8535 C

Determination of Reduced Ascorbic Acid in Small Amounts of Blood.

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We previously presented data as to the quantity of reduced ascorbic acid in blood plasma and showed its dependence upon the dietary supply of vitamin C, or of ascorbic acid administered as a medicament.[1] The data were obtained by deproteinizing plasma by means of tungstic acid and titrating immediately the reduced ascorbic acid present with 2:6 dichlorobenzenoneindophenol.

In working with infants, and when making repeated observations on the same individual, it is desirable to have a method which requires a minimum of blood. We have, therefore, developed a micromethod requiring but 0.3 ml. blood, which may readily be secured from the finger of an adult, or the heel of an infant. In the guinea pig, blood is obtained by cardiac puncture, or sometimes by incision of the marginal vein of the ear. In the latter case, a special blood pipette is required.

Collection of Blood. Collect approximately 0.3 ml. blood from a lancet wound, into a small phial (Fig. 1, B) containing sufficient powdered potassium oxalate to prevent coagulation. Stir immediately with a toothpick or small glass rod. The phial is made by sealing the end of a piece of glass tubing of approximately 10 mm. outside diameter, and, while hot, pressing it against a piece of iron to form a flat base. The tubing is cut off at about 15 mm. from the closed end. The cut end is then ground flat on a fine stone wheel and the outer wall ground to a taper for a distance of 5 mm. from the top. The grinding produces a thin edge which greatly facilitates the collection of blood from the heel of an infant. It is unnecessary to grind the side wall if the phial is used for finger blood only (which is permitted to drop directly into the phial as the finger is milked), or if blood is transferred from a syringe. The phial is stoppered (Stopper A), then placed in a suitable holder (a cork with a recess cut in it) and lowered into the metal tube of a centrifuge. It is centrifuged for a few minutes in the usual manner.

The pipette C (80 mm. x 5 mm. O.D.) is closed to a fine opening

[1] Farmer, C. J., and Abt, A. F., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1625.

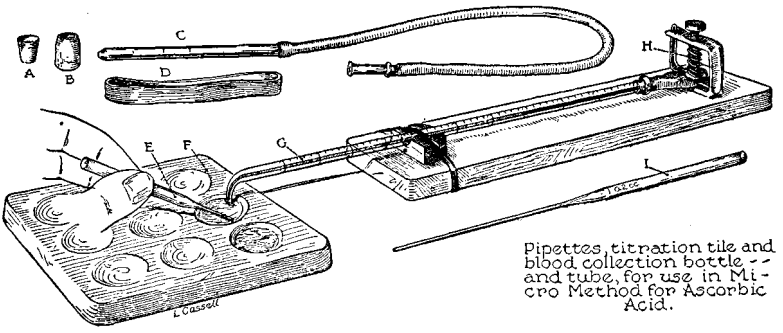

FIG. 1.

at one end and ground to a taper. It may be used to collect blood from the marginal vein of the ear in some guinea pigs having large, flat, vascular ears. A few crystals of potassium oxalate are placed in the tube, the rubber mouthpiece attached, and blood collected as when making a count. The graduations are at 0.3 ml. and 0.4 ml. capacity. When sufficient blood is obtained, detach the rubber tubing, and slip the wide rubber band D (approximately 75 mm. x 10 mm.) over both ends. Lower this assembly into the metal cup of the centrifuge, and centrifuge in the usual manner.

Deproteinization. Pipette 0.1 ml. of plasma (by means of a 0.1 ml. capillary pipette similar to I) into a 15 ml. conical centrifuge tube. With the same pipette, add 0.1 ml. distilled water, thereby rinsing the pipette. Add 0.2 ml. of a freshly prepared 5% metaphosphoric acid solution (pipette I) and mix thoroughly by tapping the centrifuge tube against the hand. Centrifuge for a few minutes. The 0.1 ml. and 0.2 ml. capillary pipettes are drawn from tubing of suitable size, and accurately calibrated.

Titration: The reduced ascorbic acid in the deproteinized plasma is titrated with a standardized solution of sodium 2:6 dichlorobenzenoneindophenol which is prepared as previously described.[1] A special pipette (G) is required (if a Rehberg microburette[2] is available, it may be used instead of the pipette described here) which is made from a thick-walled capillary tube of 0.8 mm. to 1 mm. bore. The capacity of the graduated portion is exactly 0.1 ml., and is contained in a length of about 200 mm. Each scale division is 0.002 ml., *i. e.*, the 0.1 ml. is divided into 50 parts, and calibrated at 5 points by means of mercury. The pipette is drawn out and curved downward at the end, and the tip ground to a cone by means of a grinding wheel. A thin film of vaseline is applied to the ground tip before use.

[2] Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry, Vol. II, p. 15. Williams and Wilkins, Baltimore, 1932.

The pipette is read to the nearest 0.001 ml. To operate the pipette, a rubber sac from a medicine dropper is filled two-thirds full of clean mercury and cemented or tied to the end of the glass tubing. This is compressed by means of a small Hoffman screw pinch cock which has a few coils of spring wire (H) wound loosely around the screw. The wire is attached by solder to the top bar of the clamp and to the movable bottom bar. The spring takes up lost motion in the screw and permits an even advance of the mercury in the capillary without back flow. The pipette is mounted on a lead base, the Hoffman clamp being attached by solder. A suitably grooved cork and rubber band support the protruding end of the pipette, as shown in the drawing. To fill the pipette, a few ml. of standardized dye solution are placed in a test tube. The test tube is inclined and the pipette inserted (without tilting) until the curved tip dips into the dye. The screw is now turned, compressing the rubber sac, until the mercury fills the capillary and a minute drop falls into the dye. The screw is then turned in the reverse direction until the pipette is filled with the dye to the desired amount.

The titration is carried out by pipetting 0.2 ml. of deproteinized plasma into one of the depressions of a white porcelain tile (F). Dip the curved end of the pipette into the fluid in the tile. *While stirring* with a fine pointed glass rod (E), slowly turn the screw of the Hoffman clamp until the first *faint* pink color is obtained. This is usually a permanent end point. From the reading of the pipette (minus the blank) and the standardization figure of the dye, the reduced ascorbic acid content of the plasma is calculated in milligrams per 100 ml. The blank is run by pipetting 0.1 ml. of 5% metaphosphoric acid solution and 0.1 ml. distilled water into a depression of the tile and titrating to the first permanent pink. The end point may easily be judged if 0.2 ml. of distilled water is pipetted into an adjacent depression in the tile plate. After titration, the pipette should be rinsed and left filled with distilled water.

The constancy of results obtained by this method is shown in

TABLE I.

Ascorbic Acid Solution Taken ml.	Blank ml. dye	Dye ml.	Ascorbic Acid Recovered mg.
.2	.005	.043	.00106
,,	,,	.042	.00103
,,	,,	.043	.00106
,,	,,	.043	.00106
,,	,,	.044	.00109
,,	,,	.043	.00106
Theoretical value			.00110

Table I, where a series of titrations were made on an ascorbic acid solution of known value. The ascorbic acid was dissolved in 2% metaphosphoric acid.

The use of metaphosphoric acid as a deproteinizing agent possesses many advantages over tungstic acid. Since we started to employ it (about 6 months ago), a paper appeared by Fujita and Iwatake[3] containing data on the relative stability of ascorbic acid in 2% metaphosphoric acid, 5% and 10% trichloracetic acid solutions, and water. They show no loss to occur in metaphosphoric acid for a period of 5 hours, whereas marked loss occurred in all other solutions. We have confirmed their results, and find no loss to occur in plasma filtrates standing over night when deproteinized with metaphosphoric acid. We, therefore, have abandoned tungstic acid as a deproteinizing agent, and when employing the macro-method,[1] proceed as follows:

Pipette 2 ml. of oxalated plasma into a round bottom 15 ml. centrifuge tube. Add 4 ml. of distilled water, followed by 4 ml. of 5% metaphosphoric acid solution. Thoroughly mix the contents of the tube, and centrifuge. Pipette 2 ml. portions of the deproteinized plasma into round bottom centrifuge tubes and titrate as described,[1] using a 5 ml. micro-burette. The titration may be made at any convenient time, but the plasma should be deproteinized as soon as possible.

In Table II, data are given showing the stability of ascorbic acid in plasma deproteinized with metaphosphoric acid.

TABLE II.
Ml. Dye Reduced by 0.2 ml. Metaphosphoric Acid Deproteinized Plasma.

Titrated Immediately* ml. dye	Titrated after 24 hours† ml. dye
.010	.010
.012	.012
.010	.010
.010	.010
.014	.012
.010	.010
.009	.010

*Samples taken from same person, at 30 minute intervals.
†After standing in refrigerator at 4° C. for 24 hours.

In Table III, we present data obtained on plasma, when 2 ml. samples were deproteinized by metaphosphoric acid, and the reduced ascorbic acid estimated by titration of a 2 ml. portion using a 5 cc. micro-burette, and a 0.2 ml. portion titrated by means of the micro-pipette described in this paper.

[3] Fujita, A., and Iwatake, D., *Biochem. Z.*, 1935, **277**, 293.

TABLE III.

Plasma Sample No.	——Ascorbic Acid Mg. %—— 2 ml. Portion	0.2 ml. Portion
1	1.75	1.82
2	0.70	0.84
3	0.98	1.12
4	0.54	0.53
5	1.35	1.15
6	0.86	0.76
7	0.63	0.77

Although the values are not identical, they are in as close agreement as can be expected considering the absolute amounts of ascorbic acid contained in the samples being titrated.

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8536 P

Pressor Reactions and Gastric Ulcer.

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The local effect of single and repeated spasm of the small blood vessels apparently ultimately results in alterations in the tissues that can be demonstrated objectively. This has been shown by us in production of experimental bacterial endocarditis and also observed in studies of bacterial localization in the central nervous system. Vascular spasms were induced by single and repeated injections of pitressin (betahypophamine).

Our experiments were carried out on dogs and rabbits. The dose most useful for our purpose was found to be 20 pressor units per 5 kg. of weight of the dog and 10 pressor units per 2 kg. of weight of the rabbit. The pitressin was administered intravenously from one single dose to 50 doses in dogs and from 5 to 19 doses in rabbits. The repeated injections were given twice a week. Five dogs and 2 rabbits died, the rest were killed at certain time intervals.

The gastrointestinal tract was examined and the following data obtained:

In 12 of the 35 dogs there were macroscopic pathological changes in the stomach (multiple hemorrhages, erosions and small ulcers),