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A SIMPLIFIED AND ACCURATE PROCEDURE FOR THE DETERMINATION OF ETHYL ALCOHOL IN BRAIN OR OTHER TISSUES

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The procedure widely used for the determination of ethanol in brain or other tissues is that devised by Gettler and Freireich (1). This consists of separating the alcohol from the finely minced tissues by means of a steam distillation, and then treating the distillate with an acid dichromate solution to oxidize the ethanol to acetic acid; the acetic acid is then distilled from the oxidation mixture and is titrated with a standard NaOH solution. Here, two time-consuming steam distillations are necessary, and what is even more important, 500 gm. of tissue is called for in the procedure for the required accuracy. If duplicate runs are desired, there is usually insufficient material available.

The procedure proposed here, necessitates a single distillation and requires only 100 gm. of tissue, so that if half a brain is submitted, as is usually the case, sufficient material for replicates is available.

EXPERIMENTAL

One hundred grams of brain (or other tissue) is weighed out to the nearest 0.1 gm. and is placed in a chilled Waring Blendor. Approximately 250 ml. of cold distilled water and 1 gm. of tartaric acid are added, and the mixture blended to a smooth cream. This is then transferred to a cold 1000 ml. round bottom flask, and the Blendor rinsed into this with two or three portions of 100 ml. each of cold distilled water. Five ml. of mineral oil which has previously been shown to be non-volatile at the boiling point of the mixture, is added next. (Some analysts may prefer to carry out the distillation without the addition of the mineral oil, but this necessitates slow, careful heating with constant supervision to prevent excessive foaming. We have found that the distillation can be carried out much more rapidly if the mineral oil is added.) Following this, a Kjeldahl connecting bulb (Corning #2020) is fitted via a rubber stopper to the flask, and thence to a long efficient water-cooled condenser. The condenser tip is led into a tall narrow-mouth graduated cylinder surrounded with an ice-water bath to above the 125 ml. mark. The flask and its contents are gently heated until it boils smoothly with no foaming, and is distilled until a total of 125 ml. of distillate are collected.

A modified Cavett flask (obtainable from Scientific Glass Apparatus Co., Bloomfield, New Jersey on special order) is next prepared by placing in the bottom of the flask 5.0 ml. of standard potassium dichromate and 5.0 ml. of con. reagent grade sulfuric acid. 0.5 ml. of the distillate is placed in the cup, and the apparatus assembled and the springs attached. This is then placed in a boiling water bath for 20 minutes, and the unreacted $K_2Cr_2O_7$ titrated with ferrous sulfate methyl orange solution. The solutions required are:

1. $K_2Cr_2O_7$ (reagent grade, dried over night at 105° C), 1.703 gm./liter (1.0 ml. = 0.4 mgm. of ethanol).
2. Ferrous sulfate-methyl orange solution: 33 ml. of 50% sulfuric acid (reagent grade) and 15 ml. of 0.1% methyl orange are mixed. To the resulting mixture is added 1.5 ml. of a solution of 5 gm. of $FeSO_4$ plus 3 ml. of con. H_2SO_4 made up to a volume of 20 ml.

Each time an analysis is made, the titer of the $FeSO_4$-methyl orange solution is determined in terms of the standard dichromate.

The computations then are:

$$\frac{(\text{Titer}) - (\text{Ml. of } K_2Cr_2O_7)}{\text{Titer}} \times 0.4 \times 1.25 = \% \text{ ethanol in tissue}$$

It may be observed at this point that once the initial distillate has been obtained, the alcohol content may be determined in one of several ways; the "aeration" method of Cavett (2), or a direct oxidation by means of dichromate solution followed by either distillation or titration. However, the procedure described in this paper has been found by us to give the most accurate and rapid results. The original use of the "red reducing solution" by Harger is also used in this laboratory for the determination of ethanol in blood or urine samples (3).

Discussion

Although only 100 gm. of tissue is used in the assay, it was found that 125 ml. of distillate ensured complete recovery of added ethanol. Hence the factor 1.25 in the final calculation.

As a check of the precision of the method, recoveries of alcohol added to previously assayed brain and liver were made. The results of these are shown in table I.

It will be seen from the table that recoveries are almost quantitative in the case of ethanol added to brain, but that from 10 to 16% is lost after addition to liver. The reason for this is not quite apparent at this time, but our opinion is that rapid enzymatic breakdown by liver dehydrogenases is the cause.

In addition to the series of studies reported in table I, examination of brains to which known amounts of ethanol were added, were made both by the Gettler and Freireich method and by the procedure proposed in this paper. The results of the latter method were checked by recoveries of known quantities. The results of these are shown in table II.

TABLE I

Recoveries of Added Ethanol from Brain and Liver

Specimen	Ethanol Concentration, Mgm./100 gm.	Ethanol Added, Mgm./100 gm.	Total Ethanol Found, Mgm./100 gm.	Percent Recovery
Brain #1	0	100	97	97
Brain #2	0	200	196	98
Brain #3	0	300	306	102
Brain #4	121	100	218	99
Brain #5	121	200	323	102
Brain #6	230	100	320	96
Brain #7	230	200	424	97
Brain #8	94	100	190	96
Liver #1	0	50	42	84
Liver #2	0	100	90	90
Liver #3	42	100	130	88
Liver #4	42	200	212	85

All the above are the results of averaging triplicate runs.

TABLE II

Comparison of Determinations of Ethanol by Double Distillation Method and by Proposed Procedure

Brain Number	Double Distillation, Mgm./100 gm.	Proposed Method, Mgm./100 gm.	Ethanol Added, Mgm./100 gm.	Ethanol Found, Mg./100 gm.
1	176	142	50	189
2	214	202	50	256
3	79	87	50	130
4	88	71	50	124
5	0	0	50	51

It may be seen from table II that the proposed method gives somewhat lower results than the Gettler and Freireich procedure. When recoveries of added ethanol are taken into consideration, however, the lower values appear closer to the true values. As a matter of fact, the Gettler and Freireich method proposes a correction factor for substances other than alcohol which react as acetic acid in their method. We have not found this to be the case with our procedure, since acids normally present in either fresh or moderately necrotic tissue are not titrated, and any volatile acids present from other sources do not react with dichromate solution.

Tests were made to ascertain the effect of other steam-volatile poisons in those levels commonly encountered in toxicological work. The only two that introduced an appreciable error were methanol and isopropyl alcohol. Hence, all distillates are routinely tested with Schiff's Reagent after oxidation in the usual manner. Where methanol has been found to be present, a separate assay of the distillate by any reliable method will give the dichromate equivalent, and a correction can

be applied, so that ethanol in the presence of methanol can be determined. Isopropyl alcohol can be detected in the distillate when suspected and can be determined quantitatively after oxidation to acetone. Again a correction can be applied, and the ethanol concentration obtained.

Acetone, chloroform, other halogenated volatile organic compounds, phenol, hydrocyanic acid, or acetic acid do not interfere.

The method described in this paper has not been applied as yet to putrified tissues, but from theoretical considerations, it should give reliable and valid results.

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