

## DETERMINATION OF DIETHYL ETHER IN BLOOD

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ONE of the most satisfactory and simple techniques for determining the concentration of diethyl ether in blood is the dichromate method described by Shaffer and Ronzoni (1). Dichromate is quantitatively reduced by ether in the presence of sulfuric acid, and the amount of reduced dichromate can be determined iodometrically. Recently two modifications of this method have been proposed which could further facilitate the determinations and increase the speed of analysis (2, 3). The first employs a bead tower in which ether vapor is absorbed, and thus entails drying and purifying the air which enters the tower as well as the use of special reagents which precipitate the plasma proteins and prevent foaming. This method has yielded erratic results in our hands, apparently because the oxidation of ether is not completely controllable under the conditions which the authors (2) describe.

The second modification consists of incubation of the ether-containing sample on filter paper suspended over acid dichromate solution. This method has the advantage of extreme simplicity; it does not require the use of special reagents, and the results appear consistent (3). However, the authors do not report the accuracy of their method, and they were unable to secure definite end points by iodometry.

In our experience a combination of the various modifications of the method of Shaffer and Ronzoni has yielded accurate results and these are reported in the following sections.

### METHODS

*Reagents:* 1. 0.1N  $K_2Cr_2O_7$  (2.452 Gm. made up to 500 ml. with distilled water).

2. 0.1N  $Na_2S_2O_3$  (24.83 Gm.  $Na_2S_2O_3 \cdot 5H_2O$  and 3.8 Gm.  $Na_2CO_3$  are dissolved in distilled water and diluted to 1 L.).

3. 60%  $H_2SO_4$ . (Six volumes of reagent grade  $H_2SO_4$  are added slowly with stirring and cooling to four volumes of distilled water. The final volume is approximately 5% less than the sum of its parts.)

4. 25% KI solution (25 Gm. in 100 ml. distilled water).

5. Starch solution. (Add 1 Gm. starch for iodometry to 200 ml. boiling water and boil 1 to 2 minutes until solution clears slightly. Cool and add 2 Gm. KI.)

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*Procedure.* Clean all glassware in acid-dichromate cleaning solution overnight. Rinse thoroughly in tap and distilled water before using.

Into a 250 ml. Erlenmeyer flask pipe exactly 5 ml. 0.1N  $K_2Cr_2O_7$ , and add 25 ml. 60%  $H_2SO_4$ . Swirl to mix. Roll a piece of filter paper (Whatman No. 1, 11 cm. diameter) to form a cylinder about 1 cm. in diameter, then fold it one-third the distance from one end. Fasten the folds together near the bottom so that the short end of the tube is open and lies close to the longer end. Select a rubber stopper which fits the flask mouth, and bore a hole in its center. Fit the hole with a glass rod long enough to be grasped with the fingers. Compress the long end of the filter paper between the index finger and the side of the stopper, and insert stopper and paper into the flask. The stopper should fit tightly in the mouth, and support the filter paper in the flask so that no part of it touches either the dichromate solution or the lower walls of the flask. It is simple to construct a glass support for the filter paper but it has not been found necessary.

Fill a calibrated 2 ml. hypodermic syringe anaerobically from the syringe used for blood sampling (it may be convenient to draw the samples initially in 2 ml. syringes, being careful to fill them past the mark), affix a 20 gauge 2-inch needle, and expel air bubbles. Express blood until the plunger is exactly at the 2 ml. mark, and wipe the needle. Holding the syringe plunger steady with the index finger, advance the needle through the hole in the rubber stopper of the flask and *into* the open end of the filter paper. Eject the blood and immediately close the hole with the glass plug.

Blank flasks are set up using blood drawn before the administration of ether. Incubate all flasks for two to two and a half hours at  $40\text{ C.} \pm 2$  degrees, swirling the flasks once or twice during this period. After incubation remove the stoppers and filter paper, add 40 ml. distilled water to each flask, and place the flasks in a pan containing ice and water.

Just prior to titrating each sample add 2.0 ml. 25% KI solution. Using approximately 0.011N  $Na_2S_2O_3$  (dilute stock 1:9), titrate until the solution becomes light green. Add 1.0 ml. starch solution.\* The solution in the flask should turn black on the addition of starch. Titrate until the color becomes dark purple and starts to lighten; then continue very slowly until the first persistent light blue color appears. Most unpracticed persons will pass the end point because of titrating too rapidly.

*Calculation of Results.* The analysis is based on the quantitative oxidation of diethyl ether to acetic acid. The valence change in this reaction is 8; therefore 1 millimol of ether can reduce 8 milliequivalents

\* Samples containing ether will require less thiosulfate solution in the titration than the blanks, and the total volume at the end point will therefore be less. This will reduce the accuracy of the titration. Therefore, add enough distilled water to make the volume approximately equal to what it would have been at this point in a blank titration.

of dichromate, or 80 ml. of 0.1N solution. One milliliter of dichromate solution is thus equivalent to the weight of 1 millimol of ether divided by 80, or 0.9265 mg. ether. The quantity of 0.1N dichromate remaining after oxidation of ether is equal to the normality of the thiosulfate times the volume used in titration times 10. Thus: Mg. ether found =  $9.265 X$  thiosulfate normality  $X$  (ml. thiosulfate solution used to titrate blank — ml. thiosulfate solution used to titrate unknown).

Thiosulfate normality is determined by titrating a reagent blank containing 5 ml. 0.1N  $K_2Cr_2O_7$  and 25 ml. 60%  $H_2SO_4$ , and diluting as described above.

Approximately 2 per cent of the  $K_2Cr_2O_7$  in the blanks is oxidized during the incubation. For most purposes it is sufficiently accurate to subtract 2 per cent from the value of the reagent blank and use this figure instead of the blood blank to calculate the amount of ether pres-

TABLE 1

Date	Mg. Ether		Average % Recovery	Range	Observations
	Added	Found (Avg.)			
6/15	2.43	2.38	97.9	96.4- 98.7	6
6/17	2.53	2.54	100.4	99.9-100.8	4
6/20	2.45	2.43	99.1	98.5- 99.6	3
6/21	2.45	2.40	98.1	97.2- 98.8	3
Mean $98.7 \pm 1.35$ (S.D.)					16
6/17	1.26	1.28	101.5	100.8-102.3	4
6/20	1.29	1.25	97.3	97.0- 98.0	3
6/21	1.29	1.30	100.7	98.4-103.0	3
Mean $100.0 \pm 1.99$ (S.D.)					10

ent in the sample. This procedure leads to serious error if other substances besides ether (for example ethyl alcohol) are present in the "blank" blood which are volatile and which reduce dichromate.

### RESULTS

The results are summarized in table 1. Known concentrations of ether in blood were made up gravimetrically in vaccine bottles, the ether being added by tuberculin syringe and hypodermic needle through the rubber stopper. Samples were withdrawn in calibrated 2 ml. syringes and analyzed as described above. Not more than 1 per cent of the ether was present in the air phase (1).

### COMMENT

The method described is satisfactory for the analysis of ethyl alcohol as well as diethyl ether, but not for divinyl ether.

Increasing the strength of the sulfuric acid will speed the oxidation of diethyl ether, but concentrations greater than 70 per cent must be avoided. Above this concentration the reaction proceeds past the stage desired, and some acetic acid is oxidized to water and carbon dioxide.

## REFERENCES

1. Shaffer, P. A., and Ronzoni, E.: Ether Anesthesia; Determination of Ethyl Ether in Air and Blood, and Its Distribution Ratio between Blood and Air, *J. Biol. Chem.* **57**: 741, 1923.
2. Andrews, E.; Potter, R. M.; Friedemann, T. E., and Livingstone, H. M.: Determination of Ethyl Ether in Blood, *J. Lab. & Clin. Med.* **25**: 966 (June) 1940.
3. Knox, K. D., and Pask, E. A.: Modification of Dichromate Method for Estimation of Diethyl Ether and Ethyl Alcohol, *Brit. J. Anaes.* **22**: 102 (April) 1950.



## CORRECTION

Foldes, F. F., Davis, D. L., and Plekss, O. J.: Influence of Halogen Substitution on Enzymatic Hydrolysis, *Anesthesiology* **17**: 187 (Jan.) 1956.

The title of the article noted above was erroneously abbreviated. It should have read THE INFLUENCE OF HALOGEN SUBSTITUTION ON THE TOXICITY OF LOCAL ANESTHETIC AGENTS.