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Studies on Putrefactive Ethanol Production. I: Lack of Spontaneous Ethanol Production in Intact Human Bodies

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ABSTRACT: The possibility of the production of ethanol by bacterial metabolism of blood glucose was investigated by studying a series of 26 hospital autopsies. The patients died from a wide variety of nontraumatic causes and the bodies were transported within 4 h of death to the morgue, where they were refrigerated for 0 to 28 h before samples of vitreous humor were collected for ethanol analysis. In addition, right heart blood was obtained using sterile technique for cultures as well as ethanol and glucose determinations. Ethanol was measured using gas-liquid chromatography, and glucose values were determined with a glucose oxidase method. In 13 cases, one to three species of microorganisms were isolated, but in no case was a blood or vitreous ethanol value greater than 10 mg/dL recorded. Ethanol values were also less than 10 mg/dL in the 13 cases with negative culture results. These data strongly suggest that de-novo production of ethanol by microbial metabolism does not occur in intact bodies which are refrigerated within 4 h of death. The detection of ethanol under such conditions is therefore probably indicative of antemortem consumption.

KEYWORDS: alcohol, toxicology, postmortem examinations

There is much confusion in the literature concerning the formation of ethanol after death by bacterial action on the glucose present in the blood of human bodies. For example, a recent textbook cautions that ethanol concentrations of less than 200 mg/dL may represent putrefactive alcohol production, while concentrations in excess of 200 mg/dL probably indicate antemortem alcohol consumption [*1*]. The prevailing hypothesis seems to be that bacteria enter the blood after death and metabolize glucose through the Embden-Meyerhoff pathway to form ethanol; no less than 13 different microorganisms have been implicated in this process [*2-9*]. Early studies of the putrefactive formation of ethanol used relatively nonspe-

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cific reducing methods to detect and quantitate ethanol [*10*], but more recent experiments have substituted either gas-liquid chromatography or alcohol dehydrogenase methods [*2,3,11,12*]. Unfortunately, the experimental systems studied have had little similarity to the conditions found in intact human bodies after death [*2-5,11-15*].

The purpose of the present study is to evaluate the possibility of de-novo production of ethanol by bacterial action on glucose in the blood of intact human bodies that are refrigerated soon after death.

Materials and Methods

Bodies were transported to the morgue within 1 h of death and stored at 6 ± 2°C for 3 to 27 h before autopsy, when specimens were collected in a uniform manner by one pathologist [*16*]. Specifically, vitreous humor was obtained by puncturing the lateral scleral canthus with an 18-gage needle and gently aspirating with a 10-cm^3 syringe. The vitreous aspirates were then stored in tightly stoppered glass tubes at −25°C until analyzed. Blood was collected in a sterile fashion by opening the pericardium, reflecting the heart cephalad, and sterilizing the intrapericardial portion of the inferior vena cava with a red-hot spatula. Blood was then drawn through a sterile 14-gage needle into a sterile 30-cm^3 syringe. The 14-gage needle was replaced with a sterile 18-gage needle and 5 cm^3 of blood was injected into an aerobic and an anaerobic Bactec[3] culture bottle. The remainder of the blood was stored in tightly stoppered glass tubes at −25°C until analyzed.

The Bactec culture bottles were incubated with agitation at 37°C and checked for growth at 8-h intervals for seven days before being discarded as negative. Positive cultures were subcultured onto appropriate media for speciation and identification of organisms. Blood and vitreous ethanol concentrations were determined with a Perkin-Elmer Model 900 gas chromatograph equipped with flame ionization detectors and 1.8-m by 4-mm internal diameter glass columns containing Porapak Q 80-100 mesh. Gas chromatographic analyses were performed with helium carrier gas at a flow rate of 40 mL/min and column, injector, and detector temperatures of 200, 220, and 250°C, respectively. *n*-Propanol was used as an internal standard. Glucose concentrations were determined by using a glucose oxidase method on a centrifugal fast analyzer. The method employed is linear between 20 and 450 mg/dL; values outside the linear range were reported as either "less than 20 mg/dL" or "greater than 450 mg/dL."

Results

Ethanol was not detected in either the vitreous humor or blood of any of the 26 cases studied. There were 13 cases with negative blood cultures and 13 cases with positive cultures which grew one to three organisms per case on isolation media (Tables 1 and 2).

Discussion

Evidence in the literature for the postmortem bacterial production of ethanol is largely based on studies of experimental systems not using intact human bodies (Table 3). One study used isolated human tissue samples but measured ethanol with a very sensitive but nonspecific method [*10*]. Davis et al [*2*] detected the formation of ethanol in putrefying mice; when germ-free mice were killed and kept in a sterile environment, neither putrefaction nor ethanol production occurred. Davis et al therefore concluded that ethanol was produced by the bacteria causing the decomposition of the dead mice but did not identify the responsible microorganisms. To test the hypothesis that bacteria produce ethanol by the

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TABLE 1—*Cases with negative blood cultures.*

Postmortem Interval, h	Cause of Death	Blood Glucose mg/dL	Sex	Age, Years
6	lymphoma	...[a]	m	50
7	myocardial infarction	270	m	72
10	metastatic carcinoma	270	f	37
10	perforated duodenal ulcer	20	f	99
12	metastatic carcinoma	...[a]	m	66
12	myocardial infarction	330	m	73
12	hepatoma, ruptured	80	f	76
14	metastatic carcinoma	70	f	17
16	metastatic carcinoma	...[a]	m	54
16	myocardial infarction	110	m	67
20	adult respiratory distress syndrome	...[a]	f	83
22	lymphoma	...[a]	m	60
28	metastatic carcinoma	220	m	39

[a]Less than 20 mg/dL.

TABLE 2—*Cases with positive blood cultures.*

Postmortem Interval, h	Cause of Death	Organisms Isolated from Blood	Blood Glucose, mg/dL	Sex	Age, years
4	metastatic carcinoma	*Staphylococcus epidermidis*	...[a]	m	66
4	ruptured esophagal varices	*Klebsiella pneumoniae, Staphylococcus aureus*	...[a]	m	49
5	aspiration pneumonitis	*Escherichia coli*	...[a]	f	76
5	sepsis	enterococcus, *Citrobacter freundii*	170	f	65
5	aspiration of gastric contents	anaerobes[b]	>450	m	84
7	myocardial infarction	α-hemolytic *Streptococcus*, γ-hemolytic *Streptococcus, Escherichia coli*	...[a]	m	62
10	bronchoalveolar carcinoma	*Staphylococcus aureus, Escherichia coli*	60	m	80
14	bronchopneumonia	*Escherichia coli*	...[a]	m	39
17	myocardial infarction	enterococcus, *Klebsiella pneumoniae, Serratia marcescens*	140	m	81
20	radiation pneumonitis	*Staphylococcus epidermidis*, β-hemolytic *Streptococcus*	330	f	68
21	metastatic malignant melanoma	*Klebsiella pneumoniae*, α-hemolytic *Streptococcus*, β-hemolytic *Streptococcus*	50	m	72
22	sepsis	*Pseudomonas aeruginosa, Candida albicans*	...[a]	m	87
23	myocardial infarction and pneumonia	*Enterobacter cloacae*	40	m	64

[a]Less than 20 mg/dL.
[b]Anaerobes were not speciated.

TABLE 3—*Summary of literature data on putrefactive production of ethanol.*

Conditions of Experiment	Highest Concentration Ethanol Produced, mg/dL or mg/100 g	Ethanol Detection Method	Reference
Human brain, liver, and blood incubated in vitro up to 13 days	420	titration as ethyl iodide	*10*
Rabbits and mice allowed to decompose	430	GLC[a]	*11*
Mice allowed to decompose	75	GLC	*2*
Mouse brain, liver, lung, and muscle incubated in vitro	100	GLC	*2*
Germ-free mice incubated in vitro after death	no ethanol produced	GLC	*2*
Rabbits allowed to decompose	164	GLC	*3*
Paired samples incubated up to 6 days	no increase over baseline values	GLC ADH[b]	*12*
Postmortem blood glucose adjusted to 450 mg/dL and then incubated aerobically	282	GLC	*4*
Paired samples with sodium fluoride and mercuric chloride added	no ethanol produced	GLC	*4*
Postmortem blood incubated anerobically	80	ADH	*13*
Glucogenic substrates added to blood and mixtures incubated	no ethanol produced	GLC	*14*
Blood from blood bank incubated with bacteria	107	GLC	*5*
Blood from autopsies incubated at room temperature	357	GLC	*15*

[a]GLC = gas/liquid chromatography.
[b]ADH = alcohol dehydrogenase.

metabolism of glucose in the Embden-Meyerhoff pathway, Bogusz et al [*14*] added metabolic intermediates of glucose to samples of nonsterile human autopsy blood samples. Neither ethanol nor pyruvate, its metabolic precursor, could be detected. It appears that the most that can be concluded from the above studies is that under certain sets of in-vitro conditions ethanol is formed in blood samples by an unspecified metabolic function of bacteria that is inhibited by fluoride.

In the present study, glucose in excess of 20 mg/dL was available for fermentation by bacteria in 7 of 13 cases with positive blood cultures. In these instances as well as in the six cases where glucose was less than 20 mg/dL, however, ethanol was not detected. All the microorganisms isolated in the cases with positive cultures have been previously associated with putrefactive ethanol production (Table 4). Under this set of conditions, namely, refrigeration of a body within 4 h of death, bacteria did not produce ethanol even when glucose was available. The lack of ethanol production may be due in part to the relatively prompt refrigeration of the bodies after death and the lack of an advanced state of decomposition. The experimental conditions may at first seem to have little applicability to the practice of forensic pathology, but many bodies coming to autopsy are discovered soon after death and promptly refrigerated (for example, occupants of vehicles involved in accidents). This study strongly suggests that the detection of ethanol in the blood of bodies refrigerated within 4 h of death represents antemortem consumption of ethanol rather than a by-product of bacterial metabolism.

TABLE 4—*Organisms associated with postmortem ethanol production.*

Organisms	References	Isolated in Current Series
Gram-negative		
Proteus vulgaris	*2,4–8*	no
Escherichia coli	*2–9*	yes
Pseudomonas species	*6–8*	yes
Enterobacter species	*5,6*	yes
Klebsiella pneumoniae	*3,6,7,9*	yes
Gram-positive		
Bacillus species	*6*	no
Staphylococcus aureus	*6,7*	yes
Streptococcus faecalis	*6,7*	no
α-hemolytic *Streptococcus*	*5*	yes
β-hemolytic *Streptococcus*	*7*	yes
Clostridium perfringens	*6*	no
Yeasts		
Candida albicans	*2,3,5–9,17,18*	yes
Saccharomyces cerevisiae	*3,6,17*	no

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