A problem with enzymatic determination of free fatty acids in rat and mouse blood

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Miles et al. (1) have described a sensitive and relatively economical method for assaying free fatty acids in human plasma which involves fluorometric measurement of changes in NADH. A nearly identical method was independently developed in this laboratory but serious problems arose when the procedure was applied to serum and plasma from rats. Rat and mouse serum and plasma, when mixed with substrates and enzymes needed for the fatty acid assay, cause a rapid decomposition of NADH. The rate of decomposition is often much greater than the rate of the intended enzymatic reaction. Initial attempts to modify the assay to eliminate this problem were unsuccessful. These included changing the buffer to phosphate or triethanolamine, varying pH between 7.5 and 8.0, altering concentrations of reagents and enzymes, using different grades of lactate dehydrogenase, waiting until decomposition stopped, 5-min heating of serum in boiling water, adding fluoride, and preincubating of serum in NaOH. Interference could be eliminated by preliminary extraction with heptane–isopropanol (2) but the results obtained were highly variable.

It was discovered that preincubation of samples with HCl reduced the rate of NADH decomposition by rat and mouse serum to approximately the same rate measured in serum- or plasma-free standards. Three experiments were conducted to validate this method. Serum samples (5 μl) from rats were incubated with 50 μl of 0.3 N HCl for 5–20 min, neutralized with 30 μl of 0.5 N NaOH, and assayed using the reaction system described by Miles et al. (1). The only significant difference between the assay used in this laboratory and the one reported by Miles et al. is the smaller reaction volume (0.6 ml) in accord with the recommendations of Shimizu et al. (3).

The first two experiments attempted to determine whether acid incubation altered fatty acid values. In the first, aliquots from two pooled rat serum samples (one from Charles River CD rats and one from inbred OM rats) were incubated with HCl for 5, 10, or 20 min, neutralized, and assayed. The samples incubated for 5 and 10 min gave identical results (mean of 0.34 mM), while the samples incubated for 20 min gave a slightly higher value (0.37 mM), presumably due to acid hydrolysis of triglycerides upon prolonged incubation. In contrast, samples not treated with acid gave values greater than 2.6 mM even when coenzyme A (needed to initiate the intended reaction) was eliminated from the reaction mixture. In the second experiment, a pooled rat serum sample was assayed in duplicate by a titrimetric method (3) with and without prior acid treatment and neutralization. The acidified samples gave a value of 0.298 ± 0.0028 (mean ± SE) mM which was not significantly different from the value of 0.282 ± 0.034 mM given by the nonacidified sample. It may be concluded that 5–10 min of acid treatment does not greatly alter fatty acids.

As a final test, 16 samples of rat serum were assayed by both the titrimetric method and the acid-enzymatic method. The serum samples were obtained from rats subjected to a variety of treatments designed to maximize variability between animals. The two methods correlated highly (r = 0.993) but the acid-enzymatic method gave higher values; means and standard deviations (variability between rats) for the titrimetric method were 0.416 ± 0.319 and for the acid-enzymatic method were 0.438 ± 0.267. Duplicates for the acid-enzymatic method correlated well (r = 0.97) indicating that the method is reliable.

In summary, the previously published method (1) for assay of free fatty acids is unsuitable for rat or mouse serum or plasma unless samples are treated to remove or inactivate NADH-destroying substances, prior to initiation of the enzymatic reaction. Pretreatment with acid appears to solve this problem.

This work was supported by National Institutes of Health grant AM 31612. I thank S. Greeley and A. Ramirez for their comments. C. Wysocki generously provided mice.

Manuscript received 8 August 1983.

REFERENCES

1. Miles, J., R. Glasscock, J. Aikens, J. Gerich, and M. Haymond. 1983. A microfluorometric method for the determination of free fatty acids in plasma. *J. Lipid Res.* **24:** 96–99.
2. Trout, D. L., E. H. Estes, Jr., and S. J. Friedberg. 1960. Titration of free fatty acids of plasma: a study of current methods and a new modification. *J. Lipid Res.* **1:** 199–202.
3. Shimizu, S., R. Inoue, Y. Tani, and H. Yamada. 1979. Enzymatic determination of serum free fatty acids. *Anal. Biochem.* **98:** 341–345.

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