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ASCORBIC ACID PLASMA LEVELS AND GINGIVAL HEALTH IN PERSONNEL WINTERING OVER IN ANTARCTICA

by

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SUMMARY PAGE

THE PROBLEM

To determine the relationship between ascorbic acid plasma levels and gingival health as studied in men exposed to the stress of wintering over in Antarctica during the International Geophysical Year, 1957-58.

FINDINGS

Determinations of the ascorbic acid plasma levels of the twenty-six men who were the subjects of this study revealed a significant difference between the thirteen outdoor workers and the thirteen who were indoor workers. However, no significant differences were found in the gingival tissue health of the two groups. Several possible explanations are suggested for the lack of correlation between the two items.

APPLICATION

The information gained in this study will be of interest to those concerned with the effect of the stresses of an extremely cold and hostile environment, such as that experienced in Antarctica.

ADMINISTRATIVE INFORMATION

This investigation was undertaken as a part of Bureau of Medicine and Surgery Research Project MR005.14-5220, under Subtask (2), Study of Oral Health in the Antarctic. The present report is No. 6 on this Subtask. It was published in the Journal of Dental Research, Vol. 40, No. 4, July-August 1961.

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Ascorbic Acid Plasma Levels and Gingival Health in Personnel Wintering Over in Antarctica

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Studies have shown that animals exposed to the stress of extreme cold have an increased need for ascorbic acid.[1–3] The objective of this paper is to present the relationship between ascorbic acid plasma levels and gingival health as studied in men exposed to the stress of wintering over in Antarctica during the International Geophysical Year, 1957–58. Gingival tissue was selected as an indicator of tissue health, since it seems to reflect ascorbic acid deficiencies.

EXPERIMENTAL METHOD

This study was based on the monthly clinical examination, by the same investigator, of 26 men ranging in ages from twenty-one to thirty-seven years. The experimental group of 26 men was composed of 13 outdoor workers and 13 indoor workers. The age range of the outdoor group was twenty-one to thirty-five years, with an average age of 27.6 years. The age range of the indoor group was twenty-one to thirty-seven years, and the average age was 28.4 years.

Beginning in January, 1958, and until November, 1958, the subjects of the study volunteered 30 cc. of blood monthly for routine hematologic and blood chemistry analyses.†

Ascorbic acid determinations were made on the plasma of each blood sample taken from all subjects on the same day before breakfast. The analyses were completed the same day that the samples were drawn. The method used was that of Roe and Kuether,[4] in which the ascorbic acid, contained in the trichloroacetic acid filtrate of the blood is oxidized to dehydroascorbic acid by shaking with activated charcoal and coupled with 2,4 dinitrophenylhydrazine to form an osazone. The resulting osazone is treated with sulfuric acid to produce a red color, which is measured photometrically.

At monthly dental appointments, intraoral colored photographs (Kodachrome) were taken of each subject. A careful clinical examination was then conducted, with particular attention given to the degree of gingival inflammation, which was recorded using modifications of the P.M.A. Index (papilla, marginal, and attached gingiva), after the method of Massler, Schour, and Chopra.[5] The criteria used for assessing

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† The specific blood studies done were: hematologies: red-cell count, white-cell count, differential, hemoglobin; hematocrit, sedimentation rate. Sera electrolytes: sodium, potassium, chloride, phosphorus, calcium. Blood chemistries: blood glucose, non-protein nitrogen, blood urea nitrogen, ascorbic acid, total protein, creatinine, uric acid, fibrinogen, prothrombin time.

the degree of gingival health were the same as those used by the latter authors. However, in all evaluations the attached gingiva (A) was not considered; only the papillary (P) and marginal (M) gingiva were scored.

A monthly health diary was kept on all men by the dental officer. This diary was designed by the Personnel Assessment Branch of the Medical Research Laboratory, U.S. Naval Submarine Base, New London, Groton, Connecticut. The diary consisted of seventy-five questions which each subject was required to answer each month. The answers to the questions provided an indication of the subjects' physical well-being and psychological adjustment. Data obtained from the health diary included a monthly record of weight, cigarette consumption, supplementary vitamin intake (tablets and capsules), and dietary desires and preferences.

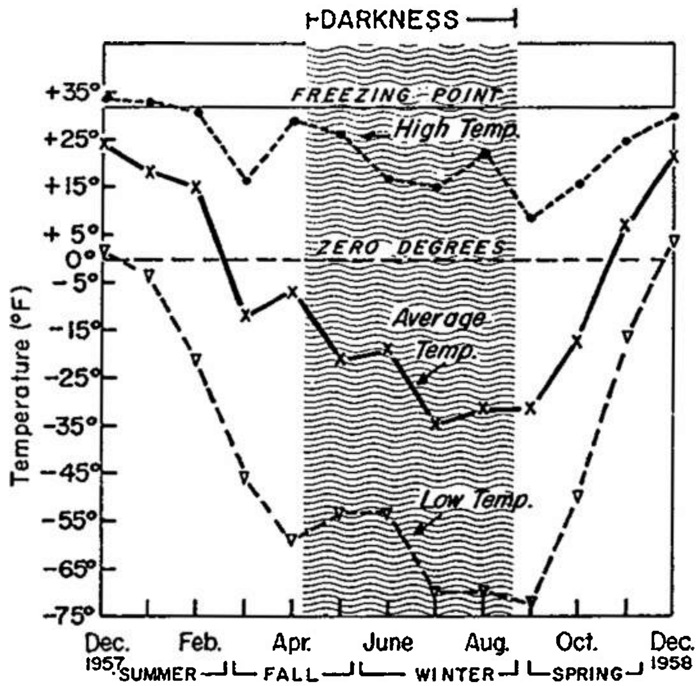

FIG. 1.—High, low, and average monthly temperatures recorded at Little America V, Antarctica, during the IGY, 1957–58.

Daily outdoor temperatures were provided by meteorologists of the U.S. Department of Commerce, Weather Bureau. These temperature readings served as a basis for establishing the possible effects that temperature variation had upon ascorbic acid plasma levels. The high, low, and average monthly temperatures recorded at Little America V are summarized in Figure 1.

RESULTS

The monthly ascorbic acid plasma level (AAPL) determinations on each subject (expressed in mg per cent) were tabulated (see Table 1). From these data two average AAPL's were calculated, one for the group at the end of each month and one for the individual at the end of the eleven-month period.

It is obvious at a glance that the average individual AAPL's of the outdoor group were generally lower than those of the indoor group. This table also shows that the average individual AAPL's for the year of the indoor group ranged from approximately 0.6 to 1.2 mg per cent (see *left side,* Fig. 2). This range falls within what is generally accepted as normal. However, the average individual AAPL's for the outdoor group over the same period ranged from approximately 0.5 to 0.7 mg per cent, which is generally accepted as low normal.

In order to examine the reliability of the differences between the two groups, a

TABLE 1

MONTHLY ASCORBIC ACID PLASMA LEVEL (AAPL) DETERMINATIONS (EXPRESSED IN MG PER CENT)

Subject	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Av. Indiv. AAPL
						Indoor Group						
1........	0.65	0.62	0.60	0.65	0.65	0.60	0.52	0.50	0.65	0.53	0.55	.593
2........	0.87	0.85	0.90	0.86	0.85	0.80	0.75	0.83	0.75	0.73	0.90	.826
3........	0.80	0.85	0.92	0.87	0.85	0.80	0.80	0.85	0.95	0.93	0.95	.870
4........	0.83	0.80	0.88	0.92	0.95	0.95	0.90	0.85	0.80	0.85	0.85	.870
5........	0.92	0.90	0.87	0.88	0.85	0.85	0.88	0.85	0.80	0.82	0.95	.870
6........	0.96	0.90	0.91	0.85	0.87	0.85	0.80	0.85	0.81	0.85	0.95	.873
7........	0.95	0.85	0.87	0.90	0.92	0.95	0.90	0.94	0.95	0.93	0.95	.919
8........	0.90	0.95	0.90	0.95	0.90	0.95	0.97	0.95	0.90	0.95	0.87	.926
9........	0.94	0.93	0.95	0.95	0.90	0.95	0.90	0.90	0.95	0.97	1.10	.952
10........	1.05	1.05	0.95	0.87	0.85	0.85	0.81	0.85	1.10	1.00	1.10	.953
11........	1.10	1.10	0.97	0.98	0.93	0.87	0.80	0.85	0.95	0.92	1.10	.961
12........	1.20	1.25	0.95	0.97	0.95	1.10	1.25	1.10	1.20	1.10	1.15	1.11
13........	1.10	1.05	1.10	1.25	1.10	1.20	1.20	1.25	1.10	1.20	1.25	1.16
Monthly group average AAPL.	.944	.931	.905	.915	.890	.902	.883	.890	.916	.906	.975	.913
						Outdoor Group						
14........	0.67	0.65	0.60	0.60	0.60	0.55	0.50	0.46	0.45	0.43	0.45	.541
15........	0.67	0.60	0.65	0.60	0.64	0.60	0.56	0.46	0.45	0.40	0.45	.553
16........	0.65	0.65	0.68	0.65	0.60	0.61	0.53	0.45	0.47	0.45	0.40	.558
17........	0.75	0.70	0.70	0.72	0.60	0.55	0.50	0.45	0.45	0.40	0.43	.568
18........	0.72	0.70	0.70	0.63	0.65	0.60	0.51	0.46	0.48	0.45	0.40	.573
19........	0.74	0.75	0.70	0.75	0.63	0.60	0.55	0.43	0.40	0.43	0.40	.580
20........	0.65	0.60	0.65	0.65	0.61	0.62	0.58	0.51	0.50	0.53	0.55	.586
21........	0.75	0.72	0.75	0.70	0.65	0.56	0.55	0.49	0.46	0.45	0.40	.589
22........	0.75	0.70	0.80	0.75	0.71	0.66	0.65	0.51	0.50	0.50	0.45	.634
23........	0.77	0.80	0.75	0.75	0.65	0.60	0.57	0.52	0.55	0.50	0.55	.637
24........	0.75	0.77	0.70	0.70	0.62	0.61	0.60	0.61	0.60	0.62	0.65	.657
25........	0.85	0.80	0.75	0.70	0.72	0.70	0.65	0.60	0.57	0.55	0.50	.672
26........	0.84	0.80	0.84	0.80	0.75	0.71	0.65	0.50	0.52	0.55	0.50	.678
Monthly group average AAPL	.735	.711	.713	.692	.648	.613	.569	.496	.492	.482	.472	.602

statistical analysis of the data was performed.* The use of an experimental design which involves successive measurements of the *same* subjects necessitates the utilization of statistical techniques that take into account the effects of correlation between the monthly measurements. Consequently, an analysis-of-variance technique designed for this purpose was employed.[6] By this method, a stringent statistical test of the significance of the differences between the indoor and outdoor groups, in terms of varying ascorbic acid plasma levels, over an eleven-month period was provided. This analysis is presented in Table 2.

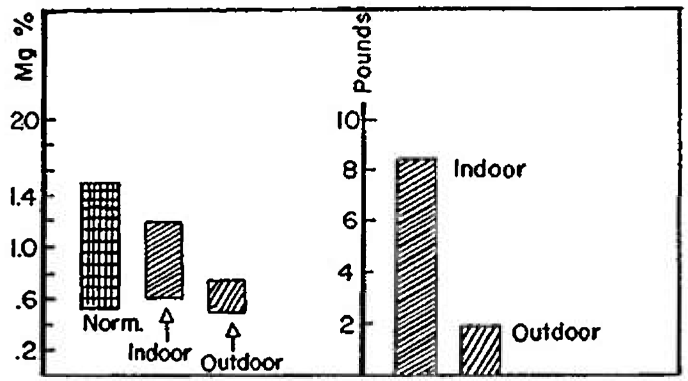

FIG. 2.—(*Left*) Comparison of the range of average AAPL's of indoor-outdoor groups with normal AAPL range. (*Right*) Comparison of average individual weight gained by indoor-outdoor groups.

TABLE 2

VARIANCE ANALYSIS OF ASCORBIC ACID PLASMA DATA FOR INDOOR-OUTDOOR GROUPS

Source of Variation	Sum of Squares	Degree of Freedom	Mean Squares	*F* Ratio	*P*
A. Between groups	69,644.63*	1	69,644.63	60.43	<0.001
B. Between subjects	27,657.43	24	1,152.39	....	....
C. Between months	7,516.41	10	751.64	257.41	<0.001
D. Interaction: groups×months	7,497.18	10	749.72	256.75	<0.001
E. Interaction: total subjects × months	701.26	240	2.92	....	....
Total	113,016.91	285	369.55	....	....

* Each raw score was multiplied by a constant (100) to eliminate decimals. This manipulation does not, of course, affect the *F* ratios.

Line A in Table 2 examines the question, "Do the mean ascorbic acid plasma levels differ between the indoor and outdoor groups over the eleven-month period?" The answer is "Yes," as the odds are less than one in a thousand for the differences being accounted for by chance. Line B answers the question, "Are there systematic differences over the subjects of both groups?" The obvious answer is "Yes." This step gives preparatory data for interpretation of lines C and D. Line C examines statistically the question, "Considering both indoor and outdoor groups together, do the AAPL's

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differ from month to month," The answer is "Yes," as the odds are again less than one in a thousand for the difference being accounted for by chance. Line D tests the most important hypothesis, examining the question, "Does the difference between the two groups change significantly from month to month?" In this case, once again, the answer is "Yes," as the odds are less than one in a thousand for the differences being accounted for by chance.

To represent graphically the differences in AAPL's between the two groups, the monthly group average AAPL's are presented in a bar graph (see Fig. 3). This graph shows clearly the relatively high and comparatively constant AAPL's of the indoor

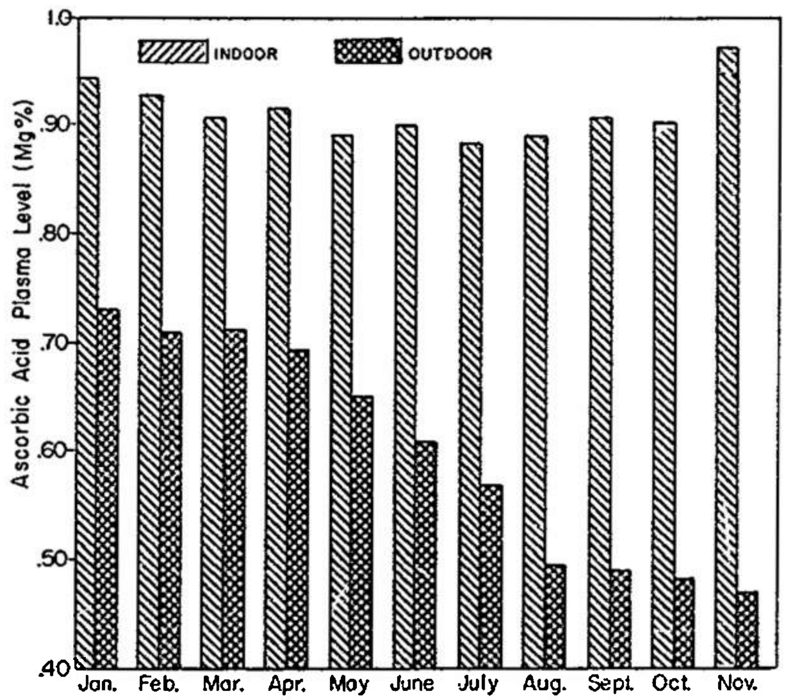

Fig. 3.—Comparison of the average monthly ascorbic acid plasma levels of the indoor and outdoor groups.

group. The AAPL's of the outdoor group were lower from the beginning and became progressively lower throughout the entire period. It is interesting to note that in Figure 4, which compares the average group AAPL's with the average monthly outdoor temperatures, the indoor group levels increased when the temperatures rose, whereas there was merely a reduction in the rate of decline for the outdoor group.

Two modifications of the P.M.A. Index were used in the analyses of the data obtained for the evaluation of gingival health. First, the number of inflamed areas, regardless of intensity, was considered. Then the data were re-evaluated and the intensity of inflammation included. Intensity was scored on a scale using normal = 0, mild gingivitis = 1, moderate gingivitis = 2, severe gingivitis = 3, and Vincent's infection = 4.

Since the number of susceptible areas varies from one subject to another, depending on the number of teeth present, the subjects were compared on a percentage basis. Table 3 presents a comparison of the indoor-outdoor individual and group average percentages on the basis of inflamed areas and intensity of inflammation. This table shows the total number of teeth of each subject, which established the total possible number of P + M areas susceptible to inflammation. Each subject's monthly P.M.A. score for the entire experimental period was analyzed to yield an individual average number of inflamed areas. These individual averages were used to compute the average

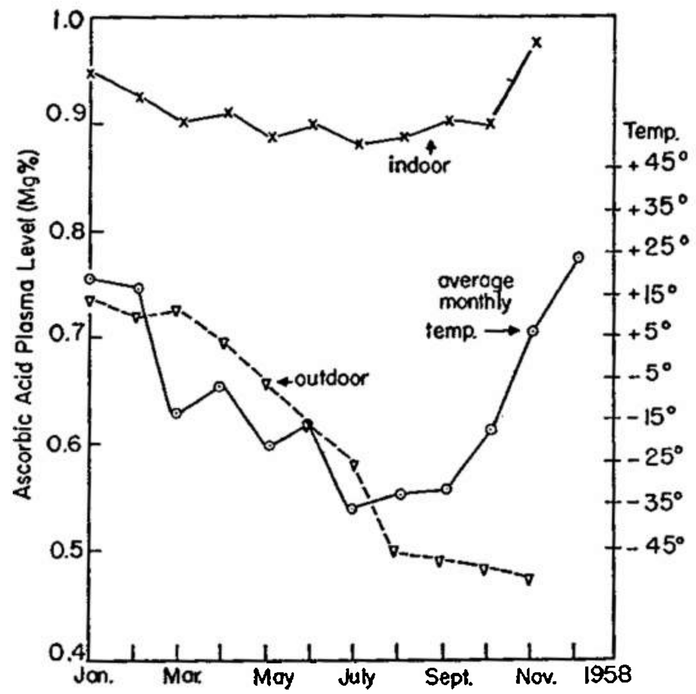

FIG. 4.—Comparison of the average monthly ascorbic acid plasma levels of the indoor and outdoor groups with the average monthly temperatures.

fraction of the total number of areas inflamed in each individual on a percentage basis, using the following formula:

$$\frac{\text{Individual average number of P + M inflamed areas}}{\text{Total individual P + M areas}} \times 100 = \text{Percentage of inflamed areas} .$$

Each subject's monthly P.M.A. score was then reanalyzed by taking the sum of the numbers assigned to each inflamed area according to the scale indicated above. These individual monthly total P + M scores were averaged to obtain the individual average P + M scores for the entire experimental period (see Table 3).

In order to compare the gingival health of both groups on a basis of intensity of inflammation, it was decided to compute a hypothetical highest possible P + M score that any subject could attain, which was represented by the product of four times the total number of susceptible areas (total P + M) in any particular subject's mouth. The fraction of the hypothetical P + M score actually observed in each mouth was computed, using the following formula:

$$\frac{(P+M)\,\text{score}}{4\,(\text{total}\ P+M)} \times 100 = \text{Percentage of highest possible}\ (P+M)\ \text{score}\ .$$

From the individual average percentages of the highest possible P + M scores, a group average percentage figure was obtained. This figure was used to represent the average intensity of gingival inflammation for each group over the entire experimental period (see Table 3).

The data on ascorbic acid plasma levels clearly show a significant difference be-

TABLE 3

COMPARISON OF INDOOR-OUTDOOR INDIVIDUAL AND GROUP AVERAGE PERCENTAGES ON BASIS OF INFLAMED AREAS AND INTENSITY OF INFLAMMATION

Subject	Total No. of Teeth	Total P+M Areas	Average No. of Inflamed Areas	Average Per Cent Inflamed Areas	Highest Possible P+M Score	Average P+M Score (Intensity)	Per Cent of Highest Possible Score
	Indoor Group						
1	21	39	38	97	156	83	53
2	24	46	46	100	184	92	50
3	23	39	39	100	156	60	38
4	25	46	46	100	184	71	39
5	26	50	39	78	200	59	30
6	28	54	0	0	216	0	0
7	28	54	18	33	216	21	10
8	28	54	39	72	216	43	20
9	23	43	2	5	172	3	2
10	26	49	4	8	196	4	2
11	26	48	42	88	192	51	27
12	27	51	0	0	204	0	0
13	28	54	2	4	216	3	1
	333	48	24	53	....	38	21
	Outdoor Group						
14	21	32	28	88	128	30	23
15	28	54	47	87	216	63	29
16	28	54	29	54	216	33	15
17	22	40	31	78	160	35	22
18	23	44	11	25	176	11	6
19	21	40	40	100	160	73	46
20	25	45	44	98	180	66	37
21	25	48	1	2	192	1	5
22	26	49	48	98	196	82	42
23	27	52	3	6	208	3	1
24	26	48	17	35	192	23	12
25	28	54	21	39	216	28	13
26	23	43	39	91	172	44	26
	323	46	28	62	....	38	21

tween the indoor and outdoor groups. The question now arises, "Will this difference be manifest in the degrees of gingival health of the two groups?"

The answer, based on the number of inflamed areas in both groups, was, as shown in Table 3 and also in Figure 5, that there were 9 per cent more inflamed areas in the outdoor group, 62 per cent, as compared with the indoor group, 53 per cent. A non-parametric test applied to these two distributions showed no significant differences.[7] The answer to the above question based on the intensity of inflammation (P + M score) is that there was no significant difference in oral health of the groups, since they both presented equal scores.

A review of the monthly health diary indicates that the subjects of this study utilized supplementary vitamin sources to a varying degree. Table 4 indicates the frequency with which the outdoor-indoor subjects utilized supplementary vitamin sources.

It appears from Table 4 that the indoor group utilized supplementary vitamin sources to a greater extent than did the outdoor group. The question arose, "Did this account for the significant difference in the AAPL's between the two groups?" Grouping the frequency data in Table 4 into two groups—those never taking vitamins and those taking vitamins once a week or more often (1 degree of freedom)—resulted in a χ^2 of 0.6, reaching significance only at the 50 per cent level of confidence.

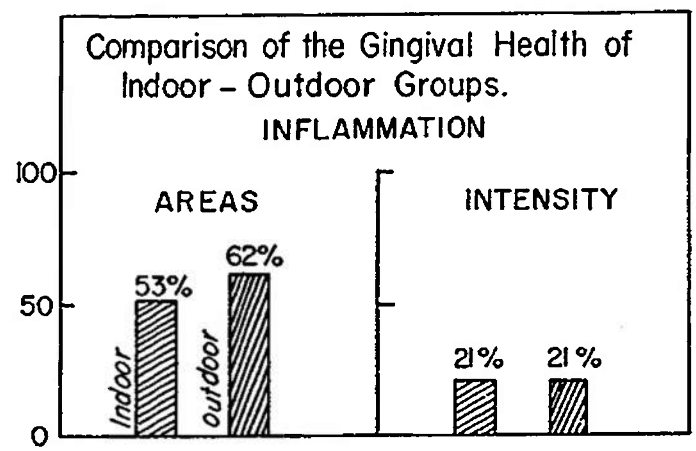

FIG. 5.—Comparison of the gingival health of indoor-outdoor groups. Group average percentages compared on basis of inflamed areas and intensity of inflammation.

TABLE 4

SUPPLEMENTARY VITAMIN HABITS OF INDOOR-OUTDOOR SUBJECTS

Frequency	Outdoor	Indoor
1. Each day	0	3
2. Three times a week	0	1
3. Two times a week	3	2
4. Once a week	3	2
5. Never	7	5

It is interesting to note in this nutritional study (*right side*, Fig. 2) that the average weight gain for the indoor group was 8.4 pounds, as compared with 1.9 pounds for the outdoor group.

The data from the health diaries indicated that both groups smoked to approximately the same extent. The indoor group averaged 18 cigarettes per man per day, and the outdoor group averaged 17 cigarettes per man per day. Lack of a significant difference in smoking between the two groups is of interest, as it has been suggested that smoking increased the need for ascorbic acid.[8]

DISCUSSION

Why was there not a larger clinical difference in the gingival health of the two groups, despite the significant difference in their ascorbic acid plasma levels? There may be several explanations for this lack of correlation. First, it may well be that a physiological difference in oral health did exist but that our gross clinical methods for evaluating it were not adequate to reflect these fine changes. It may be that the use of a tooth mobilometer to indicate changing degrees of tooth mobility[9] or the plotting of rewarming curves[10] to measure gingival circulation would give a more sensitive measure of the degree of oral health.

Second, it may be that, since the AAPL's of both groups were within the wide range of normal, that is, between 0.50 and 1.5 mg per cent, no difference in gingival health should be expected. When the plasma contains less ascorbic acid than 0.50 mg per cent, the body concentration may be designated as suboptimal. Plasma values below 0.15 mg per cent are invariably associated with clinical scurvy.[11] None of the subjects of this study had these low extremes of AAPL's, and therefore it may have been unwarranted to anticipate a dramatic difference in the gingival health of the two groups.

Third, as these results would seem to indicate, it may be that the degree of oral health and ascorbic acid plasma levels are not directly related. Perhaps, *tissue* levels of ascorbic acid are more significant than *plasma* levels. Cheraskin[8] suggests that what is needed is a laboratory test that measures ascorbic acid at the cellular level. The tissue levels of vitamin C may have been maintained at the expense of the plasma levels. Had tissue levels of ascorbic acid been recorded, using the intradermal dye decolorization test, perhaps more agreement with the oral-health findings would have resulted. Such a study is being performed in Antarctica at the present time.

It has been reported that there is a relationship between smoking and ascorbic acid requirements.[8] But in this study both groups smoked to the same extent, so that this factor can be disregarded.

The trend of the curves of the AAPL's (Fig. 4) of both groups are particularly interesting in terms of the adequacy of the vitamin C intake. The dietary sources of vitamin C available to all men at Little America were primarily from fresh-frozen citrus juices. Supplementary vitamin tablets, Hexavitamins and Decavitamins, and capsules, Viaquamin Therapeutic, containing vitamin C were also available and recommended but were taken strictly on a voluntary basis. Unfortunately, no record was kept as to how much fruit juice was consumed by each man. However, observations of the general dietary habits of the men indicated that a large majority drank at least three glasses (8 ounces per glass) of fresh-frozen citrus juice per day. The juice was served at each meal three times a day. All other frozen sources of vitamin C were cooked prior to serving and therefore lost most of their ascorbic acid content.

Figure 4 shows that the average AAPL's for the indoor group over the experimental period started out at 0.944 mg per cent and decreased slightly as the outdoor temperature dropped, then recovered to a figure slightly above the initial level when the outdoor temperatures rose. On the other hand, the average AAPL for the outdoor group at the beginning of the study was 0.735 mg per cent, and, as the temperatures dropped, the monthly group average AAPL's declined markedly. When the outdoor temperature rose again, the monthly group average AAPL's did not improve but merely decreased at a slower rate. This leads to the conclusion that the ascorbic acid intake of the outdoor group was insufficient to meet their needs.

The fact that a significant difference in AAPL's did occur suggests that the working environment of the two groups may have been responsible. It is well documented that the caloric requirements of men exposed to extreme cold is markedly increased.[2] This fact is substantiated by observations during the period of this study. The men of the outdoor group consumed substantially more food, estimated as at least twice as much as the indoor group, but gained only one-fourth as much weight as the indoor group. It seems that, despite the increased caloric intake, their rigorous outdoor labor, in an extremely cold environment, required a greatly increased metabolic rate.

Since body nutritional requirements were higher for the outdoor group, it is reasonable to assume that the ascorbic acid requirements would also be proportionately higher. It has been estimated that the ascorbic acid intakes of both groups were approximately equal; therefore, one would expect to find a lower plasma level in the group with the higher requirement as is shown in the results of this study.

SUMMARY

A report is presented of the dental research program conducted at Little America V, Antarctica, during the International Geophysical Year, 1957–58. The program was initiated to study blood elements and their possible relationship to the etiology of cold-weather oral-health problems. The blood studies conducted are listed. This paper reports the relationship of one of the blood components—ascorbic acid plasma levels to gingival tissue health. Twenty-six men, thirteen outdoor workers and thirteen indoor workers, were the subjects of the study. Determinations of the ascorbic acid plasma levels of these men revealed a significant difference between the indoor and outdoor groups. The gingival tissue health was evaluated by observations of the relative degrees of gingival inflammation presented by the subjects of both groups, and it was found that no significant differences existed in the gingival tissue health between the groups. Several possible explanations are suggested for the lack of correlation between the ascorbic acid plasma levels and the gingival tissue health.

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