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Postmortem Production of Ethanol in Different Tissues Under Controlled Experimental Conditions*

ABSTRACT: The aim of this study was to follow the postmortem ethanol production phenomenon under controlled experimental conditions (temperature, time interval) in different tissues. Specimens of blood, liver, skeletal muscle and kidney were taken from 30 corpses and no chemical preservatives were used in the specimens collected. Ethanol concentrations were detected by gas chromatography. All specimens stored at –20°C and 4°C did not show any change in ethanol concentration in an eight-day time interval. At 20°C and 30°C, all tissues, except blood, showed statistically significant ethanol production over the time interval tested. However, blood sample kept at 30°C, showed statistically significant increase in ethanol production on the 2nd and 4th day comparing to the controls. Thus, we can state that postmortem ethanol production occurs in different tissues, and is increased at higher temperatures and, in general, it is in accordance with the course of time.

KEYWORDS: forensic science, forensic toxicology, experimental conditions, postmortem production, ethanol

Postmortem synthesis of ethanol was described for the first time many decades ago (1–3). It had been found as the product of putrefaction in a few cases where it could not have been ingested premortem (1–3). It is thaught that *Candida albicans* is the main cause of ethanol production (2). However, there are at least 58 species of bacteria, 17 species of yeast and 24 species of molds, which can produce ethanol under different conditions (4). There are many microorganisms suitable for ethanol production, and a number of available substrates (4–6). Glycolysis is considered the principal process for ethanol production, but some other metabolic processes are also possible during using substrates as glucose, lactate, ribose and amino acids (4,6,7). All microorganisms producing ethanol possess alcohol dehydrogenase (4), which can play an important role in the process. In the majority of tissues there is a high concentration of lactate, which increases after death because of oxygen shortage, and it can serve as the additional source of ethanol (4). Tissues rich in glucose (glycogen) are the sites of the greatest postmortem ethanol production as glucose is the main source of endogenous ethanol. The liver has the highest glucose storage capacity in the form of glycogen that can be converted into glucose postmortem (5).

Along with ethanol, other volatiles may be produced postmortem, for example: methanol, n-propanol, isopropanol, n-butanol, sec-butanol, isoamyl alcohol, isobutanol, isopentanol, acetaldehyde, acetone, ethyl ether, formaldehyde, phenylethanol and p-hydroxyphenylethanol (4,7,8).

The amount of ethanol-produced postmortem depends on the species of microorganisms, the available substrates, the body temperature before storage, the specimens collected at autopsy and the presence of preservatives added to the specimens (9). The postmortem time interval for is also an important factor (10).

The aim of this survey was to measure postmortem ethanol production under controlled experimental conditions of temperature and time interval in different tissues. Ethanol concentration was measured by gas chromatography.

Materials and Methods

Choice of Specimens

The study was performed at the Department of Forensic Medicine, Clinical Center Novi Sad, Serbia and Montenegro, on 30 corpses of individuals aged between 20 and 50 years and whose death has occurred 6–12 h before autopsy. Those included in the study died of either natural or violent causes. Approval of families to use specimens for the study was not needed. Deaths related to medical interventions (treatment and death in the hospital, or other medical institutions), and those caused by intoxication were excluded. Blood from *a. femoralis*, liver, skeletal muscle (*m. iliopsoas*) and kidney specimens were collected.

Specimens were divided into two control and three experimental groups. The first control group was analyzed immediately and the second stored at –20°C. The first experimental group was stored at 4°C, the second at 20°C, and the third at 30°C.

All experimental groups, as well as the second control one, were divided into four subgroups according to storage duration at the given temperature. The first subgroup was stored at the designated temperature for 24 h, the second for 48, the third for 96 and the fourth for 192 h.

Specimen Preparation

Five mL of blood was used in each group. One gram of tissue was used for the first control group and of 100 g for the other groups. Specimens of all groups, except the first control group, were

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then separated into plastic containers and stored at the appropriate temperature, and after the designated time tested. Specimens of the first control group were perpetrated and analyzed immediately after autopsy (without prior storage).

Preparation of the blood samples was initially carried out by measuring 0.1 mL of blood into the glass container-vial (volume 20 mL), and then by adding 0.75 g NaCl and 0.1 mL 0.2% solution of *n*-propanol (propyl alcohol pro analysi, MERCK) as the internal standard.

All other specimens (tissue samples) were measured in 0.5 g portions, cut into small pieces and placed into glass vials before adding 0.75 g NaCl and 0.1 mL of internal standard (0.2% solution of *n*-propanol).

Analysis

Ethanol analysis was performed in duplicate, by gas-chromatography method. Blood calibrations were used.

Vials containing blood and organ tissue samples were warmed at 70°C for 60 min in headspace sampler 19395 A (Hewlett-Packard). After incubation, samples were injected into gas chromatograph 5790 A series (Hewlett-Packard) with flame ionization detector (FID). Separation was achieved utilizing capillary column HP-20M Carbovax 20M (capillary length 25 m, ID 320 μm, Film 0.25 μm) at constant column temperature of 60°C, and the signals were processed by HP GC ChemStation, with Windows REV. A.09.01 (1206) system software.

Statistics

All experimental results were given as mean values and processed by SPSS 10.0 for Microsoft Office XP, using repeated measures ANOVA, $p < 0.05$. A-priori (planned) two-independent-samples t-test ($p < 0.05$) was used for mutual comparison of designated time points.

Results

The mean concentration value of produced ethanol for a particular tissue over specific time interval and at a given temperature is the difference between the mean concentration value of measured ethanol (Table 1) for the designated conditions, and the mean concentration of the first control group (day 0. in Table 1). Repeated measures ANOVA with days and tissue samples as independent variables was used to establish statistical significance of difference of ethanol concentration in the particular tissue, at the appropriate temperature after eight days.

Statistical significance in the mentioned time interval was not observed in any tissue examined at –20°C and 4°C (Figs. 1–4) and for blood at 20°C and 30°C (Fig. 1). However, statistical significance was observed in kidney (Fig. 2), muscle (Fig. 3) and liver (Fig. 4) tissues at these temperatures and after eight days. Nevertheless, a-priori t-test showed that in blood sample kept at 30°C, statistically significant increase in ethanol production ($p < 0.05$) was obtained on the 2nd and 4th day comparing to the first control group, while statistically significant decrease of the production ($p < 0.05$) was

TABLE 1—*Mean values (n = 30) of measured ethanol concentrations (g/kg) for each tissue, at experimental temperatures (°C) and time points (day).*

Day	0.	1.				2.				4.				8.			
Temp. (°C)	–	−20	4	20	30	−20	4	20	30	−20	4	20	30	−20	4	20	30
Blood	0.24	0.24	0.23	0.27	0.31	0.24	0.28	0.34	0.52	0.24	0.23	0.26	0.40	0.24	0.20	0.29	0.27
Kidney	0.30	0.30	0.34	0.34	0.71	0.30	0.33	0.57	0.95	0.30	0.32	0.80	0.92	0.30	0.29	0.90	0.86
Muscle	0.31	0.31	0.32	0.39	0.97	0.31	0.32	0.72	0.87	0.31	0.29	1.03	0.85	0.31	0.30	0.94	0.77
Liver	0.22	0.22	0.18	0.23	0.92	0.22	0.19	0.67	1.68	0.22	0.16	1.20	2.28	0.22	0.13	1.97	2.20

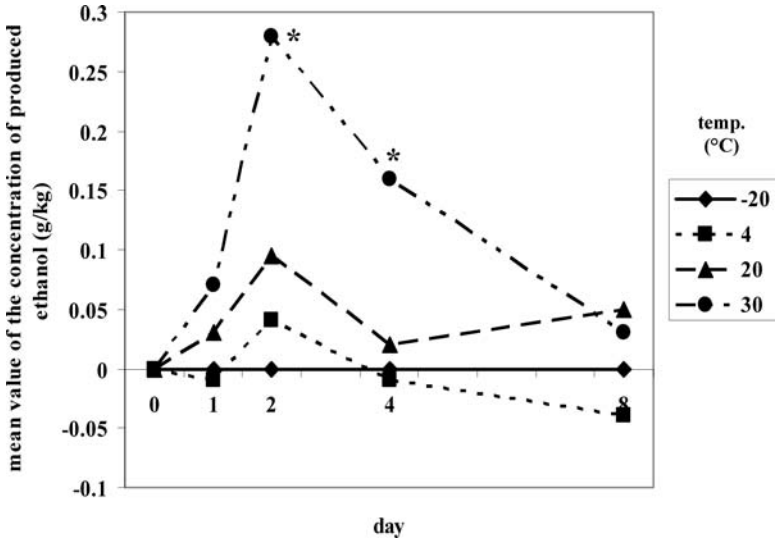

FIG. 1—*Mean values of concentration of produced ethanol in BLOOD (g/kg) within time points (days), at the experimental temperatures (°C).* * *– Statistical significance, p < 0.05 (designated time point vs 0 time).*

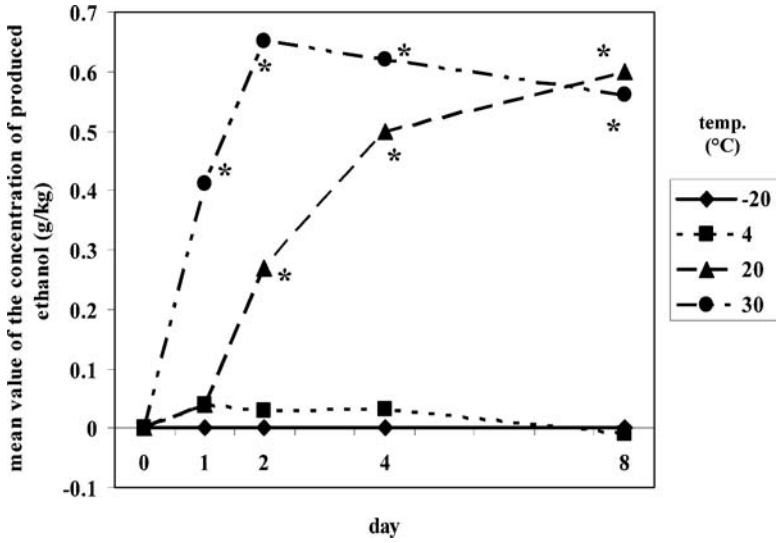

FIG. 2—*Mean values of concentration of produced ethanol in KIDNEY TISSUE (g/kg) within time points (days), at the experimental temperatures (°C).* * *– Statistical significance, p < 0.05 (designated time point vs 0 time).*

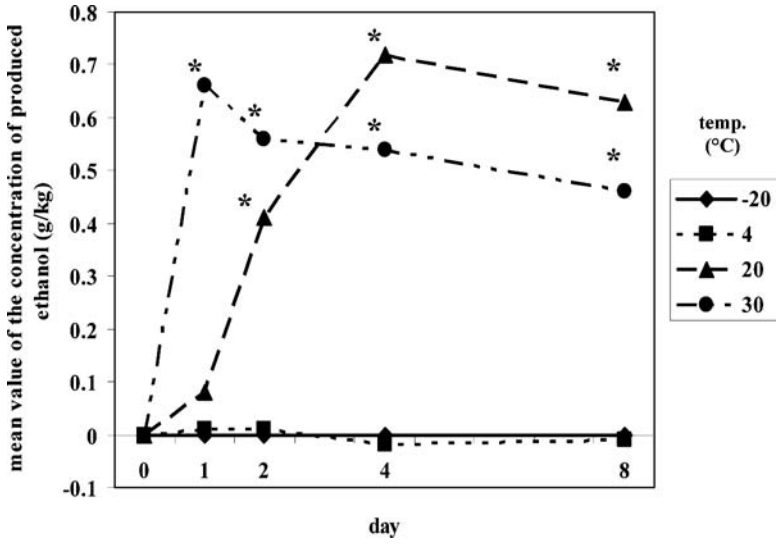

FIG. 3—*Mean values of concentration of produced ethanol in MUSCLE TISSUE (g/kg) within time points (days), at the experimental temperatures (°C).* * *– Statistical significance, p < 0.05 (designated time point vs 0 time).*

observed on the 8th day comparing to the 4th day. In the other tissues, statistically significant ethanol concentration change started on the 2nd day at 20°C and on the 1st day at 30°C. Maximum of produced ethanol at 20°C was measured on the 4th day in muscle and on the 8th day in kidney and liver tissue, while at 30°C it was noticed on the 1st, 2nd and 4th day in muscle, kidney and liver tissue, respectively. The decrease in ethanol concentration noticed after the maximum value was not statistically significant.

TABLE 2—*Mean values with ranges of premortem ingested ethanol concentrations (g/kg) in the examined specimens of 6 cadavers.*

	Blood	Kidney	Muscle	Liver
Mean value	1.67	1.27	1.20	0.91
Range	0.53–1.96	0.48–1.91	0.53–1.74	0.16–1.65

Discussion

In this survey, out of 30 dead bodies, six contained ethanol in their blood and tissues initially, with the range of concentrations shown in Table 2. However, ethanol detected in the first control sample group is assumed to correspond to premortem ingested ethanol, and not to the postmortem production. This assumption is supported by literature data showing that the body temperature has to be 5°C for at least 4 h for endogenous production to occur (10). In our research, autopsies were performed 6–12 h after death and corpses kept in refrigerator at the Department of Forensic Medicine in Novi Sad; for less than 4 h before autopsy. We are confident that no endogenous ethanol had occurred in the 30 corpses at the moment of autopsy. In addition, each corpse was carefully examined during the autopsy for the presence of decomposition changes; none were found. On the contrary, a statistically significant difference between time 0 and eight days and at the designated temperature was considered to be evidence of endogenous (postmortem) ethanol production. The

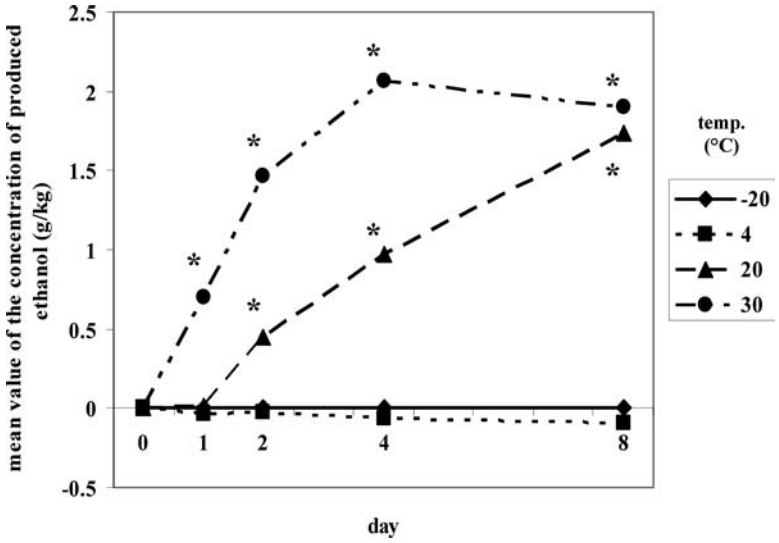

FIG. 4—*Mean values of concentration of produced ethanol in LIVER TISSUE (g/kg) within time points (days), at the experimental temperatures (°C).* * *– Statistical significance, p < 0.05 (designated time point vs 0 time).*

fact that there was no endogenous production in any tissue at –20°C and 4°C was to be expected and consistent with other (10).

The absence of endogenous ethanol production at 4°C allows for blood specimens to be kept refrigerated for long periods. It should also be noted that the development of microorganisms and the synthesis of the postmortem ethanol can be also blocked by adding 1% NaF to the specimens after autopsies (10). In our study the mentioned preservative was not added to any of blood samples (likewise to any other examined tissue) in order to create the most appropriate experimental conditions for postmortem ethanol production.

In vitro studies on postmortem ethanol production in blood specimens kept at 20°C over different time intervals, have shown a significant increase of ethanol production from the 3rd to the 13th day with a maximum concentration of 0.82 g/kg (11) and one study showed an increase up to the 5th day, stabilization to the 15th day and then an abrupt decrease (6). Apparently, these data are not congruent with ours regarding neither time intervals, nor the maximum concentration of ethanol production. Increase of ethanol production in blood specimens has also been observed after glucose addition to specimens (12). As already mentioned, the blood sample used in our investigation was taken from the femoral artery. Some studies have shown the different sites of blood sampling e.g., blood from heart and femoral vessels (13,14) with significant ethanol concentrations being produced in blood from heart, because of high glucose concentration and existing microorganisms. Taking all of this into account, we think that in our investigation, the produced ethanol concentration would have been significant, if the blood had been taken from heart, and/or if glucose had been added to the specimens. On the contrary, investigating the concentration of the ethanol produced at 30°C during four days in blood in rabbits (15,16) the authors have noticed a significant increase of the concentration from 2,5 days and on, after sacrificing. So, it is interesting to remark the coincidence of time interval when statistically significant increase of postmortem produced ethanol is evident in blood tissue (2nd day in our study), irrespective of the fact that these studies were related to samples of rabbit cadavers, and this one to samples of human ones.

From time to time, forensic practice deals with the problem of inadequate (at the room temperature) preserving of blood samples taken from living persons. The obtained results imply that blood sample on the 2nd day at 20°C shows the increase of ethanol, in average 0.1 g/kg, but at 30°C this increase is significant –0.28 g/kg (Fig. 1). So, these results could focus our attention to complexity of accurate interpretation regarding the real ethanol level at the moment of taking samples from living persons kept in inadequate conditions, especially when determined values are about low breaking limits (in our legislation 0.5 g/kg).

Results on ethanol production in kidney tissue for the designated temperatures agree with studies performed on animal cadavers (15,16), as well as human ones (11). It is not surprising that significant postmortem ethanol production was noticed in muscle tissue, muscle is a noted storage site for glycogen (4). Starvation or physical exertion of the skeletal muscles premortem, can lead to microbiological proliferation and increased postmortem ethanol production (5).

Comparing Fig. 4 with others, the greatest mean values of produced ethanol are notable the liver tissue at 20°C and 30°C and at all the time points. This is in agreement with the fact that liver has the largest capacity for glucose storage in the form of glycogen. Hence, as glucose is the basic substrate of endogenous ethanol production, this tissue has the highest potential for production (4).

The decrease of mean concentration values of produced ethanol after the initial increase, which is not statisticaly significant, can be seen in Figs. 2–4 at 20°C and/or 30°C within the time function. This is likely due to the balance acheived betwen ethanol production and degradation, probably, caused by different microorganisms.

In our survey, the measured ethanol values were considered to be accurate, despite the use of n-propanol as the internal standard. N-propanol has been detected postmortem, however, in the literature there are high ratio values (17–19), as well as ranges (20) of produced ethanol and n-propanol in cases of more advanced decomposition process. Thus, in our survey, we consider that concentrations of potentially produced n-propanol would not affect the accuracy of our determination of produced ethanol.

In this study, only controlled experimental conditions were used, and as we know, their resemblance to the conditions in nature is very limited. The samples of tissues were taken in small quantities, which under these conditions, became putridly changed. However, the degree of this putrid change cannot be equivalent to the level of

cadaver decomposition in whole, primarily for the difference in the mass. These obtained mean values of produced ethanol under these experimental conditions may not correspond to values that could have been obtained if whole cadavers had been exposed to the same experimental conditions. They may be even less analagous to those obtained if cadavers had been placed in the natural environment.

Conclusions

On the basis of the results obtained during this survey, we can state the following:

1. It is established, by measuring the level of ethanol in different tissues (blood, kidney, muscle, liver), that the ethanol production occurs postmortem in the controlled experimental conditions.
2. The postmortem ethanol production does not appear at –20°C, as well as at 4°C in any tissue, in eight days time interval.
3. Statistically significant production of postmortem ethanol is found in all tissues except in blood at 20°C and 30°C over the time interval tested**,** but the blood sample kept at 30°C, showed statistically significant increase in ethanol production on the 2nd and 4th day comparing to the control (0 day).
4. The highest level of the postmortem produced ethanol is present in liver.
5. Apart from statistically significant ethanol production, its stabilization was also noticed. It was caused by co-existing presence of degradation process, either from already existed i.e., premortem ingested ethanol, or from postmortem i.e., endogenous produced one.

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References

1. Bonnichsen R, Halstrom F, Moller KO, Theorell H. Development of ethanol in blood samples and human organs during forensic chemical practice. Acta Pharmacol Toxicol 1953;9:352–61.
2. Gormsen H. Yeasts and the production of alcohol postmortem. J Forensic Med 1954;1:170–1.
3. Gormsen H. Alcohol production in the dead body. J Forensic Med 1954;1:314–5.
4. Corry JE. Possible sources of ethanol ante- and post-mortem: its relationship to the biochemistry and microbiology of decomposition. J Appl Bacteriol 1978;44:1–56. [PubMed]
5. O'Neal CL, Poklis A. Postmortem production of ethanol and factors that influence interpretation. A critical review. Am J Forensic Med Pathol 1996;17:8–20. [PubMed]
6. Bogusz M, Guminska M, Markiewicz J. Studies on the formation of endogenous ethanol in blood putrefying in vitro. J Forensic Med 1970;17:156–68. [PubMed]
7. Blackmore DJ. The bacterial production of ethyl alcohol. J Forensic Sci Soc 1968;8:73–8. [PubMed]
8. Davis GL, Leffert RL, Rantanen NW. Putrefactive ethanol sources in postmortem tissues of conventional and germ-free mice. Arch Pathol 1972;94:71–4. [PubMed]
9. Blume P, Lakatua DJ. The effect of microbial contamination of the blood sample on the determination of ethanol levels in serum. Am J Clin Pathol 1973;60:700–2. [PubMed]
10. Clark MA, Jones JW. Studies on putrefactive ethanol production. I: Lack of spontaneous ethanol production in intact human bodies. J Forensic Sci 1982;27:366–71.
11. Varga T, Rakoczy I. Kosa Zs. Post-mortem alcohol production at different parts of human corps. Proceedings of 12th Congress of International Academy of Forensic Medicine; 1982 Sept 1–4; Wien, Austria, 1982:237–40.
12. Nanikawa R, Moriya F, Hashimoto YZ. Experimental studies on the mechanism of ethanol formation in corpses. Z. Rechtsmed 1988;101: 21–6. [PubMed]
13. Plueckhahn VD. The significance of blood alcohol levels at autopsy. Med J Aust 1967;ii:118–24.
14. Plueckhahn VD, Ballard B. Factors influencing the significance of alcohol concentrations in autopsy blood samples. Med J Aust 1968;iii:939–43.
15. Takayasu T, Ohshima T, Tanaka N, Maeda H, Kondo T, Nishigami J, et al. Postmortem degradation of administered ethanol-d6 and production of endogenous ethanol: experimental studies using rats and rabbits. Forensic Sci Int 1995;76:129–40. [PubMed]
16. Takayasu T, Ohshima T, Tanaka N, Maeda H, Kondo T, Nishigami J, et al. Experimental studies on postmortem diffusion of ethanol-d6 using rats. Forensic Sci Int 1995;76:179–88. [PubMed]
17. Nanikawa R, Ameno K, Hashimoto Y, Hamada K. Medicolegal studies on alcohol detected in dead bodies – alcohol levels in skeletal muscle. Forensic Sci Int 1982;20:133–40. [PubMed]
18. Nanikawa R, Kotoku S. Medicolegal observations on a dead body. Drawn up from the sea bed, with special reference to ethanol and diatoms. Forensic Sci 1974;3:225–32. [PubMed]
19. Felby S, Nielsen E. Postmortem blood alcohol concentration. Blutalkohol 1993;30:244–50. [PubMed]
20. Mayes RW. The post-mortem production of ethanol and other volatiles. In: GR Jones, PP Singer, eds. Proceedings of the 24th International Meeting of the International Association of Forensic Toxicologists. Edmonton: Alberta Society of Clinical and Forensic Toxicologists, 1988:94–100.

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