.4 (however, a much slower reaction might not be detected). Attempts to identify the serum component that is responsible have been unsuccessful.

There was no indication of instability of NADH at pH 6 under these conditions. The observations reported here suggest one way in which a false impression of instability might be created. These observations may also account for past disappointments in the performance of the differential kinetic assay procedure when applied to serum: the interfering reaction would appear as a high and variable blank, making it difficult to obtain reproducible results, or even to detect the mitochondrial isoenzyme reliably at normal activities. A new modification of the differential kinetic assay, to be published soon, behaves reliably under field conditions.

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Reference

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