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A Simple Method for Determination of Ethyl Alcohol in Blood.

Julius C. Abels. (Introduced by I. Greenwald.)

From the Department of Chemistry, New York University Medical College.

While methods for the determination of ethyl alcohol in blood have been known for many years, all those sufficiently accurate for clinical or medico-legal purposes involve specialized apparatus or difficult techniques. A simple, rapid method of estimating the small amounts of blood alcohol in normal and intoxicated individuals has long been needed. The procedure that we propose is a combination of those of Widmarck[1] and Heise[2] with certain modifications. We have avoided the use of Widmarck's carefully designed desiccating apparatus, as well as his final oxidimetric titration which requires a certain amount of chemical technique, and is, besides, time consuming; we employ only his principle of isolating the alcohol from the blood by desiccation over concentrated sulfuric acid containing potassium dichromate, and this we have much simplified by using rolls of filter paper and ordinary flasks (see below). We have likewise disposed of the initial blood distillation proposed by Heise which has never worked successfully in our laboratory; his final colorimetric estimation of the EtOH by comparing a known amount of dichromate-sulfuric acid solution reduced by it with a set of standards is all that is retained.

In short, the principle of the proposed procedure is to absorb the alcohol from blood caught on a roll of filter paper into a solution of potassium dichromate in concentrated sulfuric acid. This solution is reduced and develops a green or blue color which is finally compared with a set of standard solutions made from known amounts of alcohol.

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1 Widmarck, E., *Bioch. Z.*, 1922, **131**, 473.

2 Heise, F., *Am. J. Clin. Path.*, 1934, **4**, 14.

One ml. of 0.33% potassium dichromate solution in sulfuric acid (made by dissolving 333 mg. dichromate in one ml. of water, and diluting to 100 ml. with concentrated sulfuric acid) is spread on the bottom of a 50 ml. Erlenmeyer flask. 0.5 ml. of blood are then pipetted onto a roll of filter paper prepared according to Fig. 1.

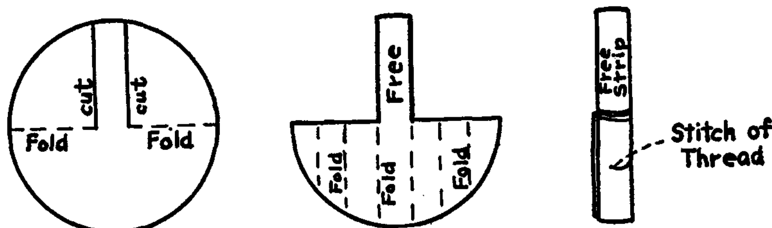

FIG. 1.

The long strip of paper left attached to the roll is inserted between the cork and neck of the flask, thus allowing the absorbed blood to be suspended over the dichromate solution. After being heated from 15 to 30 minutes in a boiling water bath or drying oven at 100°C., the flask is cooled, the cork and wad of paper are removed, and 1.7 ml. of water are added, bringing the total volume of the contents to 3.0 ml. (one ml. dichromate solution, close to 0.3 ml. water from the desiccated blood, and 1.7 mls. water now). The diluted solution is poured into a test tube (6″ x ½″) and its color finally compared with the standard set.

Fifteen standards are made containing 0, 0.05, 0.10, . . . 0.70 mg. EtOH in 3 ml. total volume from 0.01% and 0.05% EtOH stock solutions, water, and one ml. of the dichromate-sulfuric acid solution. These standard systems are heated 10 minutes in a boiling water bath, and the test-tubes (6″ x ½″) are then tightly corked and sealed. If they are exposed as little as possible to the light, they keep for at least 2 weeks.

Thirty determinations using water alone and all the types of filter paper in our laboratory failed to show any dichromate reduction. Applying the method to the determination of alcohol in the bloods of various animals, we obtained the values in Table I.

TABLE I.

Animal	No. observations	% EtOH
man	40	0.000-0.010
pig	3	0.005-0.020
dog	3	0.005-0.010
ox	3	0.010

Experiments were also made on samples of ox blood to which various amounts of alcohol were added. The results of these are shown in Table II.

TABLE II.

No. experiments	mg. EtOH added to 100 ml. blood	EtOH found (average)	Aver. blank	Recovery %
30	0.100	0.104±.004	0.010	94
10	0.200	0.204±.007	0.010	97
10	0.300	0.290±.017	0.010	93

It is quite necessary to use freshly drawn blood in this procedure, for we have found as much as 25% of the EtOH to be destroyed upon keeping the blood sample in the ice chest for 24 hours, and more than 50% after 72 hours.

Micro method: The above method has been extended to determine similar concentrations of EtOH in as little as 0.05 ml. of blood. This amount can be easily obtained from a single finger puncture; a 1% solution of picric acid is employed for sterilizing the skin.

The principle of the method is the same as above, and except for 2 minor changes, the technique is unaltered. 0.20 ml. of the dichromate-sulfuric acid solution are pipetted onto the bottom of a flask such as shown in Fig. 2; the finer the pipette is, the less

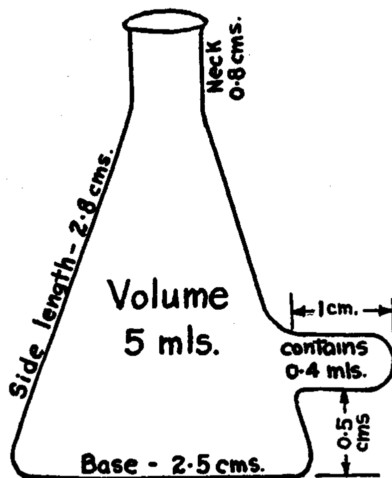

FIG. 2.

chance is there of smearing any of the oxidizing solution on the inside of the neck of the flask, with consequent reduction by the filter paper when later introduced. This source of error must, of

course, be avoided, and can be by using a fine pipette. 0.05 ml. of blood are absorbed onto a roll of filter paper (Whatman's hardened No. 5, 1" diameter) which is suspended over the dichromate solution as in the macro method. The flask is then heated in a boiling water bath or drying oven at 100°C. for 20 minutes, cooled, the cork and paper removed, and the contents of the flask finally diluted with 0.07 ml. of water, bringing the total volume to 0.3 ml. The flask is tipped on its side allowing the contents to flow into the side arm; and the color of this resulting solution is matched against a series of standards contained in small test tubes with an internal bore and wall thickness the same as those of the flask projections.

Eight standards are made containing 0, 0.20, 0.40, 1.40 mg. EtOH in 3 ml. total volume from 0.20% EtOH stock solution, water, and 2.0 ml. of the dichromate sulfuric acid solution. The systems are heated for 10 minutes in a boiling water bath, and 0.3 ml. of each are pipetted into the small test tubes which are immediately sealed.

Twenty-five determinations, using water alone on the filter paper, failed to reveal any reduction of the dichromate solution as long as a hardened filter paper was used. Soft papers are to be avoided, for in dealing with so small a volume of oxidizing mixture, a small thread of paper falling into it will invalidate the results of an experiment. Thirty-two determinations on human blood placed the EtOH concentration of human blood very near 0.00%, and certainly under 0.025%. Forty experiments on artificially prepared bloods containing from 0.10% to 0.30% EtOH gave, after subtracting the blanks, recovery values of from 100% to 107%.

We have also been able to apply both methods to a study of the blood alcohol concentrations during various stages of intoxication, and have found the two to agree within ± 3%. This latter study was undertaken in order to correlate the symptomology of such stages with the corresponding amounts of EtOH in the blood. We divided the cases into 4 groups, as:

I. Chronic alcoholics then sober; II. mildly intoxicated individuals; III. markedly intoxicated individuals; and IV. individuals showing clouding of consciousness (semi-stuperous to comatose). The results of these clinical experiments are perhaps best expressed in Fig. 3.

Although this method really measures the reducing substances in the blood volatile at 100°-105°C., the amount of these other than

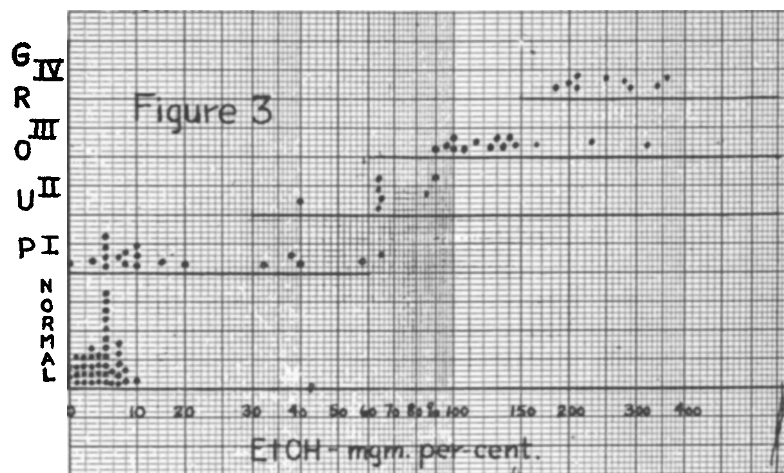

FIG. 3.

ethyl alcohol is normally small; acetone, the second largest fraction, constitutes about 20% of the total.[3] In diabetic coma, however, the amount of free acetone may reach 25 mg. %,[3] but there can be no danger of diagnosing this as an alcoholic coma where the "reducing power" of the blood is at least 8 times this value. We have run a determination on the blood of a patient in terminal diabetic ketosis, and have found the sample to possess a reducing power equivalent to only 0.015% EtOH.

Paraldehyde, a substance much used in the treatment of alcoholics, also does not interfere with the results. Bloods of patients in comas from paraldehyde poisoning show normal alcohol values.

Small mistakes in dilution in the macro method introduce no significant error, for what is really measured is the quality of the resulting solution. The macro method lends itself better to a quantitative study of alcohol in the blood, as might be required in problems on its metabolism; the micro method is sufficiently convenient and accurate for clinical diagnoses of alcoholism.

I wish to express my gratitude to Dr. I. Greenwald for having suggested this work, and for his very kind interest in, and many helpful suggestions to the problem. I also am indebted to Dr. R. K. Cannan and Dr. N. Jolliffe for the ready cooperation they extended to me at all times.

Summary: A method is presented for the determination of EtOH in blood. We have applied this method to the estimation of alcohol in the bloods of various animals and the bloods of men

3 Marriott, W. M., *J. B. C.*, 1914, **18**, 507.

in different stages of intoxication. This procedure, we believe, should prove useful in many problems involving alcoholic intoxication.

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Hemorrhagic Necrotic Skin Lesions in Rabbit Produced by *Hemophilus influenzæ* and *Hemophilus pertussis*.*

E. WITEBSKY AND H. SALM.

From the Bacteriological and Pediatric Divisions of Beth Israel Hospital, New York City.

When injected into the skin of rabbits, cultures of *H. influenzæ* can readily be distinguished from cultures of *H. pertussis*. While *H. influenzæ* causes slight inflammation, characterized by redness and swelling, *H. pertussis* produces bluish-violet discoloration in the involved part of the skin, frequently followed by necrosis.[1, 2] The observations to be reported are concerned with the influence of intravenous injection of *H. influenzæ* and *H. pertussis* on the skin lesions caused by the intradermal injection of the respective organisms.

For our experiments *H. influenzæ* is cultivated on Levinthal agar, *H. pertussis* on Bordet-Gengou medium, containing 25% rabbit blood. Twenty-four or 48-hour cultures of *H. influenzæ* and 48-hour cultures of *H. pertussis* are suspended in saline. The bacterial content of the suspension varies between 2 to 4 billion microbes per cc. Rabbits are injected intradermally with 0.25 cc. each of suspensions of *H. influenzæ* and *H. pertussis* and reinjected intravenously 24 hours later with 1 to 2 cc. of a suspension of *H. influenzæ*. Three to 6 hours later the areas prepared intradermally with *H. influenzæ* are transformed into a bluish-black lesion, and necrosis follows. The influence of the intravenous injection on areas inoculated with *H. pertussis* is only moderate, mostly none. The intravenous injection of *H. pertussis* produces the same results as the intravenous injection of *H. influenzæ*. It also may lead to hemorrhagic-necrotic lesions in the areas of the skin previously inoculated with *H. influenzæ*, while the areas inoculated with *H. pertussis* are usually not affected. We have the

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1 Takagi, Y., cited by Kasahara, M., *Klin. Wochenschr.*, 1933, 1609.

2 Gundel, M., and Schlueter, W., *Z. Blatt for Bact.*, Orig. 1933, **129**, 461.