

Total Ascorbic Acid Content of Human Blood.

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The fact that generalized infections are more common in patients with a low tissue ascorbic acid content and that ascorbic acid may be related to this and other tissue phenomena have led to the suggestion that the determination of the ascorbic acid content of blood might be of diagnostic value.¹ We wish to report some values obtained in normal and pathologic patients by a method slightly modified from that of Emmerle and Van Eekelen.² This method determines the total content of ascorbic acid present in the reduced form, any oxidized material being converted to the reduced form by the procedure. We used 5 cc. instead of 10 cc. of blood and were able to increase the ease of obtaining satisfactory duplicate determinations by using acetic instead of trichloroacetic acid for the final titration.

Our procedure is as follows: To 5 cc. of oxalated whole blood in a 50 cc. Erlenmeyer flask, add 5 cc. of 10% trichloroacetic acid, shake and then add 5 cc. of 16.6% mercuric acetate; mix thoroughly and allow to stand for 5 minutes. Then add about 0.25 gm. calcium carbonate and mix until neutral to Congo Red. Transfer to a 15 cc. test-tube and centrifuge. Without decanting, allow H₂S to bubble through the supernatant solution for a few minutes and then filter into another 15 cc. test-tube. Through this filtrate again bubble H₂S until all the air in the tube is displaced; then stopper the tube and allow the solution to remain overnight in contact with the gas in the tube. On the following day bubble nitrogen through the solution for 15 minutes in order to remove the H₂S.

The 2:6 sodium dichlorophenolindophenol used for titration of the ascorbic acid is extracted with hot water (about 25 mg. in 50 cc.) and diluted until 12 cc. of this solution is equivalent to 1 mg. ascorbic acid as determined by standardization with a solution of pure ascorbic acid. To 5 cc. of the H₂S-free filtrate, 1 cc. of 10% acetic acid is added; the solution is mixed and titrated into a tube containing 0.1 cc. of the indicator solution. For the titration we have found it convenient to use a 1.0 cc. pipette calibrated in one-

¹ Yavorsky, M., Almaden, P., and King, C. G., *J. B. C.*, 1934, **106**, 525.

² Emmerle, A., and Van Eekelen, M., *Biochem. J.*, 1934, **28**, 1153.

TABLE I.

Amount of Ascorbic Acid added to 100 cc. blood mg.	Ascorbic Acid found per 100 cc. blood mg.	Recovery %
0	1.68	—
1.2	2.92	105
1.8	3.33	93

hundredths. The titration should be complete within 3 minutes. This procedure allows for sufficient filtrate to permit 2 titrations. Table I indicates the degree to which added ascorbic acid may be recovered from blood.

Allowing the blood to stand at room temperature for varying periods up to 24 hours has no appreciable effect on its ascorbic acid content.

All figures on which this report is based were obtained from bloods taken in the post-absorptive state and represent observations on about 100 different individuals. The values in apparently normal individuals ranged from 1.19 to 2.66 mg. %. Those in patients suffering from a variety of chronic diseases (including diabetes mellitus, hyperthyroidism, rheumatic heart disease, arteriosclerosis, acromegaly and chronic glomerular nephritis) ranged from 1.11 to 2.88 mg. %. No correlation between the diseases investigated and the ascorbic acid values obtained is apparent, although cases of coronary sclerosis were almost uniformly grouped at the upper limit of the above range. We have also been unable to make any correlation between the total ascorbic acid content of the blood and the dietary regime.

8000 P

A Biliary Precipitate Characteristic of Cholelithiasis.

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We have been unable to find any previous report of a chemical test by which bile drawn from the gall-bladder or bile ducts of patients suffering from cholelithiasis may be distinguished from

* This work was started at the Richard Morton Koster Laboratory, Brooklyn, New York.