27. A Simple Method for the Clinical Estimation of Ascorbic Acid in Whole Blood

By James Deeny, Eric T. Murdock and John J. Rogan, *Lurgan*

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The procedures suggested for the determination of ascorbic acid in whole blood [e.g. Emmerie & van Eekelen, 1934; Butler & Cushman, 1940; Keuther & Roe, 1941] are generally complicated or require apparatus which is not commonly available. In the course of work on ascorbic acid deficiency we have devised a technique which we have found suitable for clinical use. It is a modification of the method of Butler & Cushman [1940] and is based on the fact that saturation of whole blood with CO prevents the oxidation of ascorbic acid when blood is haemolysed [Kellie & Zilva, 1935; Butler & Cushman, 1939; Fujita *et al.* 1939].

METHOD

Reagents. (1) 2:6-Dichlorophenolindophenol. Make up a solution in freshly boiled and cooled glass-distilled water of such strength that 1 ml. is equivalent to 0.01 mg. ascorbic acid. This will keep 18–24 hr. without appreciable loss of strength, if in a dark container. (2) Metaphosphoric acid, 32 % solution in glass-distilled water. At room temperature this solution does not keep longer than 36 hr. (3) 2*N* sodium acetate (CH_3COONa , $3\text{H}_2\text{O}$). (4) Octyl alcohol.

Procedure. Place 2 ml. oxalated whole blood in a test-tube of 2 cm. internal diameter and add 2 drops octyl alcohol. Bubble coal gas through blood for 10 min., add 4 ml. glass-distilled water, continue passage of gas for 5 min. more and then add 2 ml. 32 % metaphosphoric acid and mix by gassing for 30 sec. Add 2 ml. sodium acetate solution and mix by tilting the tube. Transfer to a centrifuge tube and centrifuge. Titrate 2 ml. portions of the supernatant fluid, which should be crystal clear, in the centrifuge tubes, using a standard white light and white background. The indicator is measured from a 2 ml. microburette. A blank consisting of a 2 ml. sample of a mixture of 6 ml. water, 2 ml. metaphosphoric acid and 2 ml. sodium acetate solution, is titrated to a faint pink colour at the same time as the unknown; this facilitates the reading of the end-point of the unknown.

Calculation. If X = titration figure for unknown and Y = titration figure for blank,
$$\frac{5(X - Y)}{2} = \text{mg. ascorbic acid/100 ml. blood.}$$

Notes on method

Estimations should be carried out as soon as possible after the withdrawal of blood and care must be taken to avoid haemolysis. Test-tubes of 2 cm. internal diameter are advised because in smaller ones some bloods froth too much and in larger ones oxidation occurs, which can be detected by the alteration in colour of the precipitate from a bright red to a brownish tinge. In passing the gas through blood we have employed a 'wash-bottle' arrangement, using quill tubing. The issuing gas may be burnt. Since some of the sulphur compounds which occur as impurities in coal gas may reduce the indicator, it is advisable to test the gas for H_2S by means of lead acetate paper; if a positive reaction is found the gas should be passed through a KOH solution before it is used.

The accuracy of the method is illustrated by the results of two series of estimations recorded in Tables 1 and 2. Table 1 shows 'recovery' results obtained when ascorbic acid was added to samples of blood of determined ascorbic acid content. Table 2 shows the relative concentrations in plasma and cells and compares the sum of these values with

Table 1. *Recoveries of ascorbic acid added to whole blood*

Sample no.	Ascorbic acid originally present mg./100 ml.	Added mg./100 ml.	Amount found mg./100 ml.	Calculated amount mg./100 ml.	Error %
1	0.83	0.91	1.79	1.74	2.9
2	0.93	0.94	1.84	1.87	-1.6
3	0.93	1.42	2.39	2.35	1.7
4	0.77	0.44	1.20	1.21	-0.8
5	0.20	0.48	0.63	0.68	-7.4
6	0.20	0.97	1.14	1.17	-2.6
7	0.15	0.95	1.14	1.10	3.6
8	0.86	0.93	1.76	1.79	-1.7
9	0.47	0.97	1.45	1.44	0.7
10	0.25	0.75	1.03	1.00	3.0
11	0.29	1.07	1.37	1.36	0.7
12	1.18	0.94	2.12	2.12	0.0
13	0.70	1.02	1.65	1.72	-4.1
14	0.55	1.06	1.62	1.61	0.6
15	0.27	0.96	1.19	1.23	-3.3
16	1.11	0.69	1.73	1.80	-3.9
17	0.92	1.02	1.97	1.94	1.5
18	0.86	0.45	1.37	1.31	4.6
19	0.41	0.91	1.39	1.32	5.3
20	0.93	0.38	1.29	1.31	-2.3

Table 2. *Whole blood determinations checked against plasma and cell concentrations*

Ascorbic acid, mg. found in 100 ml.			Total cell volume/100 ml.	Calculated ascorbic acid mg./100 ml. whole blood			Difference between calculated whole blood value and that found by new method %
Whole blood	Plasma	Cells		In plasma	In cells	Total	
0.68	0.44	0.91	42.1	0.26	0.38	0.64	-5.9
0.20	0.01	0.48	40.1	0.00	0.19	0.19	-5.0
0.77	0.47	0.93	57.3	0.20	0.53	0.73	-5.2
0.66	0.48	0.82	41.6	0.28	0.34	0.62	-6.1
1.00	0.63	1.40	46.0	0.34	0.64	0.98	-2.0

those obtained by estimations carried out on the whole blood. Plasma values were determined by a modified form of the method of Farmer & Abt [1936] suggested to us by Dr H. M. Sinclair. The centrifuged cells were made up to their original volume and 2 ml. of the resulting solution were analysed by our method.

SUMMARY

A simple clinical method for the estimation of ascorbic acid in whole blood has been described.

An advantage of the method is that only 2 ml. of blood are required.

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