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Studies on Reduced Ascorbic Acid Content of the Blood Plasma.*

L. D. GREENBERG, J. F. RINEHART AND N. M. PHATAK.

From the Departments of Pathology and Pharmacology, University of California Medical School.

Recently Farmer and Abt[1] described a method for the determination of the reduced ascorbic acid in the blood plasma. Briefly, the procedure consists in deproteinizing the blood plasma with tungstic acid and titration with 2:6 dichlorphenolindophenol. They reported that the values obtained by this method parallel the intake of vitamin C and are an accurate index of the nutritional state relative to vitamin C. We have made many determinations of the reduced ascorbic acid in the blood plasma using essentially the technique of Farmer and Abt. We find it rapid and reliable and our data lead us to concur with their opinion of its significance.

It is the purpose of this communication to present briefly some of our own studies which indicate that the plasma level of reduced ascorbic acid is an accurate index of the immediate nutritive level with regard to vitamin C and in health parallels the intake of the vitamin. Equally convincing data in studies of rheumatic fever and rheumatoid arthritis is reported separately. With very few exceptions our study is based upon post absorptive blood specimens. All data presented refer to the ascorbic acid in the blood plasma present in the reduced form.

Influence of Small and Large Doses of Vitamin C upon Plasma Levels. The plasma ascorbic acid levels of 7 apparently normal adults were determined before and after ingestion of 6 oz. of orange juice. The results, which are summarized in Table I, show an appreciable rise of the plasma level 2 to 4½ hours after the administration of this relatively small dose of vitamin C. The increases ranged from 15% to 69%. For this reason we believe that fasting blood specimens are essential for comparative data.

In Fig. 1, the plasma ascorbic acid curves of 4 individuals who received large doses of ascorbic acid (0.5 gm. per 100 lb.) are shown. The curves are similar in type to those for blood sugar with

* This work was aided by a donation from the California Fruit Growers Exchange and by the Christine Breon Fund for Medical Research. We are indebted to Hoffmann-La Roche, Inc., for supplies of ascorbic acid.

[1] Farmer, C. J., and Abt, A. F., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1625; Abt, A. F., Farmer, C. J., and Epstein, I. M., *J. Pediat.*, 1936, **8**, 1.

TABLE I.
Increase in Ascorbic Acid of Blood Plasma After a 6-oz. Dose of Orange Juice.*

Name	Plasma Ascorbic Acid		Time After Ingestion of Orange Juice, hr.	Increase, Mg. %
	Before, Mg. %	After, Mg. %		
L. L. G.	0.94	1.04	2½	.10
		1.15	4	.21
L. L. G.	1.01	1.39	4⅓	.38
A. H.	0.77	1.07	2	.30
L. D. G.	0.64	0.90	3¾	.26
J. F. R.	1.12	1.29	4⅓	.17
P. N.	0.54	0.82	4	.28
T. J. R.	0.82	1.02	3⅓	.20
F. A.	0.32	0.54	3½	.22

* 6 oz. orange juice used by us contained on the average 108 mg. of vitamin C.

the peak of the curve occurring much later; *i. e.*, 2 to 4 hours after ingestion.

Plasma Levels in "Normal" Adults—Correlation with Urinary Excretion and Diet Habit. Determination of the post-absorptive plasma vitamin C concentration was made on 55 medical students. The levels ranged from 0.25 to 1.48 mg. %, with an average of 0.72 mg. %. Twenty-five, or approximately 45%, gave values below 0.70 mg. %, a figure which is considered to be the lower range of normal by Farmer and Abt.[1] Fasting specimens probably account

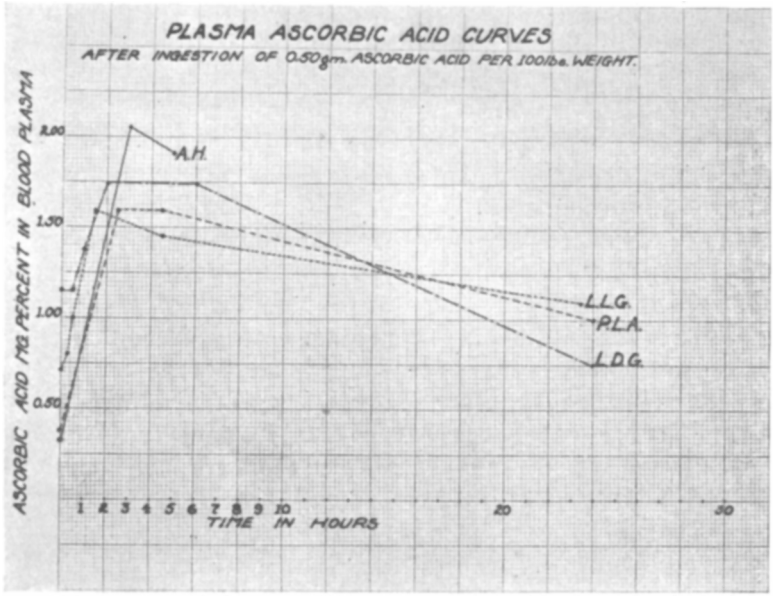

FIG. 1.

for lower levels in our series. It is also true that the nutritional habits of a rather large number of this group were inadequate. Using the test dose excretion procedure described by Harris and co-workers,[2] we find a close parallelism between the plasma ascorbic acid values and the urinary excretion following test doses of 250 to 300 mg. of vitamin C, either as orange juice or ascorbic acid. Of 25 students who excreted more than 50 mg. of ascorbic acid in the urine in the 24-hour period following the test dose, the average plasma value was 0.92 mg. %. The average value of 13 students who excreted less than 50 mg. was 0.44 mg. %. The recent dietary habit of 38 students was estimated with respect to vitamin C intake by an experienced nutritionist; 11 of these were rated as low in their vitamin C intake. In this group the average plasma ascorbic acid level was 0.55 mg. %. Of 19 cases considered fair in their vitamin C intake, the average plasma level was 0.75 mg. %. Eight students rated as good gave an average level of 1.1 mg. %. Complete data on this and a more extended "normal" group will be reported later. Seven students, who had low plasma vitamin C values initially, were given daily supplements of 100 mg. of ascorbic acid and subsequent determinations were made at various intervals. The results are summarized in Table II. It will be seen that 6 of the group responded with good or fair plasma concentrations of the vitamin in 5 to 7 days. The seventh student had a particularly poor nutritional history relative to vitamin C. Sixteen days were required to raise the plasma vitamin C to a correspondingly high level. These data clearly indicate a close parallelism between vitamin C intake and the value of reduced ascorbic acid in the blood plasma.

Further evidence is afforded in the following controlled dietary experiment on rhesus monkeys. Prior to beginning this experiment, 3 of the monkeys, (Nos. 1001, 1027 and 1032) had been main-

TABLE II.
Increase in Plasma Ascorbic Acid After Administration of 100 mg. of Vitamin C Daily.

Name	Initial Plasma Ascorbic Acid, Mg. %	Duration of Treatment, Days	Final Plasma Ascorbic Acid, Mg. %
J. D. H.	.46	5	1.06
B. O. K.	.43	5	1.07
Simard.	.42	11	0.80
F. O. D.	.26	16	0.88
		22	1.23
J. N. C.	.31	7	0.78
J. D.	.40	7	1.20
L. L. H.	.58	7	0.86

[2] Harris, L. J., Ray, S. N., and Ward, A., *Biochem. J.*, 1933, **27**, 2011.

tained on a sub-optimal vitamin C intake (approximately 6 cc. orange juice daily) for 13 months. The fourth, a control animal (No. 1030), had received a daily supplement of one-half an orange during this same period. Plasma analyses for ascorbic acid were made during the week of January 10. The diets of 3 of the monkeys were then altered as follows: No. 1030 now received no supplement of vitamin C; No. 1027 and No. 1032 were given one-half an orange daily. Monkey No. 1001 was allowed to continue on an unchanged regime. Periodic determinations of the ascorbic acid of the plasma were carried out for 5 months. The plasma levels of the monkeys over this period of time are represented graphically in Fig. 2. Note the very prominent drop in curve of monkey No. 1030 after withdrawal of the supplement and again the rapid rise after restoring the supplement on May 14. In the case of monkey No. 1001, the curve of the plasma ascorbic acid remained low. Although monkeys No. 1027 and No. 1032 exhibited increases in the plasma vitamin C after being placed on one-half an orange daily, the levels fluctuated considerably. The reason for this is unknown. It is possible that the prolonged period of deficiency to which these animals were previously subjected may have rendered the mechanism controlling vitamin C labile.

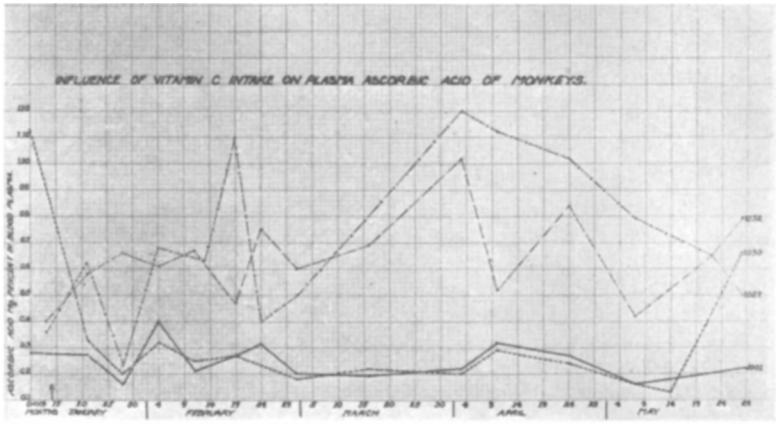

FIG. 2.

It is pertinent to note that the estimation of the reduced plasma ascorbic acid is only a measure of the immediate nutritive or metabolic level relative to vitamin C, and is dependent upon recent dietary habit to a large degree. Although it is an index of the vitamin C nutrition at the time of the test, in a single case a low level does not imply tissue injury or scurvy (either clinical or sub-clinical). The

latter results from the operation of sub-optimal or low metabolic levels over some period of time. Conversely, a good or high level would not indicate that deficiency had not operated to produce tissue injury in the past. A more accurate index of the degree of deficiency existing at the time in any given case can be had by serial determinations following administration of known vitamin C supplements. This is apparent in Table II and Fig. 2.

Summary. Experimental data is presented confirming the work of Farmer and Abt that the reduced ascorbic acid content of the blood plasma in the "normal" individual parallels the vitamin C intake. Confirmation of this is presented in a controlled dietary experiment using rhesus monkeys. For satisfactory comparative data, determinations should be made on fasting blood specimens. On the basis of the studies recorded, we believe that fasting plasma ascorbic acid levels below 0.7 mg. % are probably sub-optimal. Levels ranging between 0.7 and 0.9 mg. % would appear adequate. Optimal levels probably lie above this range. Reduced ascorbic acid plasma levels below 0.5 mg. % must be considered low.†

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"Three Dimension" Graphs for Correlating "Age-Weight-Gland" Relationships.

J. H. CLARK. (Introduced by L. G. Rowntree.)

From the Laboratories of the Philadelphia General Hospital.

In biometric studies of rats, it is desirable at times to compare simultaneously and graphically (1) Various organs by weight with (2) age and (3) body weight of the animal. By ordinary two dimension graphs, relationships between any 2 of these data can be well illustrated, but only by "three dimension" graphs is it possible to illustrate simultaneously the interrelationship of all 3—age, body-weight and weight of any given organ.

The construction of such graphs is simple (Chart 1): Ordinate AB forms with abscissae BC and BD an angle of 120°. AB represents age, the only constant uniform with all rats; BC body-weight and BD gland-weight. The "age-weight" is easily plotted, the line B_1C_1 paralleling BC and forming an angle of 120° (AB_1C_1). Gland-

† We wish to acknowledge our indebtedness to Dr. Nina Simmonds for the dietary surveys made upon the group of medical students.