Henry Ford Hospital Medical Journal

Volume 26 | Number 2 Article 12

6-1978

Letters to the Editor

Henry Ford Hosp Med Journal
Vol 26, No 2, 1978

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Rigorous Alkaline Hydrolysis of Bile Acids

To the Editor:

Analytical determination of fecal bile acids is a complex process because the extracts used contain many different bile acids, lipids, and colored impurities. Bile acids must be separated from most other materials before quantification can be effected. A certain percentage of the bile acids in the extracts is conjugated; that is, they are combined with glycine or taurine by an amide linkage. These amides must be hydrolyzed before separations are possible, a process that requires rigorous alkaline hydrolysis (2 M NaOH @ 250°F for three hrs). Although this process has little effect on the recovery of hydroxy-bile acids, major quantities of keto-bile acids are destroyed[1,2] resulting in underestimation of the size of the fecal bile acid pool. To achieve accurate fecal bile acid determinations, keto-bile acid losses obviously must be minimized.

We have developed a simple method which prevents keto-bile acid destruction and should be applicable to most existing methods of fecal bile acid determination. In this method, easily destroyed keto-bile acids are reduced to stable hydroxy-bile acids before alkaline hydrolysis. The reduction utilizes sodium borohydride and is effected at room temperature.

The effectiveness of the method is illustrated by the results of the following study. An alcohol solution containing from 0.2 to 0.6 μMoles of a hydroxy, keto- or keto-hydroxy bile acid is placed in a nickel crucible and evaporated to dryness. The residues are dissolved in 0.5 ml of ethanol, and 0.2 ml of 0.1 M ethanolic sodium borohydride is added to samples to be reduced; the mixture is then allowed to stand overnight. Control samples are not reduced. The solutions are then evaporated to dryness, the residue dissolved in 2 ml of 2 M NaOH, and the mixture autoclaved for three hrs @ 250°F (15 psi). When hydrolysis is complete, the contents of the crucibles are transferred to 25 ml screw-capped (teflon-lined) test tubes. The solutions are acidified (pH 1) with 12 M hydrochloric acid and extracted three times with 3 ml of diethyl ether. The combined ether extracts are washed once with water and then evaporated to dryness. The residues which contain bile acids are dissolved in pyrophosphate buffer (0.1 M pH 10) and assayed for bile acid content using 3-hydroxysteroid oxidoreductase coupled with nicotinamide dinucleotide.[3]

The results in the table below show that a) hydroxy-bile acids which contain no keto groups are stable under the conditions imposed by rigorous alkaline hydrolysis; treatment of these acids with sodium borohydride has no effect on recoveries; b) recoveries of keto- and hydroxy-keto-bile acids are very poor following alkaline hydrolysis; c) reduction of keto- and hydroxy-keto-bile acids with sodium borohydride before alkaline hydrolysis completely protects these compounds from destruction.

Effect of Rigorous Alkaline Hydrolysis* on Bile Acid Recoveries

5β-cholan-24-oic acid	% Loss during alkaline hydrolysis**	
	No reduction	BH_4^- reduction
3α-hydroxy-	2 ± 1†	1 ± 1
3α,12α-dihydroxy-	0 ± 1	2 ± 1
7α-hydroxy-3-oxo-	44 ± 5	0 ± 1
Taurine conjugate of		
3-oxo-	27 ± 2	4 ± 1
3,7-dioxo-	40 ± 3	5 ± 4
Glycine conjugate of		
3-oxo-	25 ± 2	0 ± 1
3,7,12-trioxo-	29 ± 2	0 ± 1

* 2 M NaOH @ 250°F for 3 hrs

** 10 determinations on each sample

† Standard deviation

We believe that borohydride reduction can be applied to most of the existing methods used in determining fecal bile acids and will improve the accuracy of these methods in most cases.

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Acknowledgment

This work was supported in part under Project R-58820 by funds from Henry Ford Hospital through a Ford Foundation bequest and partially from funds derived from NIH Grant AM 20522.

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