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Calibration of 3-Hydroxybutyrate Assays

To the Editor:

McMurray et al. recently described (1) an automated, enzymatic method for 3-hydroxybutyrate in plasma or serum for the Hitachi 705 or Gilford 103 analyzers, using kinetic methodology similar to that described by Custer et al. (2) for centrifugal analyzers. The description of standard preparation was slightly confusing and warrants a comment in light of the fact that, as we have pointed out previously (3), several authors (2, 4, 5) have incorrectly described standardization of kinetic 3-hydroxybutyrate assays.

All enzymic methods for 3-hydroxybutyrate are based on that of Williamson et al. (7, 8), developed in 1962 after isolation of a soluble, reasonably pure preparation of 3-hydroxybutyrate dehydrogenase from Rhodopseudomonas spheroides. In their original assay and most early modifications, the reaction was allowed to go to completion (equilibrium) and the 3-hydroxybutyrate concentration in the biological specimen was calculated from the total increase in absorbance at 340 nm. More recently, several automated kinetic assays, which are calibrated by comparing the rate of increase in 340-nm absorbance for an unknown with that for a standard, have appeared for instruments used in clinical laboratories. However, assignment of the 3-hydroxybutyrate concentration in the standard has caused problems, in that 3-hydroxybutyrate is a chiral (optically active) compound with two enantiomers (optical isomers), (+)-3-hydroxybutyrate and (-)-3-hydroxybutyrate. Only the (-) enantiomer is found to any extent in mammal, and only it is oxidized by the 3-hydroxybutyrate dehydrogenase from R. spheroides. However, optically pure (-)-3-hydroxybutyrate is commercially unavailable, and all standards for the kinetic assays have been racemic sodium D,L-3-hydroxybutyrate, a 50:50 mixture of sodium D(-)- and L(+)-3-hydroxybutyrate. McMurray et al. (1) state that the sodium salt of racemic D,L-3-hydroxybutyrate was 41% D-isomer. This is incorrect; the figure 41% accounts not only for the inactive L-isomer but also for the sodium weighed into the solution. They go on to describe, correctly, weighing 307 mg of D,L-3-hydroxybutyrate standard. Thus, their assay is standardized correctly.

An additional note of caution in calibrating a kinetic, enzymic method for a chiral metabolite with a racemic D,L-mixture: in some cases, the non-substrate enantiomers may inhibit the enzyme. In such cases, a given weight concentration of racemic standard actually gives less than half the reaction rate that the same concentration of optically pure standard gives (3). Preuveneers et al. (9) carefully investigated whether L(+)-3-hydroxybutyrate was a significant inhibitor for the R. spheroides 3-hydroxybutyrate dehydrogenase and found it was not. Thus, L(+) enantiomer inhibition is not a problem in standardization of D(-)-3-hydroxybutyrate kinetic enzymic assays.

References

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