8205 C

Ascorbic Acid Content of Blood.

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While numerous papers have appeared dealing with the ascorbic acid content of various plant and animal tissues, and urine, only a few refer to the quantity present in the blood. Van Eekelen, Emmerie, Josephy and Wolff,[1] and Emmerie and Van Eekelen[2] deproteinize blood, blood plasma, or serum with trichloracetic acid, precipitate interfering substances, chiefly SH compounds, with mercuric acetate, then treat with H_2S, which not only precipitates the mercury but also reduces that portion of ascorbic acid which in blood occurs in a reversibly oxidized state. The H_2S is later removed by a stream of nitrogen, and the ascorbic acid estimated by titration with 2:6 dichlorobenzenoneindophenol.[3] Gabbe[4] claims that loss of ascorbic acid occurs if a solution of pure ascorbic acid is treated with H_2S in the presence of mercuric acetate, and therefore, omits this step. Tauber and Kleiner[5] describe a method generally applicable to plant and animal tissues (and to blood) in which the essential features of deproteinization, removal of interfering substances, and H_2S reduction are preserved. They estimate the ascorbic acid present either by titration or by its ability to reduce potassium ferricyanide, with subsequent development of Prussian-blue upon the addition of the ferric gum ghatti reagent of Folin and Malmros.[6]

Our experience with several of these methods disclosed a number of difficulties. Some of the methods require considerable quantities of blood. Mercuric acetate solutions must not be over two weeks old, or filtrates containing colloidal sulphides may be obtained, particularly when the method is applied to urine. Any colloidal material in the filtrate makes it impossible to obtain a satisfactory

[1] Van Eekelen, M., Emmeric, A., Josephy, B., and Wolff, L. K., *Klin. Wschr.*, 1934, **13**, 564.

[2] Emmerie, A., and Van Eekelen, M., *Biochem. J.*, 1934, **28**, 1153.

[3] Tillmans, J., Hirsch, P., and Hirsch, W., *Zeitschr. f. Untersuch. d. Lebensmittel*, 1932, **63**, 1.

[4] Gabbe, E., *Klin. Wschr.*, 1934, **13**, 1389.

[5] Tauber, H., and Kleiner, E. S., *J. Biol. Chem.*, 1935, **108**, 563.

[6] Folin, O., and Malmros, J., *J. Biol. Chem.*, 1929, **83**, 115.

endpoint during the titration with the dyestuff. The endpoint with trichloracetic acid filtrates is not sharp.

In the present study, we are chiefly concerned with finding some method whereby the relative ascorbic acid level of blood in infants and children may be determined as an aid in studying subclinical scurvy and following its course under treatment. Since the amount of blood obtainable is frequently limited, the following method has been employed: Five or more cubic centimeters of blood are drawn and oxalated. The blood is centrifuged immediately, the plasma removed, and a tungstic acid filtrate prepared. Usually 2 cc. of plasma are pipetted into a round bottom 15 cc. centrifuge tube, 4 cc. distilled water added, followed by 2 cc. of 5% sodium tungstate, the contents mixed, then 2 cc. of ⅓ normal sulphuric acid added, in the usual manner.[7] After *thorough* mixing, the tube is allowed to stand for 1 to 2 minutes, then centrifuged. About 6 to 7 cc. of clear fluid are obtained. Two cubic centimeter portions are pipetted into round bottom centrifuge tubes and titrated immediately to the first faint pink color (compared with the untitrated solution) as rapidly as possible. A 5 cc. microburette (div. 0.01 or 0.02 cc.) is used to measure the required volume of dye solution.

The dyestuff is prepared as follows: Approximately 0.1 gm. of sodium 2:6 dichlorobenzenoneindophenol (E. K. Co. No. P 3463) is twice extracted with scalding hot water (for maximum solution). Each extract is poured through a small filter paper, combined, and diluted with freshly boiled and cooled water to a volume of 50 cc. Reject the insoluble residue. This stock solution is good for about 3 weeks. Dilute 10 cc. of this stock solution to 100 cc. with boiled and cooled distilled water. This diluted solution after standardization is used for titration of the deproteinized plasma fluid.

The diluted dyestuff is standardized as follows: A definite amount (approximately 60 mg.) of ascorbic acid (Cebione, Merck, 0.1 gm. ampules, vacuum sealed) is weighed out and dissolved in 5% acetic acid solution (made from freshly boiled and cooled water) and diluted to 100 cc. Two cubic centimeters of this solution are transferred to a 50 cc. volumetric flask and diluted to the mark with 5% acetic acid solution. (This solution approximates in ascorbic acid content that of deproteinized plasma.) The dilute dyestuff is now titrated against 2 cc. portions of dilute ascorbic acid solution, in the manner prescribed for titration of the deproteinized plasma

[7] Folin, O., Laboratory Manual of Biological Chemistry, 5th Ed., Appleton and Co., 1934, p. 263.

solution. We check the ascorbic acid solution further by titration against a 0.01 N iodine solution (1.14 cc. 0.01 iodine = 1 mg. ascorbic acid) which has previously been standardized against potassium iodate (Merck's Reagent). The ascorbic acid solution must be used immediately after standardization.

Table I shows the recovery of various quantities of ascorbic acid added to the plasma before deproteinization.

TABLE I.
Recovery of Added Ascorbic Acid (on Basis of 2 cc. Deproteinized Plasma).

Plasma	Initial Ascorbic Acid mg.	Added Ascorbic Acid mg.	Total Ascorbic Acid mg.	Recovered mg.	Recovered % of total A. A.
Dog*	.0045	.1838	.1883	.1782	94.64
"	.0045	.1838	.1883	.1800	95.59
Human	.0067	.0010	.0078	.0074	93.25
"	.0067	.0021	.0088	.0076	86.36
"	.0054	.0008	.0063	.0063	100.00

* In this series 2 additional determinations were run on the same plasma deproteinized by means of trichloracetic acid. The recovery values were 102.28 and 97.29 %, respectively.

Ascorbic acid values of plasma deproteinized by trichloracetic acid (2 cc. plasma, 5.5 cc. distilled water, 2.5 cc. 20% CCl_3COOH) have in many instances been determined on the same specimen as deproteinized by tungstic acid. The values in trichloracetic acid fil-

TABLE II.

Student	Age, Yrs.	Ascorbic Acid — Urine mg./cc. Single Specimen†	Ascorbic Acid — Blood Plasma mg./100 cc. Tungstic Acid Filtrate	Customary inclusion or absence of citrus fruit from diet before starting experiment
B.I.	32			Oranges daily
Initial value		0.0068	1.375	
O.J. for 1 week		.1150	1.463	
R.M.	22			Citrus fruit once a day
Initial value		.0015	1.030	
O.J. for 1 week		.0022	1.350	
A.N.	21			" " occasionally
Initial value		.0041	0.687	
O.J. for 1 week		.0049	1.350	
R.R.	22			2 or more oranges daily
Initial value		.0047	2.290	
O.J. for 1 week		.1200	2.360	
F.S.	21			Occasional orange
Initial value		.0011	0.916	
O.J. for 1 week		.0044	1.463	
E.W.	22			Citrus fruit daily
Initial value		.0016	1.145	
O.J. for 1 week		.0227	1.350	

† Estimated according to method of Harris and Ray, *Lancet*, 1935, **228**, 71.
O.J. = Orange juice.

trates are usually a few milligrams percent higher due to 2 causes: the endpoint of the titration is less definite, and trichloracetic acid apparently contains some foreign substance which reduces the dyestuff a little less rapidly than ascorbic acid.

Table II shows data obtained on a group of medical students receiving one pint of orange juice daily for one week.

Table III presents data taken at random on children attending the pediatrics clinic.

TABLE III.

Name	Age, Yrs.	Ascorbic Acid—— Urine mg./cc. Single Specimen	Ascorbic Acid—— Blood Plasma mg./100 cc. Tungstic Acid Filtrate	Customary diet: fruits, vegetables
B.M.	13½	.0083	0.819	Citrus fruit, green vegetables
E.R.	1	.0029	0.671	None
,,	1	—	0.513	Diet unchanged
,,	1	—	1.687	After receiving 30 mg. Cebione (Merck) daily for 1 week
K.P.	9	.0450	1.374	1 orange daily
M.W.	7	.0111	0.859	Green vegetables
E.M.	14	.0001	0.458	No fruit or tomato juice for years. (Allergic asthma)
M.J.	5	.0112	1.220	Orange or tomato daily
D.M.	1	.0049	1.168	Orange daily

Mirsky, Swadesh, and Soskin[8] in a recent study state that they have been unable to make any correlation between the *total* ascorbic acid content of blood and the dietary regime. Our data presented in the tables clearly indicate that the ascorbic acid percent in the blood in the *reduced* form follows closely the Vitamin C intake. Assuming the correctness of the observations of Mirsky, Swadesh, and Soskin, the conclusion is unavoidable that for clinical purposes the reduced ascorbic acid content of blood is of the greater significance. We are collecting additional data on this question.

Plasma deproteinized by tungstic acid cannot be treated with H_2S in order to effect a reduction of any ascorbic acid occurring in the oxidized form. Such treatment leads to the formation of a colloidal yellow tungsten sulphide, which up to the present has been impossible to remove. We have, however, subjected trichloracetic acid deproteinized plasma to H_2S reduction, and obtained enhanced ascorbic acid values. Several values are shown in Table IV.

[8] Mirsky, A., Swadesh, S., and Soskin, S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1130.

TABLE IV.
Ascorbic Acid Values in mg. per cc. Plasma.

Subject	D.N.	I.L.	V.W.	J.D.	J.F.
Tungstic Acid Filtrate Ascorbic Acid in reduced form	0.422	1.346	1.010	1.262	0.926
Trichloracetic Acid Filtrates after H_2S reduction Total Ascorbic Acid	0.802	1.683	1.978	1.569	1.123

These data show no constant relationship between reduced and total ascorbic acid values in the various plasma samples.

In conclusion, we wish again to state that this method is presented with full knowledge that it estimates only that portion of ascorbic acid occurring in plasma in the reduced form. It requires but small amounts of blood, and as far as we have been able to determine to date, accurately indicates the nutritional state of the body relative to Vitamin C.

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8206 P

Studies on Non-bacterial Cholecystitis. A Non-Traumatizing Technique for Study of Chemical Cholecystitis.*

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To produce an experimental cholecystitis from which conclusions can be drawn concerning human cholecystitis, 3 basic postulates must be fulfilled. First, the cholecystitis must be a direct result of the material used, and not even partially due to an inherent defect in the technique. Second, the material introduced must be such as is normally found either in the body fluids or in the ingested food, and should not be artificial and foreign to the body. Third, the reaction produced in the gall-bladder by this material must be similar in appearance to human cholecystitis.

The ideal method for introducing any substances into the gall bladder is by way of its natural passages, *e. g.*, the common duct.

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