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The Incidence of Ethanol and Acetone in
the Blood and Urine
of Victims of Sexual Assault

by
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THE INCIDENCE OF ETHANOL AND ACETONE IN THE BLOOD AND URINE OF VICTIMS OF SEXUAL ASSAULT¹

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ABSTRACT

The blood and urine from 255 sexual assault cases, divided into visible and no visible physical trauma groups, were examined for the presence of ethanol and acetone. The incidence of acetone was compared with the incidence in blood samples from 200 ethanol negative Red Cross blood donors and 158 ethanol negative samples from natural, non-traumatic deaths. The incidence of acetone in the blood and urine of sexual assault victims was approximately ten times that of the comparison groups. The visible trauma group was approximately three times more likely to exhibit detectable acetone levels than the no visible trauma group. The presence of ethanol in the blood reduced the frequency of acetone detection in the sexual assault victims.

RÉSUMÉ

On a tenté de déceler la présence d'éthanol et d'acétone dans des échantillons de sang et d'urine de 255 victimes d'agressions sexuelles réparties en deux groupes: traumatismes physiques évidents et non évidents. La fréquence pour l'acétone a été comparée à celle dans 200 échantillons de sang de la Croix Rouge sans teneur d'éthanol et à celle de 158 échantillons sans teneur d'éthanol provenant de personnes décédées de causes naturelles. La présence d'acétone dans le sang et l'urine des victimes d'agressions sexuelles était 10 fois plus fréquent que chez les personnes faisant partie du groupe de contrôle. Le groupe à traumatismes évidents a trois fois plus de chance d'accuser des niveaux détectables d'acétone. La présence d'éthanol dans le sang a diminué la fréquence de la détection d'acétone chez les victimes d'agressions sexuelles.

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INTRODUCTION

The Centre of Forensic Sciences provides a uniform sexual assault kit to all police agencies in Ontario for the collection of evidence from the victims of sexual assault. Among the samples collected are blood and urine which are routinely analyzed for volatile drugs by headspace gas chromatography. The only volatiles commonly detected are acetone and ethanol.

This paper describes the incidence of acetone and ethanol in a one year study of sexual assault victims.

METHODS AND MATERIALS

Determination of Acetone and Ethanol Concentration

A Perkin-Elmer Multifract F-45 headspace gas chromatograph equipped with a flame ionization detector was used to determine the acetone and ethanol concentrations. The column was a 1.8 m \times 3.0 mm steel column packed with Carbopack C 60/80 mesh coated with 0.2% Carbowax 1500.

The operating temperatures were; oven: 90°C, injector port: 150°C, dosing system: 170°C and automatic turntable: 50°C. The carrier gas (nitrogen) and hydrogen pressures were 1.2 bar.

Tertiary butanol was added to each sample as an internal standard. Samples were allowed to equilibrate in the automatic turntable for at least 30 minutes prior to analysis. All samples were analyzed in duplicate and the mean result was reported in each case.

The coefficient of variation for the aqueous standards of ethanol or acetone, analyzed under the described conditions, was approximately 1.5%.

The blood or urine was reported as negative if the ethanol concentration was less than 10 mg/dL or if the acetone concentration was less than 1 mg/dL.

The blood and urine results were considered separately.

Sexual Assault Cases

Approximately 286 sexual assault cases were examined in the Toxicology section of the Centre of Forensic Sciences over a one year period. Of these, 31 were not investigated further by the police and no analyses were required, leaving a total of 255 cases. In some cases, both blood and urine were not submitted. A total of 252 blood samples and 235 urine samples were analyzed.

The blood was normally collected in a grey top Vacutainer (XF 947) containing 100 mg of sodium fluoride and 20 mg of potassium oxalate. Urine was collected in a 15 mL screw top vial which contained 120 mg of sodium fluoride and 60 mg of sodium citrate.

The blood and urine were collected 5.4 ± 5.1 hours after the assault (mean \pm SD). The victims of the sexual assault were 252 females and 3 males whose ages ranged from 3 to 84 years. The mean age and standard deviation were 24 ± 13 years.

The cases were divided into two groups. "visible" and "no visible" trauma, according to the police case submission form and the medical form completed at the hospital. The visible trauma group included all individuals with some obvious physical injury such as cuts, bruises or abrasions. The no visible trauma group were victims who had no reported visible physical injuries. According to these criteria, 153 cases were classified as no visible trauma and 102 cases as visible trauma. In some cases the information from the police regarding the sexual assault was incomplete and minor visible injuries may not have been recorded. Some visible trauma cases, therefore, may have been classified as no visible trauma.

Comparison Samples

The incidence of acetone in the sexual assault victims was compared with the incidence of acetone in blood samples obtained from 200 Red Cross blood donors. This blood was collected in standard blood collection bags (CPDA-1).

Other comparison samples included 158 blood and 52 urine samples from natural, non-traumatic deaths. Both urine and blood were collected in 15 mL screw top vials which contained 120 mg of sodium fluoride and 60 mg of sodium citrate. Subjects with reported diabetes or liver disease and samples which were obviously putrefied, were excluded. Samples were also excluded from the comparison groups if the concentration of ethanol was greater than 10 mg/dL. The incidence of physical trauma in the comparison group was considered negligible.

RESULTS

The incidence of acetone and ethanol in sexual assault cases is shown in Table 1. Approximately 25% of the blood and urine samples had an ethanol concentration greater than 10 mg/dL. Acetone could be detected in 19% of the urine samples and 4% of the blood samples.

Acetone positive cases were observed less frequently in samples containing ethanol (Figure 1). When ethanol was present 1.6% of the blood samples were positive for acetone. In the absence of ethanol, 5.3% of the blood samples were positive for acetone. Similarly 7.6% of the urine samples containing ethanol were positive for acetone while 26.6% of the ethanol negative urine samples were positive.

Table 2 shows the incidence of acetone in the comparison groups, the total sexual assault victims, and the visible and no visible trauma groups. Ethanol positive samples were excluded in order to eliminate the effects of any ethanol-acetone interactions.

The incidence of acetone in the blood and urine of sexual assault victims was approximately 10 times that of the comparison groups (Table 2). The visible trauma group was approximately three times more likely to exhibit detectable acetone levels than the no visible trauma group.

Age had no effect on the incidence of acetone in the urine of sexual assault victims (Figure 2). Approximately 22% of the urine samples were positive for acetone in each age group except for the 0-9 years age group. In this age group the

TABLE 1
DISTRIBUTION OF ETHANOL AND ACETONE IN VICTIMS
OF SEXUAL ASSAULT

	BLOOD		URINE	
	NUMBER	PERCENT ¹	NUMBER	PERCENT
NEGATIVE	180	71.4	124	52.8
ETHANOL ONLY	61	24.2	61	26.0
ACETONE ONLY	10	4.0	45	19.1
ACETONE AND ETHANOL	1	0.4	5	2.1
TOTAL	252	100.0	235	100.0

¹ — percentage of total within the group

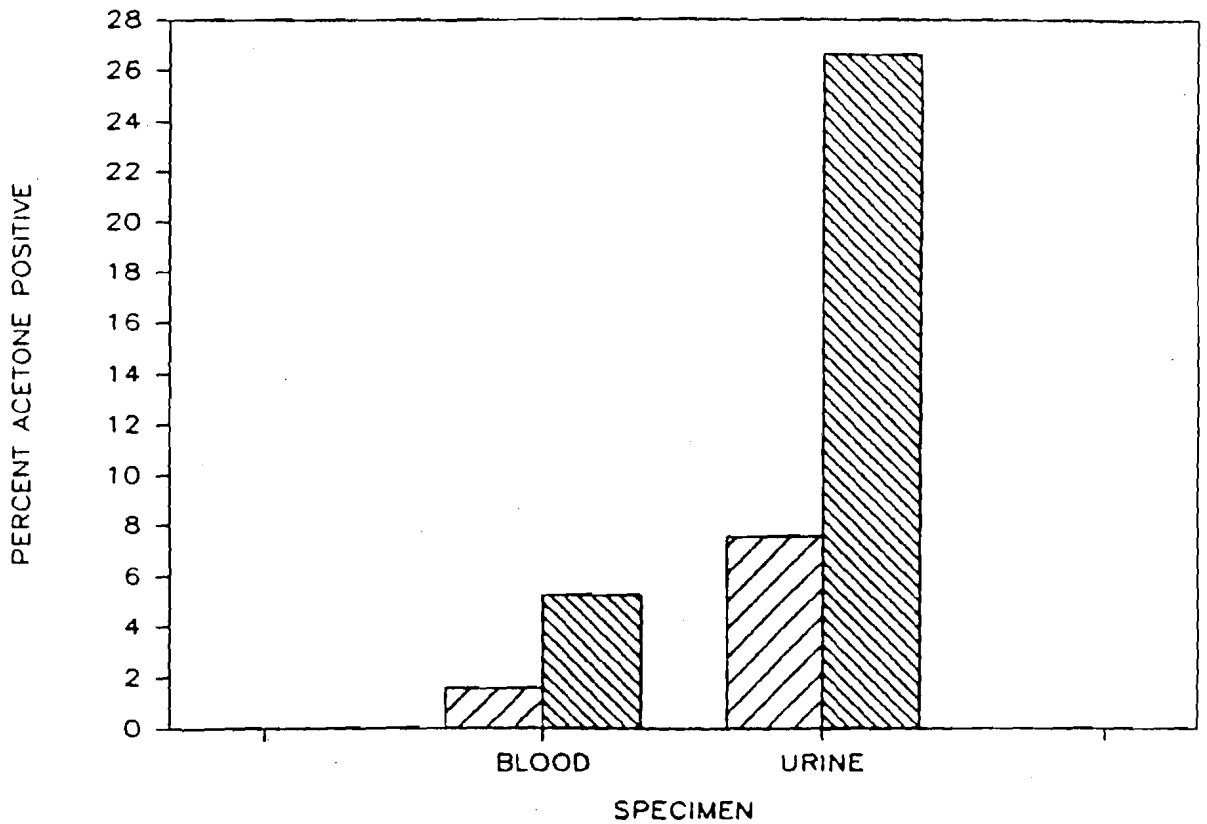




Figure 1 The percentage of blood and urine samples, either positive () or negative () for ethanol, exhibiting detectable acetone.

TABLE 2
FREQUENCY OF ACETONE AT CONCENTRATIONS > 1 mg/dL

A — BLOOD¹

SAMPLE	INCIDENCE ²	PERCENTAGE
RED CROSS BLOOD	1/200	0.5
NATURAL DEATH	1/158	0.6
SEXUAL ASSAULT VICTIMS		
TOTAL	10/190	5.3
NO VISIBLE TRAUMA	3/114	2.6
VISIBLE TRAUMA	7/76	9.2

B — URINE¹

SAMPLE	INCIDENCE ²	PERCENTAGE
NATURAL DEATH	2/52	3.8
SEXUAL ASSAULT VICTIMS		
TOTAL	45/169	26.6
NO VISIBLE TRAUMA	16/102	15.7
VISIBLE TRAUMA	29/67	43.3

¹ — ethanol positive samples excluded

² — number positive / total cases

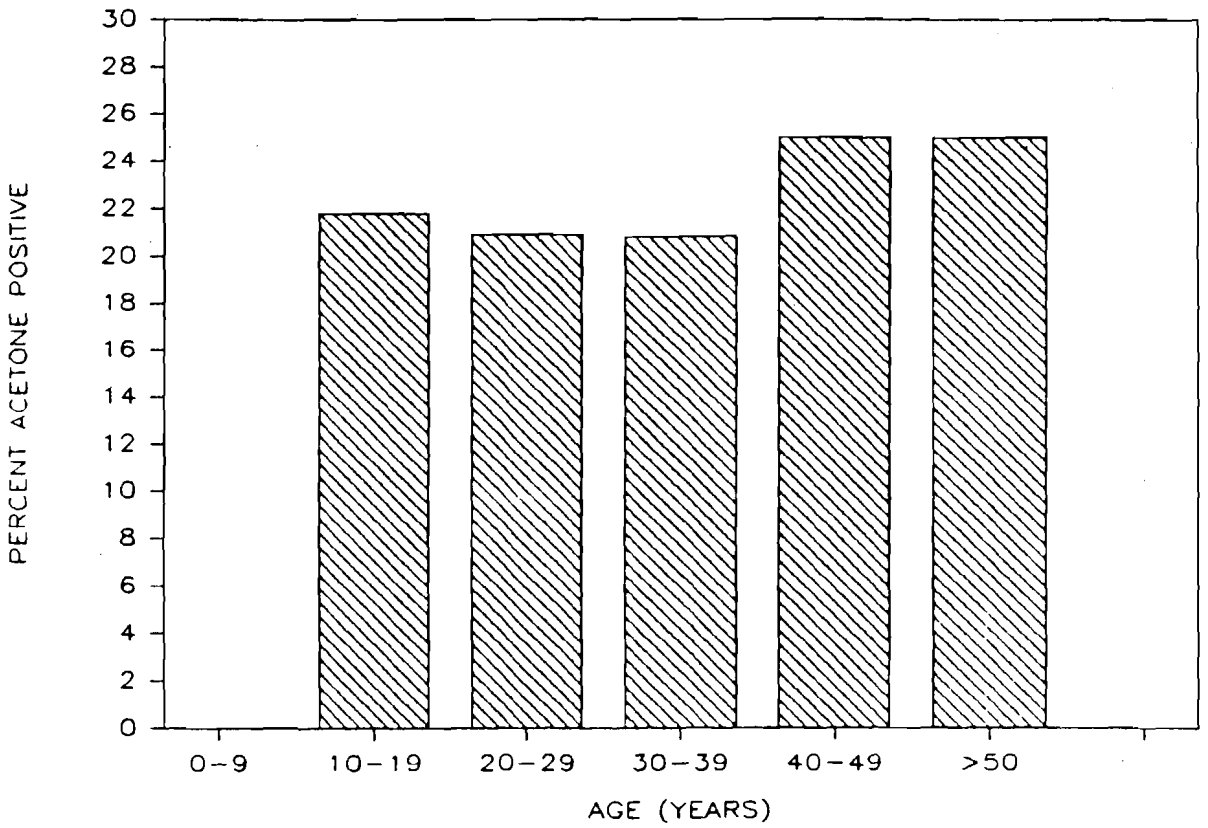


Figure 2 Age distribution of detectable acetone in the urine (all cases).

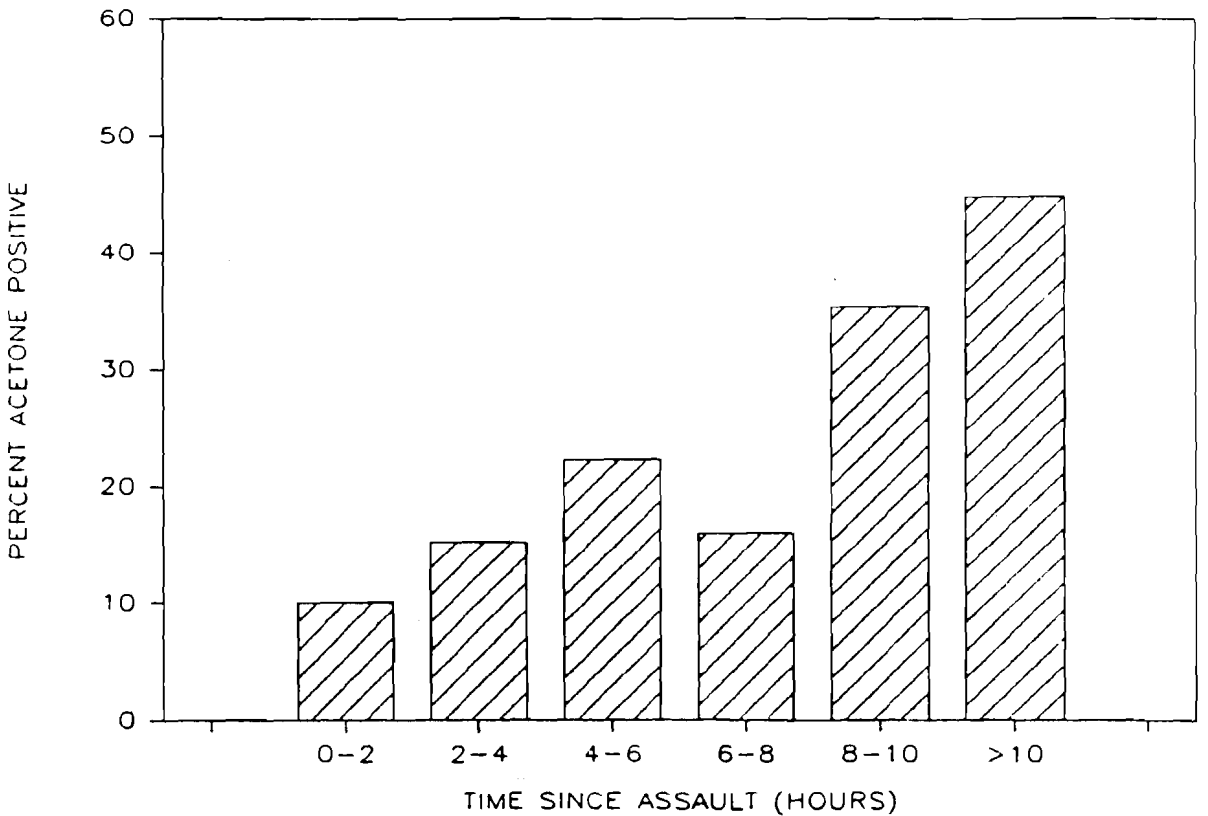


Figure 3 Percentage of urine samples exhibiting detectable acetone levels with increasing time since assault (all cases).

limited sample size ($n = 3$) may be responsible for the result.

Figure 3 shows the relationship between the frequency of acetone detection in the urine and the time since the assault. The incidence of acetone increased with increasing time from the assault. At 0 to 2 hours after the assault 10% of the samples were positive for acetone. The percent positive increased to 44.8% when the urine was collected more than 10 hours after the time of the assault. The time since assault in the visible and no visible trauma groups was examined (Figure 4). Acetone appeared in the urine of the visible trauma group at an earlier time and with a greater frequency than in the no visible trauma group.

The low number of acetone positive blood samples ($n = 10$) precluded a detailed study of age and time versus the acetone frequency in blood.

DISCUSSION

Acetone concentrations in healthy non-fasting subjects have been found to range from 0.00 to 0.28 mg/dL in plasma and 0.02 to 0.25 mg/dL in urine (1, 2, 3). The cutoff value for acetone used in this study (1 mg/dL) is above expected normal concentrations.

The acetone concentration may be elevated due to fasting (4), uncontrolled diabetes (5), isopropanol poisoning (6), or disulfiram use (7).

These conditions cannot account for the 10 fold increase in acetone incidence in the victims of sexual assault compared with the comparison groups (Table 2) and do not explain why the visible trauma sexual assault victims had a greater incidence of acetone than the no visible trauma group (Figure 4).

The ketone bodies, acetoacetate, 3-hydroxybutyrate and acetone, are pro-

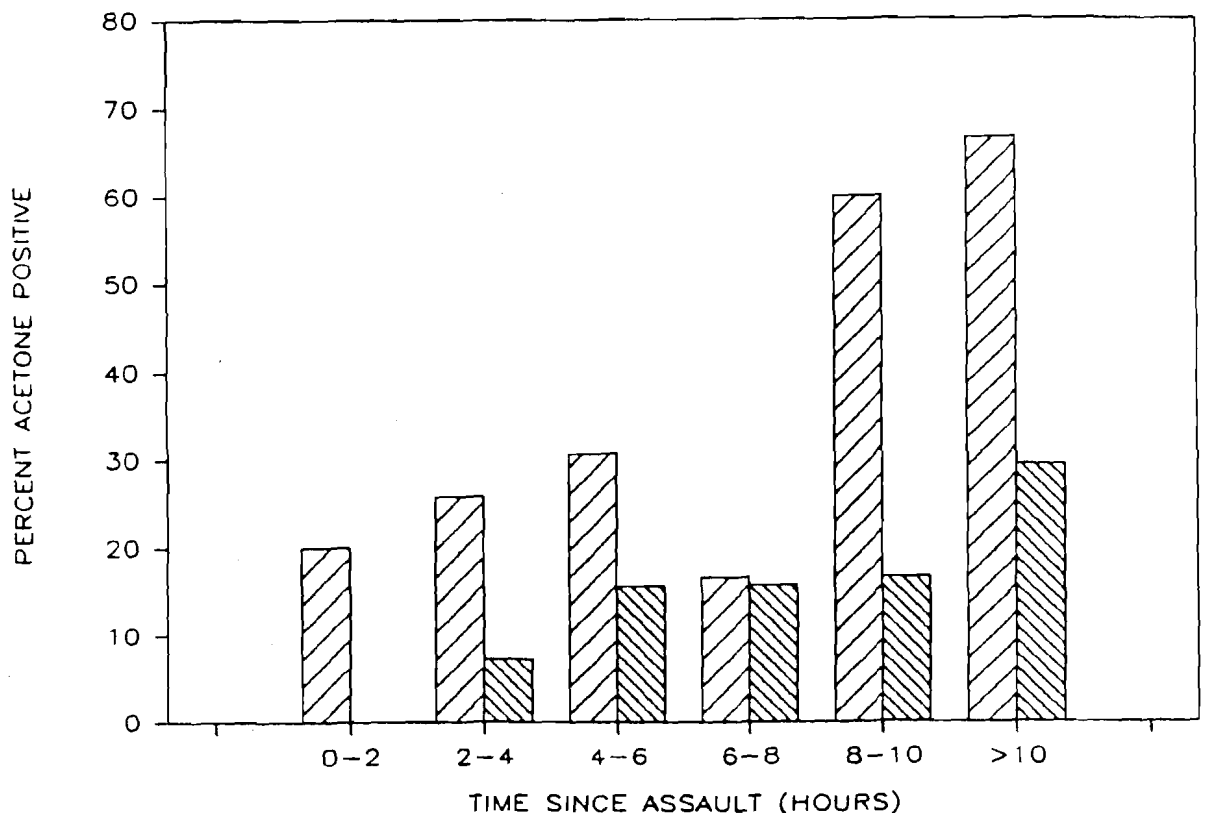


Figure 4 Percentage of urine samples exhibiting detectable acetone levels, with increasing time since assault, in victims with 'visible' (▨) and 'no visible' (▩) trauma.

duced in the liver mainly from the partial oxidation of fatty acids (8, 9) (Figure 5). The various components of trauma such as pain, fear, haemorrhage and tissue damage can significantly increase the concentration of the stress hormones, especially the catecholamines (10). Fear and stress can also elevate the free fatty acids concentration in blood (11, 12). It has been reported that intravenous infusions of epinephrine or norepinephrine can significantly increase plasma acetone and ketone body concentrations (13-18). Physical injury can also increase ketone body concentration (19) and elevated acetone concentrations have been found in victims of trauma other than sexual assault (20).

The incidence of acetone is, therefore, related to the incidence of trauma, the lowest incidence of acetone occurring in the comparison group with negligible trauma. The highest incidence of acetone was found in the visible trauma sexual assault victims.

Ethanol may influence the incidence of detectable acetone due to an indirect action on ketone body synthesis (Figure 5). The metabolism of ethanol by the liver produces excessive amounts of NADH (21, 22). The increase of NADH in the liver causes ketone body production to shift from acetone and acetoa-

cetate to 3-hydroxybutyrate (8, 23, 24, 25). Detection of acetoacetate and 3-hydroxybutyrate requires specialized headspace gas chromatography techniques (1, 26, 27) which were not utilized in this study. Since the individual components of the ketone body pool were not examined the apparent decrease in acetone expression with ethanol could be the result of a redistribution of elements within the pool and not due to a decrease in free fatty acid release. Other ethanol induced events, such as inhibition of fatty acid oxidation (28, 29) or a decrease in the release of acetylcholine in the brain (30), may also contribute to the apparent decrease of detectable acetone.

The frequency of acetone appearance changed with the time the samples were collected after the assault. The low incidence of acetone in the urine and blood taken less than 2 hours after the assault was expected, since the release of stress hormones does not produce an immediate increase in acetone concentration. Peak ketone body concentrations in blood occur 40 to 90 minutes or more after the start of catecholamine infusion (13, 15, 18).

Acetone, once produced, is eliminated primarily via the lungs and has a half-life of 3-5 hours (31, 32). The authors

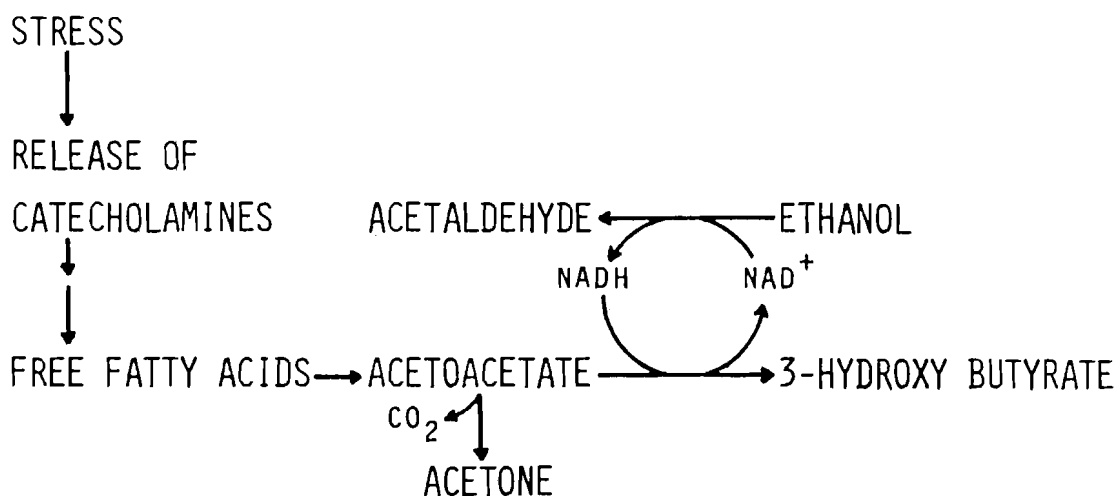


Figure 5 Pathways of stress-induced ketone body formation and ethanol metabolism.

expected the percentage of individuals positive for acetone to increase for several hours after the assault, a result of *de novo* ketone body production due to catecholamine release, and then to decrease gradually as the acetone was eliminated through respiration. Instead, the incidence of acetone continually increased with time (Figures 3, 4). The highest frequency occurred in samples collected greater than 10 hours after the assault.

This effect may be due to the production of acetone from starvation, if the victim had not eaten since the assault, or acetone produced from the catabolism of amino acids and protein loss in response to injury (10, 33). The latter explanation seems more likely since the frequency of acetone in urine samples taken more than 10 hours after the assault was greater in the visible trauma victims than the no visible trauma group (Figure 4). The incidence of acetone more than 10 hours since the assault was 67% for the visible trauma group versus 29% for the no visible trauma group. If starvation were the explanation the incidence of acetone in both groups should be similar.

The response of the human body to trauma is interesting and varied. Research is required to elucidate the ketone body response to trauma especially acetoacetate and 3-hydroxybutyrate. Other areas of interest forensically may be the hyperglycemic and hyperlactonemic response to trauma (34, 35).

CONCLUSION

Victims of sexual assault react to the physical and physiological stress of the assault by fatty acid mobilization and subsequent ketone body production. These individuals were ten times more likely to have an increased concentration of acetone in the urine than a normal comparison population. Within this

group acetone was detected three times more frequently in victims that experienced some degree of physical injury than in the less traumatized individuals. An increased incidence of physical trauma, therefore, produced an increased incidence of acetone. Since the ingestion of ethanol decreased the frequency of detectable acetone, possibly through a shift in the components of the ketone pool, forensic interpretation of these observations is not possible until further studies are conducted on the nature of the ketone body distribution.

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