BRIEF COMMUNICATION

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Detection of Ketosis in Vitreous at Autopsy after Embalming

REFERENCE: Steinhauer JR, Volk A, Hardy R, Konrad R, Daly T, Robinson CA. Detection of ketosis in vitreous at autopsy after embalming. J Forensic Sci 2002;47(1):221–223.

ABSTRACT: Ketosis occurs in ketoacidosis or malnourishment. When either is suspected in relation to a death, it may be important to analyze for ketosis at autopsy. We encountered a case where starvation was suspected in a deceased nursing home resident, where the body had been embalmed prior to autopsy. Gas chromatography (GC) was unable to separate acetone from formaldehyde, a component of embalming fluid. The Acetest is a simple test that can detect acetone and acetoacetate in body fluids. We validated the Acetest with GC on vitreous. The Acetest and GC were consistent except at very low levels of acetone or acetoacetate. The sensitivity of the Acetest for acetoacetate in vitreous was 10 mg/dL, consistent with early starvation. Significant interference from embalming fluid did not occur. The Acetest was negative in the described case. The Acetest is a simple and useful test for the detection of ketosis in embalmed autopsies.

KEYWORDS: forensic science, toxicology, vitreous, ketosis, ketoacidosis, acetoacetate

Ketosis, to include ketonemia and ketonuria, is the result of increased fatty acid catabolism commonly occurring in states of true or effective malnourishment, such as diabetes, alcohol intoxication, and acute or chronic starvation (1–4). In the event that diabetes, alcoholic ketoacidosis, or starvation may have been related to an individual's death, the postmortem determination of the presence of ketosis may be important. In the ketotic state, increased production and excretion of the ketones acetoacetate (AcAc), beta-hydroxybutyrate (β-OHB), and acetone occurs, and these substances are detectable in whole blood, serum, plasma, or urine (2,4). In most instances, β-OHB is present in the highest concentrations, comprising over 75% of serum or urine ketones, followed by AcAc at 20%, and acetone is present in smaller quantities. In certain states, however, such as severe hypoxemia or severe diabetic ketoacidosis, the ratio of β-OHB to AcAc can be as high as 8:1 with low acetoacetate and acetone levels (2). Ketone concentrations in serum due to acute and chronic malnourishment have been well documented. In early starvation (1 to 3 days of fasting) AcAc typically reaches 10 mg/dL or higher. In chronic starvation, the levels reach four to five times this level (3,4). AcAc levels in diabetic or alcoholic ketoacidosis can be much higher than those encountered in simple malnourishment (4,5). Recently, we encountered a case in which the family of an elderly decedent suspected she had been chronically malnourished at the nursing home where she resided, and they requested an autopsy. Unfortunately, at the time of request, the body had already been embalmed.

The embalming fluids used by the funeral home in this case were Dodge Standard Cavity and Dodge Permaglo (The Dodge Company, Cambridge, Massachusetts). Both fluids contain formaldehyde and methanol. In our forensic toxicology laboratory, the presence of acetone in urine, serum, or whole blood is routinely determined with headspace analysis by gas chromatography (GC) (6). Our method separates methanol, ethanol, acetone, 2-propanol, and n-propanol, which is an internal control. However, acetone has a retention time of 2.85 min, and formaldehyde has a retention time of 2.65 min, and our system can not resolve the two substances. GC analysis of both vascular and ocular fluid from the decedent was performed, and a methanol peak was readily identified at 1.4 min. Additionally, a single large peak with a retention time of 2.75 min was also identified, so the presence or absence of ketones could not be determined in the presence of formaldehyde by this method. The Acetest (Bayer Corporation, Elkhart, IN) is a simple, inexpensive, commercially available test for the presence of acetone or acetoacetate in urine, serum, plasma, or whole blood (7). We sought to determine if the Acetest would be useful in the determination of ketones in embalmed tissue fluid specimens.

Materials and Methods

In most instances, postmortem analysis for ketosis would be performed on urine or whole blood. However, during the embalming process, the vast majority of the blood is removed from the circulatory system and replaced by embalming fluid, and urine is drained from the bladder. Furthermore, postmortem blood samples are likely to be hemolyzed. For these reasons, we chose to analyze vitreous for the presence of ketones, since vitreous ketone levels have been shown to correlate with serum ketone concentrations in alcoholic ketoacidosis in nonembalmed forensic specimens (5). Samples of ocular fluid from known ketotic autopsy cases were ob-

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Received 29 Dec. 2000; and in revised form 14 May 2001; accepted 22 May 2001.

tained for this study to serve as positive control cases. Vitreous from nonketotic autopsy cases was pooled and used for negative controls and known dilutions for sensitivity determinations.

The Acetest consists of a single tablet containing sodium nitroprusside, glycine, and an alkaline buffer. The tablet is placed on clean white paper, and a drop of urine, serum, plasma, or whole blood is added to the tablet. Hemolyzed blood samples should be avoided, however, since hemoglobin can interfere with the test (3). Certain substances, such as bromsulfalein, sulfhydryls, phenylketones and L-dopa can also interfere, causing false positive results. The presence of acetone or acetoacetate in the sample is indicated by the production of a purple color after 30 s, 2 min or 10 min for urine, serum, or blood, respectively. A pink, tan, or yellow color indicates a negative result. We observed for the color change after 30 s with deionized water solutions and 2 min with ocular fluid. The manufacturer provides a lower limit of detection for AcAc of 5 mg/dL in urine and 10 mg/dL in whole blood or plasma. In order to determine the lower limit of detection for AcAc in ocular fluid and in water and to evaluate for interference from the embalming fluid, we performed the acetest on serial dilutions of a 200 mg/dL aqueous stock solution of AcAc. All tests were performed in parallel with positive and negative controls. When a color change was observed that was slightly purple in color, distinctly different from the negative control, but not strong enough to be comparable to the weekly positive example on the manufacturer's color chart, a designation of "detectable" was recorded, but we decided not to consider these positive. A 50 mL stock solution was created by dissolving 53 mg of 90 to 95% pure lithium acetoacetate (F.W. 108) (Sigma Chemical, St. Louis, Missouri) in deionized water. The stock solution was then serially diluted in deionized water, pooled vitreous from nonembalmed specimens, and vitreous spiked with high and standard concentrations of embalming fluid. The pooled vitreous was known to be negative for acetone or acetoacetate by GC analysis. One portion of the vitreous was spiked with a 1:1 solution of Dodge Standard Cavity in deionized water and another portion with a 1:7 solution. Sixteen ounces of Dodge Standard Cavity or Dodge Permaglo are mixed in water to make one gallon for standard embalming practices. We mixed these embalming fluid solutions at a 1:1 ratio with the pooled vitreous, and then performed the dilutions. Final concentrations of acetoacetate in each series of dilutions ranged from 20 mg/dL to 1.25 mg/dL, thus bracketing the physiologic acetoacetate levels typically encountered in early starvation.

The Acetest was compared to gas chromatography by performing the test on vitreous samples known to be positive or negative for AcAc or acetone by GC. Known positive acetone, and acetoacetate, and negative controls were also analyzed. GC by headspace analysis was performed on all specimens. Analysis was performed on a Shimadzu GC-14A gas chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, Maryland) isothermally with an oven temperature of 185°C and an injector temperature of 195°C. The column was packed with Porapak S (Supelco, Bellafonte, Pennsylvania) porous polymer. Because of its low volatility, AcAc could not be detected by our routine gas chromatography methods. Prior to analysis, AcAc was decarboxylated to acetone by heating specimens according to a method previously described by Felby and Nielsen (8).

Finally, the vitreous specimen from the case in question was analyzed using the Acetest.

Results and Discussion

The results of the sensitivity determinations are compiled in Table 1. The lower limit of detection of the Acetest for acetoacetate in water and in pooled vitreous was determined to be 5 mg/dL. This is well below the levels expected even in early starvation (3,4), and it is lower than the limit of detection of 10 mg/dL in serum provided by the manufacturer. When AcAc was dissolved in vitreous spiked with a standard embalming fluid solution, a purple color change was observed at 5 mg/dL; however, the coloration of the tablet in this case was very subtle, and was less than the small positive example on the manufacturer's color chart. This result was designated as "detectable" instead of "positive," because it was not truly negative. The result was definitely positive at 10 mg/dL, so this was determined to be the lower limit of detection. This is still sensitive enough to detect AcAc and acetone levels encountered in early starvation. When the more concentrated solution of embalming fluid was used, however, significant interference was experienced, and the lower limit of detection was 20 mg/dL. While levels between 10 and 20 mg/dL were not tested, this suggests that the embalming fluid somehow interferes with the Acetest. It may be a result of the acidity of the embalming fluid overwhelming the alkaline buffer in the Acetest tablet; the nitroprusside test requires a basic environment to occur. We do not believe this to be problematic, since under normal conditions, the concentration of embalming fluid in vitreous in practice is not likely to reach the levels present in even our low concentration specimens.

TABLE 1—*Results of Acetest sensitivity determination in different combinations of vitreous and embalming fluid.*

	Solvent			
AcAc Concentration	Deionized Water	Vitreous	Vitreous and 1:1 DSC*	Vitreous and 1:7 DSC*
20 mg/dL	Positive	Positive	Positive	Positive
10 mg/dL	Positive	Positive	Negative	Positive
5 mg/dL	Positive	Positive	Negative	Detectable
2.5 mg/dL	Negative	Negative	Negative	Negative
1.25 mg/dL	Negative	Negative	Negative	Negative

* Dodge Standard Cavity embalming fluid diluted 1:1 and 1:7 in deionized water as indicated.

The Acetest and GC results were consistent for the presence of acetoacetate and acetone in vitreous from ketotic and nonketotic decedents when significant levels were present. All except one of the samples with acetoacetate levels higher than 5.0 mg/dL that were positive by gas chromatography were also positive by the Acetest (Table 2). One of the GC positive, Acetest negative samples was considered "detectable," and the GC result was consistent with 6.8 mg/dL of acetoacetate. This would not likely be high enough to be considered evidence of starvation ketosis. The other discrepant result in a positive control case was negative by the Acetest and had only 3.4 mg/dL of acetoacetate by GC. No false positives by the Acetest were identified, and the presence of embalming fluid alone, concentrated or diluted, did not yield a positive result. Analysis of the vitreous specimen from the forensic case in question was also negative, disputing the claim of chronic starvation.

Aside from the overall size of this study, it is limited by the fact that we were unable to obtain specimens from decedents known to be acute or chronically malnourished in the absence of diabetes or alcohol intoxication at the time of death. Our positive specimens were from cases of ketoacidosis in diabetics or alcoholics. We believe it is unlikely, though, that the ketosis of starvation is significantly different enough from the ketosis of diabetes, other than

TABLE 2—*Validation of Acetest gas chromatography.*

Sample*	Acetest Result	GC Result
20 mg/dL acetoacetate	Positive	Positive
10 mg/dL acetoacetate	Positive	Positive
5 mg/dL acetoacetate	Positive	Positive
2.5 mg/dL acetoacetate	Negative	Positive
1.25 mg/dL acetoacetate	Negative	Positive
Pooled Vitreous	Negative	Negative
Control Case 1	Positive	Positive
Control Case 2	Positive	Positive
Control Case 3	Detectable	Positive (6.8 mg/dL)†
Control Case 4	Positive	Positive
Control Case 5	Negative	Positive (3.4 mg/dL)†
Deionized Water	Negative	Negative

* All cases are vitreous samples known to be positive or negative for acetone and acetoacetate by gas chromatography.

† Concentration measured by GC is given in parentheses.

maximal ketone concentrations, to invalidate our results. Additionally, since severe ketoacidosis and hypoxia can lead to ketosis with much higher levels of β-OHB and lower levels of AcAc, this test may not be applicable when those situations are present; however, other supportive tests are available for hyperglycemia or alcohol intoxication. This weakness can perhaps be overcome by enzymatic conversion of β-OHB to Acetoacetate or acetone prior to testing (9), and this may be worth further investigation when complete recovery of ketone bodies is desired; however, this conversion involves more complex and difficult enzymatic steps, and the premise of this investigation was to describe a simple method of testing for ketosis. Finally, it is also known that the onset of ketosis is delayed in obese individuals as compared to normal or underweight individuals (10), and early starvation may not be detectable in obese decedents with the Acetest. However, it is less likely that short-term fasting in an obese individual without other problems will be related to their death. We conclude, therefore, that the Acetest is a simple, inexpensive test for the presence of ketosis in forensic autopsy cases where the decedent has been embalmed prior to postmortem examination. We believe this can be a useful adjunct in combination with other evidence as to whether an individual was malnourished prior to their demise.

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