Ascorbic Acid Supplementation

II. Response of Certain Blood Constituents

MERTON P. LAMDEN, PH.D.,* SUSAN B. MERROW, M.ED.,† HAROLD B. PIERCE, PH.D.,‡
CRISTINA SCHWEIKER, M.S.,§ CHESTER A. NEWHALL, M.D.¶ AND ALICE LAUGHLIN, M.S.||

THE EFFECT of ascorbic acid intake from foods and supplements on serum and white cell levels of this vitamin has been reported by numerous investigators.[1–12] In general, where intake and blood levels have been low, increased ingestion has been followed by an elevation of the blood levels. On the other hand, when intake and blood levels have initially been relatively high, supplements as large as 1 gm. per day for three months have resulted in no progressive change in either the serum or white blood cell levels.[9]

This paper presents an analysis of the data on intake and blood levels of certain nutrients obtained during a three-year study of the response of lesions of the gums to ascorbic acid supplementation.[13] Serum levels of ascorbic acid, vitamin A, carotene and protein, and white cell ascorbic acid levels were studied in relation to the intakes of these same nutrients.

EXPERIMENTAL PROCEDURE

Duplicate determinations were made of serum and white cell ascorbic acid,[14,15] serum vitamin A, carotene[16] and protein,[17] and ar average was obtained of the analyses of three blood samples for each season. Details of this investigation, such as blood collection, selection of subjects and estimation of nutrient intake have been described in the preceding paper.[13]

A study of the urinary excretion of ascorbic acid was conducted with nine subjects. In the fall of 1952, subjects having high, medium and low serum ascorbic acid levels, according to the initial blood determinations, were selected from the prospective test group. The twenty-four–hour excretion of ascorbic acid was determined by a modification of the 2,4-dinitrophenylhydrazine method[18] one week before, on the first day, and after two, five and twenty-seven months of supplementation. Except for the first day of supplementation, values are averages of two successive twenty-four–hour urine collections. Serum and white cell ascorbic acid levels were also determined on the days urine samples were collected.

RESULTS

The data collected in the initial period, fall 1952, were used as a baseline for comparisons of changes that occurred in the five subsequent periods through spring 1955. Although interest was primarily in changes from the beginning to the end of the study, comparisons were also made from fall 1952 to fall 1954 to allow for a possible seasonal effect.

Ascorbic Acid Intake

The average daily intake of ascorbic acid from food (Table I) was approximately the same for test and control groups at the begin-

From the College of Medicine and the Vermont Agricultural Experiment Station, University of Vermont, Burlington, Vermont.

* Associate Professor of Biochemistry, College of Medicine; † Associate Nutritionist, Vermont Agricultural Experimental Station; ‡ Professor of Biochemistry and Chairman of Department; § Assistant Biochemist, Vermont Agricultural Experiment Station; ¶ Professor of Anatomy and Chairman of Department; || Assistant Biochemist, Vermont Agricultural Experiment Station. Present address: Columbia-Presbyterian Medical Center, New York, New York.

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TABLE I
Average Ascorbic Acid Intake

Season and Year	Test Group (mg.)	Control Group (mg.)	Significance of Difference Between Groups (%)
F, 52	63 ± 3*	60 ± 4*	n.s.
S, 53	412 ± 17	57 ± 3	1
F, 53	363 ± 15	50 ± 4	1
S, 54	386 ± 17	58 ± 4	1
F, 54	390 ± 17	47 ± 3	1
S, 55	340 ± 20	59 ± 5	1

NOTE: In this and other tables, F = fall; S = spring.
* Standard errors.

ning of the study. During supplementation the total average ascorbic acid intake per day of the test group was six to eight times greater than that of the control group.

Ascorbic Acid Content of Serum and White Blood Cells

Tables II and III show the average ascorbic acid contents of the serum and white cells of the test and control groups, and comparisons between the two groups in each of the six seasons. In the fall of 1952, before the supplement was started, the average values of serum and white cell ascorbic acid were in the middle of the range of average levels reported for adults.[12,19–26] At this time the average serum ascorbic acid level for the test group was significantly higher ($P < 0.05$) than that of the control group. In each of the subsequent periods, the average serum ascorbic acid levels of the test group were significantly higher ($P < 0.01$) than those of the control group and the differences between the two groups were considerably greater than in the initial period. In the fall of 1952 the average white cell ascorbic acid levels of the two groups were approximately the same, but with supplementation these levels were significantly higher for the test than for the control group in all periods except the fall of 1953.

TABLE II
Average Serum Ascorbic Acid Levels

Season and Year	Test Group (mg. %)	Control Group (mg. %)	Significance of Difference Between Groups (%)
F, 52	1.17 ± 0.07*	0.98 ± 0.05*	5
S, 53	2.03 ± 0.10	1.26 ± 0.07	1
F, 53	1.87 ± 0.11	1.29 ± 0.06	1
S, 54	2.04 ± 0.07	1.36 ± 0.07	1
F, 54	1.57 ± 0.12	1.08 ± 0.06	1
S, 55	1.58 ± 0.12	1.17 ± 0.06	1

* Standard errors.

TABLE III
Average White Blood Cell Ascorbic Acid Levels

Season and Year	Test Group (mg. %)	Control Group (mg. %)	Significance of Difference Between Groups (%)
F, 52	28.67 ± 1.12*	28.39 ± 0.71*	n.s.
S, 53	31.67 ± 1.10	28.21 ± 0.89	5
F, 53	32.95 ± 1.26	30.07 ± 1.16	n.s.
S, 54	32.40 ± 1.59	25.90 ± 1.49	1
F, 54	32.38 ± 2.52	25.32 ± 1.41	5
S, 55	36.52 ± 1.71	28.07 ± 1.08	1

* Standard errors.

The average serum levels of the test group were significantly higher in the fall of 1954 ($P < 0.01$) and the spring of 1955 ($P < 0.01$) than in the fall of 1952. The average serum level of the control group was also significantly higher ($P < 0.05$) in the spring of 1955 than in the fall of 1952 but this cannot be attributed to an increased intake of ascorbic acid.

The average white cell ascorbic acid level of the test group was higher in the two final periods than in the fall of 1952. The change from fall 1952 to spring 1955 was significant ($P < 0.01$). In the control group there was less, but not significantly less, ascorbic acid in the white cells in the final periods than in the base period.

It is apparent that the supplement taken by the test group resulted in an increase in both serum and white cell ascorbic acid levels over the entire period.

Ascorbic Acid Correlations

No significant correlations were found between ascorbic acid intake and white cell ascorbic acid level. With the exception of the control group in the fall of 1952, the positive correlations between ascorbic acid intakes and serum ascorbic acid levels were significant [r values: test group +0.436 ($P < 0.05$), +0.454 ($P < 0.05$), +0.509 ($P < 0.05$); control group +0.549 ($P < 0.01$), +0.376 ($P < 0.05$)]. Similar results were also found in nutritional status studies conducted in the northeast region[27] and by Hard et al.[28] On the other hand, Dodds and MacLeod[29] found limited association between dietary ascorbic acid and serum ascorbic acid levels. However, in their study, intakes were based on dietary records of only one or two days. Bryan et al.[1] reported a correlation between ascorbic acid intake and plasma ascorbic acid level only when this level was less than 1.0 mg. per cent.

The correlations between serum and white cell ascorbic acid levels were not significant except for the control group in the spring of 1955 ($r = +0.495$, $P < 0.01$). Morse et al.[12] found significant positive correlations when adult women subjects were receiving ascorbic acid intakes of 33 mg., 58 mg. and 83 mg. but not when they were receiving 133 mg. of ascorbic acid. In a second study, Morse et al.[30] reported no significant correlations with intakes over 47 mg. These findings suggest that significant correlations between serum and white cell levels would be unlikely at the intake levels of both the test and control groups in the present study.

Rank correlation coefficients[31] indicated there was no significant correlation between changes from the base period to the two final periods in ascorbic acid intakes and white cell levels in either group. A significant positive correlation between changes in ascorbic acid intakes and in serum ascorbic acid levels occurred only in the test group in the period from fall 1952 to spring 1955 ($r_s = +0.696$, $P < 0.01$).

There was a significant positive correlation ($r_s = +0.433$, $P < 0.05$) between changes in serum and white cell ascorbic acid levels of the test group from fall 1952 to spring 1955 and a positive correlation ($r_s = +0.405$) significant at the 10 per cent level between changes from fall 1952 to fall 1954. That is, the subjects with the greatest increase in serum ascorbic acid tended to be the subjects with the greatest increase in white cell ascorbic acid. Whereas the correlation between the serum and white cell levels in one season was not significant (with one exception), as previously noted, the rank correlation determined for *changes* in levels caused by supplementation tended to be significant.

Nutrient Interrelationships

Relations between the intakes of vitamin A, carotene, and protein and their respective levels in the serum were also investigated.

The average dietary intakes of vitamin A and carotene of both groups were essentially similar in all seasons. For the most part, the combined intake of vitamin A and carotene exceeded the recommended dietary allowance of the National Research Council. The serum carotene levels in both groups showed a consistent seasonal pattern with values for the spring lower than those of the previous fall. There was no significant difference between the serum vitamin A or carotene levels of the test and control groups in any season, and the ascorbic acid supplement seemed to have no significant effect on the average serum levels of vitamin A or carotene. Analysis of the data suggested a positive association between changes in serum vitamin A and carotene levels unrelated to the ascorbic acid supplement.

The average protein intake of each group was initially above the recommended dietary allowance of the National Research Council and decreased significantly to below this allowance during the period of the investigation. The average serum protein was the same for each group throughout the study and decreased significantly over this period, but no consistent correlation was found between protein intake and serum protein level or between changes in intake and serum level. This indicates that, although the average serum protein level of the group decreased when the average protein intake decreased, it would not be possible to predict the serum protein level from the protein intake or the extent to which a decrease in protein intake of a subject would be reflected in a decrease in the serum protein level.

In order to uncover any possible interrelations, the data were further analyzed. Rank correlation coefficients were determined for all the remaining possible associations on changes between and among the diet and blood constituents studied. Although occasional significant correlations occurred, there seemed to be no consistent pattern.

Ascorbic Acid Excretion

Urinary excretion of ascorbic acid in the nine selected subjects averaged 34 mg., 56 mg., 384 mg., 333 mg. and 332 mg. one week before, on the first day, and after two, five, and twenty-seven months of supplementation, respectively. The increase in ascorbic acid excretion after the first day of supplementation was significant ($P < 0.01$). The decreases in urinary excretion of ascorbic acid on the last two sampling dates were not significant.

During the first day of supplementation there was only a slight rise in the urinary excretion of ascorbic acid indicating that, on the average, these subjects were unsaturated with respect to ascorbic acid. Considering the average daily ascorbic acid intake of 63 mg. as indicated by seventeen-day diet records and the serum ascorbic acid level of 1.21 mg. per cent (range 0.96 to 1.60 mg. per cent), a greater increase might have been expected. Roderuck et al.,[32] however, reported that there may be a lower correlation between mean intake and either blood concentration or urinary excretion if the daily intake is irregular. The situation in this study may have been similar. The initial average daily intake from the seventeen-day diet records ranged from 42 to 74 mg. and individual ascorbic acid intakes recorded for the days during which samples were collected varied widely from the average calculated from the seventeen-day diet records.

Within the individual sampling dates there were no significant correlations between the urinary excretion of ascorbic acid and the intake or white cell levels. When the data on urinary excretion and intake before supplementation and after two, five and twenty-seven months of supplementation were pooled, there was a significant positive correlation ($r = +0.805$, $P < 0.01$). The only significant correlation ($r = +0.855$, $P < 0.01$) between the urinary excretion and serum levels of ascorbic acid occurred on the first day of supplementation.

SUMMARY AND CONCLUSIONS

The effect of ascorbic acid supplementation on the serum and white cell ascorbic acid levels, the serum levels of vitamin A, carotene and protein, and the urinary excretion of ascorbic acid of women of college age was studied for three years. Serum and white cell ascorbic acid levels of the test group which received large daily doses of ascorbic acid were significantly higher than those of the control group. There were significantly higher levels for the test group in the final periods than in the base period. The subjects in the test group having the greatest increase in serum ascorbic acid tended to have the greatest increase in white cell ascorbic acid levels. The supplement did not affect the serum levels of vitamin A, carotene and protein. In both the test and control subjects, the ascorbic acid intakes were significantly correlated with the serum ascorbic acid levels. The amount of change in one was related to the amount of change in the other only in the test group from the base to the final period.

The ascorbic acid supplement greatly increased the urinary excretion of this nutrient throughout the study. There was a significant positive correlation between urinary excretion and fasting serum levels of ascorbic acid on the first day of supplementation.

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