Can β-Hydroxybutyrate Be Detected at the Bedside by In Vitro Oxidation with Hydrogen Peroxide?

JAMES R. OSTER, M.D., BARBARA RIETBERG, B.S., ANDREW L. TAYLOR, M.D., GUIDO O. PEREZ, M.D., RAJIV CHANDRA, M.D., AND LAURENCE B. GARDNER, M.D.

The diagnosis of ketoacidosis with an inordinately high plasma and urinary concentration ratio of β-hydroxybutyrate (β-OHB) to acetoacetate (AcAc) is difficult, because only AcAc and acetone react with the diagnostic reagents used clinically to detect ketones. The purpose of this study was to assess the validity of the claim that β-OHB can be identified with a simple modification of the usual bedside test for ketones, using hydrogen peroxide (H_2O_2) and Ketostix (Ames Division, Miles Laboratories, Inc., Elkhart, Indiana). Unfortunately, the lowest detectable concentration of urinary β-OHB was 50 mmol/L, and serum β-OHB could not be detected at levels less than 100 mmol/L, a clinically irrelevant level. The relative insensitivity, the inapplicability to serum, and the potential hazard of the routine use of 30% H_2O_2 by practicing physicians or houseofficers render the method of limited value. DIABETES CARE 7: 80–82, JANUARY–FEBRUARY 1984.

As a result of a reduction-oxidation (redox) shift, the serum β-hydroxybutyrate (β-OHB)/acetoacetate (AcAc) concentration ratio in diabetic ketoacidosis (DKA) frequently increases to 3/1 or greater.[1,2] Urinary ratios are similar or somewhat higher.[1,2] In DKA of moderate severity, typical plasma levels are about 10 mmol/L for β-OHB, 3 mmol/L for AcAc, and 5 mmol/L for acetone.[2,3] Typical urinary levels are less well defined, but, for β-OHB, may reach the range of 50–100 mmol/L, or occasionally higher.[3,4]

If the redox shift in a ketoacidotic state is exaggerated by a relative excess of NADH (as may occur in association with alcoholic ketoacidosis, when DKA is complicated by lactic acidosis, or uncommonly in severe DKA), the concentration of AcAc may be so low that moderate-to-severe ketoacidosis may be present in spite of nitroprusside reactions that are inappropriately weak or even negative.[5,6] This situation has been termed β-hydroxybutyric acidosis; its importance lies in the potential risk of misdiagnosis and inadequate therapy of a severe metabolic disturbance.

The nitroprusside-based test for the bedside detection of ketones produces the greatest color change with AcAc, much less with acetone, and none with β-OHB.[7–9] Acetest tablets (Ames Division, Miles Laboratories, Inc., Elkhart, Indiana) detect both AcAc and acetone, but Ketostix (Ames) detect only AcAc.[9] The specific enzymatic or nuclear magnetic resonance assays for the measurement of AcAc and β-OHB are expensive, relatively time-consuming, and not available in most clinical laboratories.

In a recent review of the approach to a variety of disorders of fluid, electrolyte, and acid-base homeostasis,[10] it was claimed that a few drops of H_2O_2 could be added to a urine that initially gives a negative test for ketones, and that conversion to a positive reaction would confirm the presence of β-OHB. The purpose of the present study was to determine whether β-OHB could be detected by a simple method using in vitro oxidation with H_2O_2. To our knowledge, there are no published data regarding the feasibility of this procedure.

METHODS

The instructions on the manufacturer's package inserts[8,9] were followed for the use of the Acetest tablets and Ketostix (Ames). Solutions of acetoacetic acid (lithium salt, 90–95% pure, Sigma Chemical Co., St. Louis, Missouri), DL-β-hydroxybutyric acid (sodium salt, 98% pure, Sigma), and acetone (analytic reagent, Mallinckrodt Chemical Works, St. Louis, Missouri) were added to ketone-free urine provided by one of the investigators or normal pooled serum to yield the final concentrations.

Those experiments designed to detect β-OHB used either 3% H_2O_2 (Pharmaceutical Reagent, CMC, Inc., Smyrna,

TABLE 1
Reactivity of lithium acetoacetate and acetone in urine with Acetest tablets and Ketostix

Concentration (mmol/L)		AcAc*	Acetone‡§
20	S	Lg(160)†	N
	T	Lg	Mod
10	S	Lg(80)†	N
	T	Lg	Sm
5	S	Mod-Lg	N
	T	Mod-Lg	Sm
2.5	S	Mod	N
	T	Mod	Sm
1.25	S	Sm-Mod	N
	T	Mod	N
0.6	S	Sm	
	T	Sm	
0.3	S	N	
	T	Sm	
0.15	S	N	
	T	N	

S, Ketostix; T, Acetest tablets; N, negative; Lg, large; Mod, moderate; Sm, small; and Tr, trace.
*Mol wt = 108; 10.8 mg/dl = 1 mmol/L.
†The numbers in parentheses (160, 80) indicate two gradations of large reactions. According to the manufacturer's color chart these reactions are said to correspond to a level of approximately 160 mg/dl and 80 mg/dl, respectively.
‡ Mol wt = 58.1; 5.8 mg/dl = 1 mmol/L.
§For acetone, results for 50, 100, and 200 mmol/L, respectively, were negative with Ketostix, and moderate, moderate, and large with Acetest tablets. For simplicity, results not shown.

Tennessee) or 30% H_2O_2 (reagent grade, Fisher Scientific, Pittsburgh, Pennsylvania). Peroxide was added to the specimens so that the final dilution of H_2O_2 was approximately 1:10 (peroxide to specimen). The specimens were then gently stirred for a few seconds, followed immediately by exposure to the test reagents. Readings were made at the time of maximal color intensity (between 10 and 15 s), because further delay resulted in fading.

The color changes obtained with Ketostix and 30% H_2O_2 were different from those shown on the Ames color chart. Our grading scale is based on gradations of intensity rather than actual differences in hue. By our scale, trace means a limited but definite and reproducible change. Because of fading and the lack of an established standard, all assessments were made after simultaneously dipping several Ketostix into varying concentrations of ketones. At least two investigators looked at the colors and compared them with standards to decide on final readings. The data in the tables are based on triplicate determinations; repeat determinations only rarely differed from the first.

RESULTS

Control studies with commercially obtained acetoacetate and acetone in urine (Table 1). The reactivity of Ketostix and Acetest tablets with commercially obtained AcAc and acetone added to normal urine was consistent with the data provided by the manufacturer and that in the literature.[7-9] For AcAc, the tablets were slightly more sensitive than the reagent-impregnated strips. The sensitivity of the tablets to acetone was approximately 1/10 of that to AcAc. The Ketostix were nonreactive with acetone.

Studies with Ketostix and β-hydroxybutyrate, with and without hydrogen peroxide (Table 2). Without H_2O_2, both strips and tablets were nonreactive to urinary β-OHB levels as high as 800 mmol/L. Positive reactions were obtained with Ketostix and 3% H_2O_2, but these were not observed at concentrations <200 mmol/L. Levels as high as 600 and 800 mmol/L of β-OHB produced reactions of only trace-to-small and small-to-moderate, respectively. The use of 30% H_2O_2 improved the sensitivity of the procedure, but β-OHB levels of <50 mmol/L were still undetectable. Neither incubation of the β-OHB-containing specimens for 5, 20, 45, and 70 min, nor heating in a water bath altered the level of the reactions. Of interest, the addition of 30% H_2O_2 to urine containing only enough AcAc to give a Ketostix reaction of less than moderate resulted in negative reactions, allowing recognition of 67 mmol/L β-OHB.

β-OHB in *serum* could be detected with Ketostix, but a concentration of 100 mmol/L was required to give a trace reaction (Table 2).

DISCUSSION

Recognition of the severity of ketoacidosis when the level of the readily measurable ketone, acetoacetate, is low may be difficult.[2,5-7] Alberti and Hockaday[7] have stated that the most confusing cause of inaccuracy in the clinical detection of ketones is the large variation in the β-OHB/AcAc ratio among ketoacidotic patients. Furthermore, several clinical reports docu-

TABLE 2
Reactivity of sodium β-hydroxybutyrate in urine and serum with Ketostix after addition of hydrogen peroxide

Concentration of β-OHB (mmol/L)*	Urine			Serum
	Ketostix			Ketostix
	No H_2O_2	3% H_2O_2	30% H_2O_2	30% H_2O_2
800	N	Sm-Mod	Lg	Lg
600	N	Tr-Sm	Lg	—
400	N	Tr	Mod-Lg	Sm-Mod
200	N	Tr	Mod	Sm
100	N	N	Sm	Tr
50	N	N	Tr	N
20	N		N	
10	N			
5	N			

Abbreviations as in Table 1.
*Mol wt = 126.1; 12.6 mg/dl = 1 mmol/L.

menting the finding of negative tests for blood and/or urinary ketones, even though ketoacidosis is pronounced,[5,6] suggest that β-OHB/AcAc ratios may exceed 10/1.

In the present study, the addition of 30% H_2O_2 to urine allowed detection of β-OHB, but only at a concentration of 50 mmol/L or greater. Such a relatively high level would probably be associated with a sufficiently high concentration of AcAc to ensure a strong nitroprusside reaction, thus eliminating the need for H_2O_2. Serum β-OHB could not be detected at a concentration <100 mmol/L, a clinically irrelevant level.

It is of interest that the Ketostix rather than the Acetest tablets reacted to β-OHB plus H_2O_2. If the oxidation product were AcAc, we would have expected the tablets to be more sensitive, as they are to "native" AcAc. If the oxidation product were acetone, the reagent strips should have been insensitive. These findings raise the possibility that substances other than AcAc or acetone contributed to the reaction.

In conclusion, relatively high urinary levels of β-OHB can be detected at the bedside using Ketostix and 30% H_2O_2. When the reaction of blood and/or urine with Acetest tablets or Ketostix is weak or negative in a patient with an unexplained high anion gap type metabolic acidosis, a positive test with H_2O_2 would indicate the presence of relatively large amounts of urinary β-OHB and permit a diagnosis of β-hydroxybutyric acidosis. Nevertheless, the relative insensitivity, the inapplicability to serum, and the potential hazard of the routine use of 30% H_2O_2[11] render the method of limited value.

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From Medical and Research Services, Veterans Administration Medical Center, and the Department of Medicine, University of Miami School of Medicine, Maimi, Florida.

Address reprint requests to Dr. J. R. Oster, Nephrology Section (111C), VA Medical Center, 1201 NW 16th Street, Miami, Florida 33125.

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