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THE RESULTS OF STUDIES ON THE DETERMINATION OF ETHYL ALCOHOL IN TISSUES

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This paper on the determination of ethyl alcohol in tissues was read before the organizational meeting (1950) of the Academy of Forensic Sciences.—EDITOR.

There are many methods for the quantitative estimation of ethyl alcohol in tissues, some of which are quite specific (1), (2), (3), while others are based upon the relatively non-specific reaction of tissue distillates with potassium dichromate (4), (5), (6). The former methods are very time consuming so that one must sacrifice some specificity in daily routine analyses of a large number of cases such as are encountered in a Medical Examiner's Office. As a result, recourse is usually had to one of the modifications of the original Nicloux procedure, and the concentration of reducing substances in the tissue distillate is reported as ethyl alcohol after methyl alcohol has been eliminated by qualitative tests.

The authors have adopted the procedure of Muehlberger (7) for routine quantitative analysis of tissues. In this procedure, 50 grams of minced tissue are taken, tartaric acid and mineral oil are added, and the whole is steam distilled. 100 ml. of distillate are collected. To 5 ml. of distillate there are added 5 ml. of standard potassium dichromate solution and 5 ml. of concentrated sulphuric acid. This mixture is heated in a boiling water bath for fifteen minutes, cooled, and diluted to approximately 200 ml. with distilled water. 3 grams of potassium iodide are added, and the liberated iodine is titrated with standard sodium thiosulfate.

There were several points involved in this procedure which, it was felt, required investigation and clarification.

I. Information was lacking as to the percentage of alcohol recovered by the method employed. Hence, known amounts of ethyl alcohol were added to 50 gram samples of brain which were then subjected to analysis. The results obtained are recorded in Table 1.

Table 1.

RECOVERY OF ETHYL ALCOHOL

Sample No.	Mg. Alcohol Added	Mg. Alcohol Recovered	Per cent Recovery
1	51.5	52.0	101
2	51.5	52.0	101
3	103.0	101.0	98
4	103.0	102.3	99.4
5	154.5	157.0	101.5
6	154.5	152.0	98.4
7	206.0	200.0	97.2
8	206.0	198.5	96.5
9	257.5	251.0	97.4
10	257.5	246.5	95.8
11	309.0	296.5	96.0
12	309.0	296.5	96.0
		Average	98.1

It may be seen that the percentage recovery varied from 95.8 to 101.5 with an average recovery of 98.1%. This means a maximum variation of 0.01% in reported concentrations at levels of 0.40% or less and a maximum variation of 0.03% at the higher levels.

II. Distribution of ethyl alcohol in the various parts of the brain. Inasmuch as the procedure involves the use of 50 gram samples of tissue, it was deemed advisable to determine whether it was necessary to grind the entire organ, mix, and then remove a 50 gram aliquot or whether 50 grams could be removed from the intact organ for use as a sample. Accordingly, the brains were obtained from five different cases, and 50 gram samples were taken for analysis from the left frontal lobe, the right frontal lobe, the cerebellum, the left occipital lobe, and the right occipital lobe. The results of these analyses are shown in Table 2.

Table 2.

DISTRIBUTION OF ETHYL ALCOHOL IN BRAIN

Case No.	Left Frontal Lobe	Right Frontal Lobe	Cerebellum	Left Occipital Lobe	Right Occipital Lobe
1	0.20%	0.20%	0.23%	0.21%	0.21%
2	0.07%	0.08%	0.07%	0.08%	0.08%
3	0.27%	0.28%	0.27%	0.28%	0.27%
4	0.22%	0.21%	0.21%	0.21%	0.21%
5	0.23%	0.24%	0.26%	0.25%	0.24%

The maximum variation was shown in cases 1 and 5 in each of which a difference of 0.03% was shown between the concentration of alcohol

Table 3

REDUCING SUBSTANCES (AS ALCOHOL) FORMED BY PUTREFACTION

		Initial Conc.	2 days	4 days	6 days	8 days	10 days	13 days
Sample No.1								
A*	Brain	0.00%	0.07%	0.16%	0.29%	0.29%	0.29%	0.35%
	Liver	0.00%	0.01%	0.02%	0.02%	0.11%	0.09%	0.06%
	Blood	0.00%	0.01%	0.04%	0.05%	0.06%	0.05%	0.06%
B*	Brain	0.00%	0.01%	0.04%	0.03%	0.03%	0.02%	0.02%
	Liver	0.00%	0.01%	0.01%	0.03%	0.02%	0.02%	0.01%
	Blood	0.00%	0.01%	0.02%		0.01%		0.01%
Sample No. 2								
A*	Brain	0.00%	0.06%	0.12%	0.19%	0.27%	0.31%	0.31%
	Liver	0.00%	0.04%	0.03%	0.04%	0.08%	0.07%	0.05%
	Blood	0.00%	0.00%	0.02%	0.02%	0.01%	0.01%	0.03%
B*	Brain	0.00%	0.00%	0.01%	0.02%	0.02%	0.02%	0.02%
	Liver	0.00%	0.01%	0.01%	0.02%	0.07%	0.11%	0.14%
	Blood	0.00%	0.01%	0.01%		0.00%		0.01%
Sample No. 3								
A*	Brain	0.00%	0.21%	0.26%	0.34%	0.37%	0.42%	0.34%
	Liver	0.00%	0.05%	0.20%	0.20%	0.14%	0.21%	0.11%
	Blood	0.00%	0.04%	0.03%	0.10%	0.13%	0.16%	0.21%
B*	Brain	0.00%	0.01%	0.00%	0.01%	0.01%	0.01%	0.01%
	Liver	0.00%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%
	Blood	0.00%	0.01%	0.01%	0.01%	0.02%	0.01%	0.00%

*Group A represents the results on specimens kept at room temperatures, 20° to 26°C; group B the results on specimens kept in the refrigerator at 5°C.

in the left frontal lobe and the cerebellum. The concentration in the cerebellum was highest in both of these cases. The variations in the different parts of the brain were not deemed to be significant enough to necessitate the taking of aliquots from the entire minced organ.

III. Inasmuch as the work of the Medical Examiner's Office frequently involves the examination of bodies in varying stages of putrefaction, a study was undertaken to determine the effects of putrefaction upon the concentration of alcohol as determined by this method.

Analyses were conducted on fresh samples of brain, liver, and blood. With the exception of the tissues of sample 5 (Table 4), 50 gram portions of each of the aforementioned tissues were placed in tightly capped glass jars, half of which were allowed to remain at room temperatures of 20°C to 26°C while the other half were maintained at refrigerator temperature of 5°C. At various intervals of time after the original analysis, these specimens were re-analyzed.

Table 4

REDUCING SUBSTANCES (AS ALCOHOL) FORMED BY PUTREFACTION

	Initial Conc.	2 days	4 days	7 days	9 days	11 days	14 days
Sample No. 4							
A* Brain	0.16%	0.39%	0.49%	0.42%	0.41%	0.46%	0.47%
A* Liver	0.13%	0.15%	0.14%	0.02%	0.02%	0.11%	0.03%
A* Blood	0.21%	0.22%	0.22%	0.31%	0.21%	0.16%	0.13%
B* Brain	0.16%	0.16%	0.15%	0.15%	0.16%	0.14%	0.11%
B* Liver	0.13%	0.13%	0.12%	0.12%	0.11%	0.12%	0.12%
B* Blood	0.21%	0.21%	0.19%	0.16%	0.21%	0.20%	0.20%
	Initial Conc.	2 days	5 days	7 days	9 days	12 days	14 days
Sample No. 5							
A* Brain	0.22%	0.40%	0.58%	0.57%	0.56%	0.55%	0.47%
A* Liver	0.18%	0.24%	0.19%	0.21%	0.21%	0.18%	0.12%
A* Blood	0.24%	0.20%	0.26%	0.27%	0.19%	0.14%	0.02%
B* Brain	0.22%	0.22%	0.23%	0.23%	0.23%	0.21%	0.12%
B* Liver	0.18%	0.18%	0.20%	0.18%	0.18%	0.18%	0.17%
B* Blood	0.24%	0.22%	0.21%	0.23%	0.24%	0.23%	0.21%

*Group A represents the results on specimens kept at room temperatures, 20° to 26°C; group B the results on specimens kept in the refrigerator at 5°C.

In the case of sample 5, each of the tissues was minced and divided into two portions which were placed in tightly covered jars. One portion was then kept at room temperature while the other was kept at refrigerator temperature. At the indicated intervals of time after the original analysis, 50 gram samples were withdrawn from the containers for analysis.

The results of these analyses are shown in Tables 3 and 4. It may be seen that in all of the tissues kept at room temperatures, a definite increase in reducing substances (calculated as ethyl alcohol) occurs, with the increase being most marked with brain tissue. This is true whether or not the original tissue contained any alcohol.

Surprisingly, although the liver shows some increase in reducing substances, it is never as great as that shown by the brain. Considering the relatively large carbohydrate stores in the liver, one would expect that fermentation would result in a higher alcohol production in that tissue than in brain or blood. However, it is known that the liver contains an enzyme which converts ethyl alcohol to acetic acid (8). If this reaction proceeds at a rate greater than the rate of alcohol production, it might account for the lower results in the liver.

Except for the liver of sample 2 and the brain of sample 5, specimens

kept under refrigeration showed no appreciable change in alcohol concentration even after fourteen days.

Much remains to be done in the study of the effects of putrefaction on alcohol production, and it is our intention to investigate further to determine whether the reducing substances found are, in fact, ethyl alcohol. Also, it is hoped to determine whether the same order of results would ensue with putrefaction of the intact animal as occurs with tissues removed from the body.

In conclusion, the work reported here is a preliminary report of a study of one of the modifications of the Nicloux procedure for the determination of ethyl alcohol in tissues. To summarize the results:

(1) The percentage recovery of ethyl alcohol averages 98.1%, thus making it unnecessary to make any correction for this factor.

(2) There is no significant variation in alcohol distribution in the different areas of the brain.

(3) In putrefaction, apparent alcohol production is so great, even after two days, as to make the results of an analysis under these circumstances completely unreliable.

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