

A STUDY OF THE NORMAL DISTRIBUTION OF ASCORBIC ACID BETWEEN THE RED CELLS AND PLASMA OF HUMAN BLOOD

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A number of investigations (2, 3, 6, 7, 10) within the past 10 years has demonstrated the significance of determining ascorbic acid in the whole blood or white blood cells in assessing the vitamin C nutriture of individuals. These studies have shown (a) that the blood cells may contain ascorbic acid when there is no vitamin in the serum (2, 3), (b) that scurvy does not appear until the cellular ascorbic acid is zero (2, 3), and (c) that the cellular (2, 3) or whole blood (6, 7, 10) values are closely correlated with the total bodily ascorbic acid. Because of the continued widespread use of ascorbic acid determinations on serum or plasma in nutritional surveys, it seemed that a study undertaken to examine the interrelation of ascorbic acid levels in the serum (plasma), red cells, and whole blood would contribute to an understanding of the data already amassed. Such an investigation was further indicated by the lack of agreement among those who have already explored this interrelation as to whether the red blood cell or the serum contained the larger amount of ascorbic acid.

The present report deals with a study of the interrelations of the ascorbic acid concentrations in the plasma, red cells, and whole blood, as determined in the cases of twenty-six healthy adults.

Methods

Throughout this study the ascorbic acid concentration of whole blood and of plasma was determined by the 2,4-dinitrophenylhydrazine method of Roe and Kuether (9). This method assays both ascorbic and dehydroascorbic acid.

The subjects used in the study were healthy men and women living on an ordinary diet. During the course of the investigation the diet of a few of the subjects was supplemented, for experimental purposes, with crystalline ascorbic acid. In all cases venous blood was obtained by venipuncture after 12 to 15 hours of fasting. The blood was immediately heparinized.

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Hematocrits were determined with Wintrobe tubes. The ascorbic acid concentration of the cells was calculated from the formula

$$A_c = (A_{wb} \times 1 - A_p V_p) / V_c$$

where A_c , A_{wb} , and A_p represent the ascorbic acid concentration of the cells, whole blood, and plasma respectively, and V_p and V_c represent the plasma and cell volumes respectively. V_c includes the buffy layer and hence A_c refers to total (red + white) blood cell ascorbic acid content.

Results

Table I summarizes the data collected on the eighteen healthy male and eight healthy female subjects. In all, thirty-eight determinations were made on these subjects. Essentially, the range of values we studied included the range of values reported by other investigators. The results will be presented in two parts.

(a) *Relation between A_c and A_p* —Because ascorbic acid is a water-soluble substance, we felt that a truer picture of the distribution of ascorbic acid between cells and plasma would be obtained if the ascorbic acid concentrations were expressed in terms of mg. per 100 ml. of plasma and red blood cell water. In making the necessary calculations we used the data given by Bodansky (1) on the water content of human serum and red cells.

Accordingly the data in Table I were recalculated in terms of ascorbic acid per 100 ml. of H_2O and were charted. The straight line

$$A_p = A_c - 0.45 \tag{a}$$

where A_p and A_c are ascorbic acid concentrations, in mg. per 100 ml. of plasma and cell water respectively, was fitted by inspection. We felt that the application of the more refined statistical methods was unwarranted in view of the small population used in this study. This equation satisfactorily describes the trend of our data. From the equation it is seen that when the ascorbic acid concentration in the plasma and that in the cells are expressed in relation to water, the cells contain more ascorbic acid than the plasma under fasting (equilibrium) conditions over the entire range of ascorbic acid concentrations in plasma reported in this study.

When the ascorbic acid concentration is expressed in mg. per cent (as in Table I), the relation between A_c and A_p becomes

$$A'_p = 1.43 A'_c - 0.72 \tag{b}$$

Expressed in this way, the amount of ascorbic acid in the cells is greater than the amount in the plasma only when A_p is less than 1.55 mg. per cent. Above this level there is more ascorbic acid in the plasma (because of the greater amount of water in the plasma than in the cells).

TABLE I

Ascorbic Acid Concentrations in Whole Blood, Plasma, and Red Blood Cells of Twenty-Six Healthy Subjects before Breakfast

Subject	Hematocrit	Whole blood ascorbic acid	Plasma ascorbic acid	Cellular ascorbic acid	$A_p:A_c$
	<i>per cent</i>	<i>mg. per cent</i>	<i>mg. per cent</i>	<i>mg. per cent</i>	
A. R.*	43.8	0.35	0.25	0.45	1.8
	47.1	0.45	0.30	0.55	1.8
*	44.0	0.50	0.30	0.70	2.3
	47.3	0.80	0.65	0.95	1.5
J. Pe.*	53.9	0.60	0.50	0.65	1.3
*	51.2	0.60	0.50	0.65	1.3
	54.3	0.70	0.55	0.85	1.5
	50.5	1.25	1.20	1.25	1.0
	51.6	1.90	2.00	1.80	0.9
J. Po.	47.7	0.60	0.30	0.90	3.0
H. G.	47.4	0.70	0.50	0.95	1.9
W. H.	47.0	0.70	0.60	0.80	1.3
L. Z.	51.6	0.80	0.70	0.90	1.3
F. C.	46.7	0.80	0.70	0.90	1.3
P. C.	46.8	0.80	0.65	0.95	1.5
G. N.	50.6	0.80	0.75	0.80	1.1
*	47.7	1.00	1.10	0.90	0.9
M. B.	44.2	0.85	1.00	0.65	0.65
D. D.	50.4	0.85	0.90	1.00	1.1
C. E.	50.0	0.90	0.75	1.05	1.4
L. H.	48.6	0.90	0.85	0.95	1.1
L. M.	45.8	0.95	0.95	0.95	1.0
M. S.	44.5	1.05	0.95	1.15	1.2
F. S.	44.4	1.10	1.10	1.10	1.0
	48.1	1.20	1.05	1.30	1.2
	45.6	1.50	1.30	1.80	1.4
F. B.	47.2	1.25	1.30	1.20	0.9
P. R.	43.4	1.25	1.20	1.35	1.1
	44.2	1.95	1.80	2.15	1.2
M. L.	43.9	1.40	1.35	1.45	1.1
R. J.	51.1	1.45	1.70	1.20	0.7
A. H.	41.9	1.55	1.45	1.60	1.1
	42.4	1.85	1.95	1.70	0.9
N. R.	47.4	1.65	1.70	1.60	0.95
P. K.	46.4	1.65	1.60	1.70	1.1
M. P.	43.4	1.70	1.85	1.50	0.8
M. M.	46.0	1.75	1.40	2.15	1.5
R. C.	43.4	1.85	1.90	1.80	0.95

* Blood obtained after a breakfast of toast and coffee; fasting with respect to ascorbic acid.

(b) *Relation between A_{wb} and $A_c:A_p$* —From equation (a) and the normal hematocrit we can calculate what the relation between A_{wb} and $A_c:A_p$

should be. Thus from $A_p = A_c - 0.45$ and $A_{wb} = 0.62 A_p + 0.38 A_c$ (normal hematocrit corrected for water content of whole blood, plasma, and cells (1)), we obtain

$$\frac{A_c}{A_p} = \frac{A_{wb} + 0.28}{A_{wb} - 0.17} \quad (c)$$

From our original data recalculated in terms of mg. per 100 ml. of H_2O , we plotted $A_c:A_p$ versus A_{wb} and fitted a curve based on equation (c). This equation satisfactorily described the relationship. In other words, the ratio of the concentration of ascorbic acid in the blood cells to the concentration of ascorbic acid in the plasma is related to the concentration of ascorbic acid in the whole blood. Below $A_{wb} = 1.5$ mg. per 100 ml. of H_2O , the dependency of the value of the ratio $A_c:A_p$ upon A_{wb} is most significant, for in these ranges the greater concentration of ascorbic acid in the cells assumes a larger rôle in determining the value of this ratio than in the range of values where A_{wb} is greater than 1.5.

A shift in the ratio $A_c:A_p$ with changing whole blood ascorbic acid content can be demonstrated when a single subject is followed at intervals before, during, and after saturation with ascorbic acid. Subject J. Pe. (Table I) was saturated with ascorbic acid, according to the criterion of Heineman (6), taking 1500 mg. of ascorbic acid in 24 hours. After saturation, the ratio decreased. During the following month, the ratio increased again as the whole blood ascorbic acid decreased. In another subject, who altered his diet so as to obtain a large amount of ascorbic acid from natural sources, we were able to demonstrate a decreased ratio (in a test about 1 month after the dietary alteration) as the whole blood ascorbic acid content increased (Subject A. R., Table I). Thus when one individual is followed through a period of changing ascorbic acid nutrition, we find the same relation between $A_c:A_p$ and A_{wb} as when several individuals, each differing with respect to ascorbic acid nutriture, are compared.

We believe that the peculiar form of the relation between $A_c:A_p$ arises from the facts that (1) $V_p > V_c$ and that (2) when $A_p = 0$, $A_c = 0.45$. Since the cells have a greater ascorbic acid content than does the plasma, as the plasma concentration approaches zero, the ratio $A_c:A_p$ rapidly approaches infinity.

DISCUSSION

Our finding that the ratio of the concentration of ascorbic acid in the cells to that in the plasma depends upon the ascorbic acid concentration in whole blood confirms the findings of other workers who have studied this problem. In 1938 Heineman (6) reported that in fasting subjects

the ratio of ascorbic acid in the cells to that in the plasma was always greater than 1 and that this ratio increased as the whole blood ascorbic acid decreased. Butler and Cushman (2) state that the ratio $A_c:A_p$ varies with the state of vitamin C nutrition. Recently Roe, Kuether, and Zimler (10) reported that, when the whole blood ascorbic acid was less than 0.6 mg. per cent, the whole blood contained more ascorbic acid than the plasma; when the whole blood ranged between 0.6 and 0.9 mg. per cent, the ascorbic acid content of whole blood and plasma was approximately equal, and when the whole blood exceeded 0.9 mg. per cent, the ascorbic acid concentration in whole blood was less than that of the plasma.

The fact that the distribution of ascorbic acid between the cells and the plasma is related to the whole blood level of ascorbic acid in part explains the disagreement in the literature concerning whether the ratio, $A_c:A_p$, is greater or less than 1. Examination of the reported data reveals that only a few of the investigators have studied the values of this ratio over the whole range of normally occurring ascorbic acid levels. This fact is clearly brought out by plotting on one chart all the available data from the literature. In general, the observed values follow closely the theoretical curve (equation (c)) describing the relation between $A_c:A_p$ and A_{wb} . The relatively wide scatter which obtains in such a plot can be attributed reasonably to differences in methodology. Some workers (2, 8, 12) did not estimate the combined ascorbic and dehydroascorbic acid concentrations, whereas others (5, 7, 10, 11, 13) have estimated the total ascorbic acid content. Some investigators (2, 8, 12) have estimated the cellular ascorbic acid by direct analysis; others (5, 6) have calculated the cellular ascorbic acid from the hematocrit (see Butler and Cushman (2) for a critique of some of the methods used).

Clinical experience supports the present results, for it is now known that when the plasma contains no ascorbic acid (experimental scurvy) the cells contain ascorbic acid until the patient is scorbutic (2, 3). The white blood cells and platelets seem to cling even more avidly to the ascorbic acid than do the red cells (2).¹ Experiments on the nature of this cellular binding of ascorbic acid will be reported in a subsequent communication.² The results of these experiments suggest that ascorbic acid forms a relatively stable bond with a protein fraction of the red cell. In addition, studies which are not yet completed suggest that ascorbic acid forms a loose bond with a protein of the plasma.²

¹ In this connection Cuttle's (4) observation is of interest. This worker found that in leucocytosis the whole blood contained more ascorbic acid than did the plasma. As the white count returned to normal, the whole blood contained less ascorbic acid than the plasma.

² Sargent, F., and Forbes, W. H., to be published.

SUMMARY

1. Thirty-eight determinations of ascorbic acid in the whole blood, plasma, and red blood cells were made on eighteen healthy males and eight healthy females.

2. When the ascorbic acid concentration is expressed in mg. per 100 ml. of blood cells or plasma, the relation between A_c and A_p is approximately $A'_p = 1.43 A'_c - 0.72$.

3. When the ascorbic acid concentration is expressed in mg. per 100 ml. of H_2O , the relation between A_c and A_p is approximately $A_p = A_c - 0.45$.

4. The ratio of the concentration of ascorbic acid in the cells to that in the plasma depends upon the concentration of ascorbic acid in the whole blood. This relation is approximately

$$A_c: A_p = \frac{A_{wb} + 0.28}{A_{wb} - 0.17}$$

where A is expressed in mg. per 100 ml. of H_2O .

5. We conclude that the disagreement in the literature concerning the distribution of ascorbic acid between cells and plasma (serum) is largely due to the fact that the various workers have not investigated the same range of whole blood ascorbic acid concentrations.

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