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Colorimetric Determination of Urethane as Ethyl Alcohol in Blood.*

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Archer, Chapman, Rhoden and Warren[1] have described a method for the determination of urethane (ethyl carbamate) in blood in connection with urethane therapy in leukemia. Their method was based upon the hydrolysis of urethane to ethyl alcohol and determination of the latter by oxidation with dichromate and titration of excess dichromate by iodimetry. This procedure, in specimens of normal blood, gave a relatively high nonspecific "urethane" value of 3-5 mg %; the clinical levels were 10-20 mg %. No recovery experiments were reported.

For the purposes of studying the blood urethane level in patients receiving urethane locally for therapy[2] against gram-negative organisms in infected wounds, a method was needed with greater precision at lowel levels of urethane. Hemingway, Bernat, and Maschmeyer[3] have indicated the unsatisfactory results of iodimetry in micro-titrations of chromic acid, and have proposed the use of barium diphenylamine sulfonate as an indicator, and ferrous sulfate as the titrant. For convenience we have preferred a colorimetric procedure; for increased accuracy we have introduced a more concentrated blood filtrate. The ferrous titration has been, in our hands, equally precise but more time-consuming.

Reagents. 0.300% potassium dichromate. 27 N sulfuric acid. 3 vol. of concentrated acid added to 1 vol. of water. 50% sodium hydroxide (wt.-vol.). 2/3 N sulfuric acid. 10% sodium tungstate.

1:5 tungstic acid blood filtrate. 2 volumes of water are added to oxalated blood, preferably at least 12 ml for blood low in urethane, and the mixture is shaken occasionally for a few minutes. One volume each of 10% sodium tungstate and 2/3 N sulfuric acid is added. It is mixed well and allowed to stand for 5 minutes, after which it is centrifuged and the supernatant fluid is filtered.

Distillation apparatus. An all-glass distillation apparatus is used, consisting of a glass-stoppered 125 ml pyrex distilling flask with an air-cooled condenser ¼ inch in diameter and 15 inches long. This leads to a vertical delivery tube 7 inches long ending in an aeration bulb. The aeration bulb dips below the surface of the acid-dichromate solution contained in a 25 ml pyrex glass-stoppered graduated cylinder cooled in an ice water bath in a liter beaker. The ice bath rests on a wooden block high enough (about 3½ inches) so that the aeration bulb will be well above the 13 ml mark on the cylinder when the block is removed and the ice bath with the cylinder is lowered to the table.

Blank determination. 16 ml of tungstic acid filtrate and roughly 4 ml of water or less filtrate and enough water to make a 20 ml volume are added to the distilling flask containing a few glass beads. 1 ml of 0.300% potassium dichromate and 3 ml of 27 N sulfuric acid are added to a 25 ml glass-stoppered cylinder and mixed. The cylinder is placed in the ice bath with the ice water covering the 16 ml mark. The delivery tube is lowered into the cylinder so that the aeration bulb is near its bottom. The distillation is carried out with a micro-burner and must be fast enough to prevent the acid-dichromate solution from sucking back. About 8.5 ml are distilled. To stop the distillation, the ice bath with the cylinder is lowered to the table while the flask is still being heated. The outside of the delivery tube is washed with a few tenths ml of water, which is blown

* Aided by a grant of the Committee on Therapeutic Research of the Council of Pharmacy and Chemistry of the American Medical Association.

1 Archer, H. E., Chapman, L., Rhoden, E., and Warren, F. L., *Biochem. J.*, 1948, **42**, 58.

2 Howe, C. W., *Surg., Gynec., and Obstet.*, 1948, **87**, 425.

3 Hemingway, A., Bernat, L. A., and Maschmeyer, J., *J. Lab. and Clin. Med.*, 1948, **33**, 126.

into the cylinder by heating the flask. The volume in the cylinder is made to about 12.9 ml with additional distillate (or water).

The cylinder is stoppered, shaken, sealed with 27 N sulfuric acid, and heated at 85° for 60 minutes. It is cooled in water, made up to a volume of 13 ml and mixed. The contents are transferred to an Evelyn tube and read in the Evelyn colorimeter using a 440 mμ filter[4] after the instrument has been set to 100% transmittance with water. The preparation of a standard curve is described below. A similar standardization should be done by each user of the method.

Urethane plus blank determination. The 25 ml cylinder containing freshly prepared acid-dichromate solution is set up with the distillation apparatus as in the blank determination. Sixteen ml of tungstic acid filtrate or less filtrate and enough water roughly measured to make a 16 ml volume are added to the distilling flask. 4 ml of 50% sodium hydroxide are added, the flask is stoppered and the contents are mixed. A water bath is set up around the distilling flask and heated at boiling for 15 minutes. To stop this heating, first the flame under the boiling water bath is increased until the acid-dichromate solution is forced out of the delivery tube. Then the ice bath is lowered to the table, the boiling water bath is removed, the ice bath is replaced and the distillation is started immediately. The rest of the procedure is as in the blank determination. Since the dichromate color is very stable, the determination may be interrupted either before or after the 85° incubation by storage in the refrigerator. The urethane concentration is obtained by subtracting the concentration of the blank from that of the urethane plus blank.

Urethane standard curve and formula. Different amounts of urethane, varying from 0.15 to 1.3 mg, were analyzed by the above method. Each quantity was determined 4 to 10 times. The average values of the optical densities were plotted against mg urethane (Fig. 1). The relationship was linear in this range. The value of the optical density with no urethane was taken as that of a determination with 16 ml water plus 4 ml of 50% sodium hydroxide. This value, 0.4297 ± 0.0068, was slightly lower than that, 0.4436 ± 0.0056, of the acid-dichromate solution simply diluted to 13 ml. The slope of the line was taken as the average of the separate slopes, omitting that of the 1.3 mg urethane point. Amounts of urethane greater than 1.2 mg showed a consistent deviation from this line. The formula of this line is:

Mg Urethane = 3.47 (0.4297 — L).

where the absorbency of the sample, or "optical density", L = log 100 — log G, and G is the Evelyn galvanometer reading (percentage transmittancy).

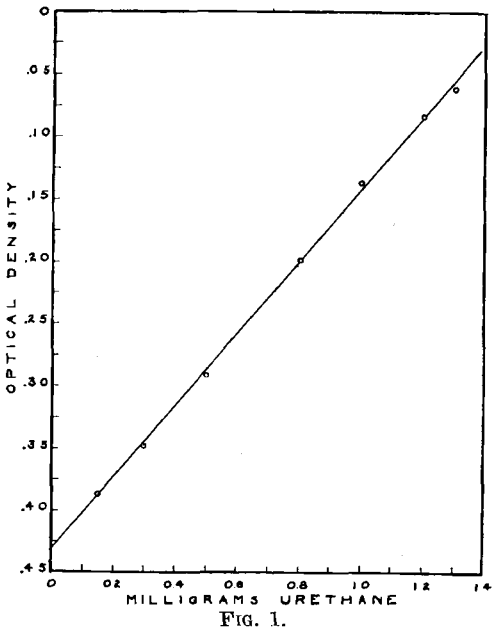

FIG. 1.
Urethane standard curve.

Precision of method. The precision of the above urethane standard determinations is given in Table I. Since the error of a single determination is within ± 2 standard errors, at 0.15 mg urethane the error is about 10% and this decreases to about 2% with 0.5 to 1.2 urethane.

Recovery of urethane added to blood. The recoveries of urethane added to whole blood at concentrations from 3.9 to 43.5 mg % using a 1:5 tungstic acid filtrate are recorded in Table II. These vary from 99 to 111%.

[4] Gibson, J. G., and Blotner, H., *J. Biol. Chem.*, 1938, **126**, 551.

TABLE I.
Precision of Urethane Standard Determinations.

Urethane, mg	0.15	0.3	0.5	0.8	1.0	1.2
Standard error, in %	5.1	2.4	0.7	1.2	0.5	0.7

TABLE II.
Recovery of Urethane Added to Blood.

Urethane added, mg %	Urethane found, mg %	% recovery
3.9	4.2	108
5.7	6.3	111
7.8	7.7	99
11.1	12.3	111
15.9	16.1	101
18.5	19.3	104
19.2	19.0	99
32.3	34.5	107
43.5	45.7	105

Such bloods gave the same urethane values when they were previously hemolyzed with one tenth volume of 1% saponin. The 1:10 tungstic acid filtrate was abandoned because the recoveries were more variable at low urethane concentrations.

Specificity of method. In 5 normal bloods we have found non-specific "urethane" values varying from 0.1 to 1.4 mg % and averaging 0.8 mg %. Five other bloods from patients receiving drugs (sulfadiazine, streptomycin, penicillin, phenobarbital and amytal) in therapeutic doses gave "urethane" values from 0 to 1.1 mg % and averaging 0.5 mg %. These "urethane" values are zero within the accuracy of the method, hence urethane values below 1.5 mg % have no significance.

Clinical blood urethane levels. Patients with wound infections receive urethane locally.[2] In 2 such cases, we have found the blood urethane to be 3.1 and 5.6 mg %, after 4 and 8 days of treatment, respectively.

Application of method for ethyl alcohol determination. The blank determination in the urethane method may be used for the determination of ethyl alcohol since the conditions of the distillation and incubation were chosen to give quantitative recoveries of ethyl alcohol in amounts yielded in the urethane determination, that is, about ½ the amount of urethane. A standard alcohol curve was constructed from dilutions of a standard alcohol solution prepared from a weighed quantity of absolute ethyl alcohol. The curve was linear up to 0.4 mg alcohol, the 0.6 mg point deviating about 5 percent from the line. The equation of the line up to the 0.4 mg point was:

Mg Ethyl Alcohol = 1.65 (0.4436 − L).

Strictly speaking the optical density at zero alcohol (0.4436 in the equation) should be that of a blank distillation with water.

Summary. A colorimetric method is described for the determination of urethane in blood, which gives 99 to 111% recoveries in the range 4 to 44 mg % urethane, and a non-specific "urethane" value of normal blood of 0.1-1.4 mg %.

A part of the method may be directly used for the determination of ethyl alcohol.

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Agglutination of Sea Urchin Eggs and Sperm by Basic Proteins.

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It is well known that basic proteins (protamines and histones) react with a wide variety of unrelated substances forming precipitates (with proteins, nucleic acids, etc.), causing cell agglutination (*i.e.* erythrocytes,[1] bacteria[1]) and exerting various physiological effects (*i.e.* parthenogenesis,[2] bacteriostasis[3]).

[1] Lajmanovich, S., and Mittelman, N., *Rev. Inst. Bact.*, "Carlos G. Malbran," 1944, **12**, 320.