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ASCORBIC ACID (VITAMIN C) AND ITS EFFECTS ON PARAINFLUENZA TYPE IH VIRUS INFECTION IN COTTON-TOPPED MARMOSETS[123]

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SUMMARY • Recent studies suggest that increased amounts of ascorbic acid (vitamin C) may increase resistance or after the clinical response of man to respiratory viral infections. To examine this question in a nonhuman primate, ascorbic odd was added to the diet of cotton-topped marmosets (Saguinus oedipus). Along with controls, these animals were inoculated intranasally with a strain of parainfluenza type III virus of marmoset origin. Increased amounts of vitamin C did not prevent virus infection or primary immunologic response in the animals, but it did delay onset of the disease, reduce clinical responses, and decrease mortality (57% v* 36%).

Studies on the microbial flora of marmosets by ourselves and others have identified some of the bacteria and viruses to which these animals are susceptible (1,2). The susceptibility of the marmoset to various respiratory viruses of presumed human origin suggested that these animals may be used to study nutrition-infection interactions. Pauling (3) has hypothesized that elevated blood levels of ascorbic acid in humans may be associated with a decreased susceptibility to respiratory illness of viral etiology. This paper reports 1) the results of a study in which cotton-topped marmosets *(Saguinus oedipus)* were used as a biomedical model to study this hypothesis, and 2) the effects of long-term ascorbic acid supplementation on these animals.

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MATERIALS AND METHODS

Marmosets: Cotton-topped marmosets *(Saguinus oedipus)* were conditioned for 2 mo prior to study. Visual health signs were recorded daily, and body weights and temperatures were recorded at specified intervals. The mean body weight of the marmosets used in this study was approximately 400 g. All marmosets were afebrile prior to virus inoculation. Animals were individually caged to assure that each animal would receive its designated amount of daily vitamin C.

Parainfluenza type III virus inoculations: Each of 14 vitamin C (ascorbic acid; L-Ascorbic Acid)[4,5] supplemented, and 7 non-supplemented (control) marmosets without detectable serum antibody to parainfluenza type III virus (dilution screening level 1:5) received intranasal inoculations. All were inoculated with 0.25 ml of 1000 $TGID_{50}$ of parainfluenza type HI 108 days after vitamin G supplementation was begun. Preliminary tests showed that 1000 $TGID_{50}$ of the virus would infect all marmosets and produce seroconversion.

Virologic examination: Routine throat swabs and blood specimens were obtained from these animals upon admission and during quarantine at monthly intervals. Throat swabs were processed as described earlier (2,4).

Viral identification and neutralization tests were made by hemadsorption (HAd) and hemadsorption-inhibition (HAd-I) tests using guinea pig red blood cells, as described by Hsiung (5). Marmoset serum antibody titers against parainfluenza type III virus were tested prior to inoculation and at 30 days post-inoculation.

Vitamin C supplementation: Marmosets in the supplemented group received orally 100 mg crystalline ascorbic acid twice a day, the equivalent of 35 g per day for a 70 kg man. This amount was used in order to study the effects of large doses of the vitamin on viral respiratory infection. Ascorbic acid supplementation was found to be most effective when the crystalline vitamin was mixed directly into banana slices which were given prior to feeding. Vitamin C supplementation was removed from the diet at 35 days post-inoculation.

Control and supplemented marmosets were bled every 2-4 wk throughout the course of the study. Bleedings of the supplemented animals were performed prior to daily administration of vitamin C in order to reduce the possibility of measuring transient concentrations of ascorbic acid. One ml samples of whole blood were taken from the femoral artery into syringes containing 0.1 ml (150 units) of heparin. Whole blood ascorbic acid (WBAA) determinations on trichloroacetic acid stabilized samples (6, 7) were performed according to the method of Roe and Kuether (8) as modified by Lowry *et al* (9).

RESULTS

The effects of vitamin C supplementation on concentrations of WBAA are shown in Fig 1 and Table 1. Concentrations of WBAA in the supplemented animals appeared to reach their maximum levels during the first month of supplementation (10) and did not further increase with continued supplementation. Small variations in WBAA concentrations, however, were seen in both supplemented and control groups throughout the course of supplementation. Vitamin C supplementation was discontinued 35 days post-inoculation, and concentrations of WBAA were monitored in order to test the hypo-

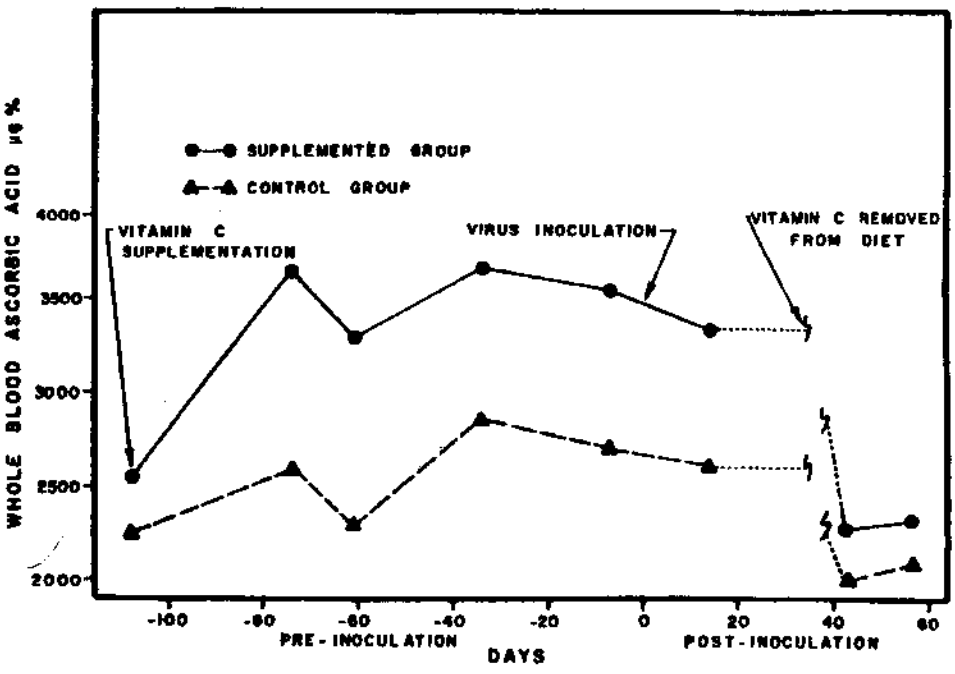

Fig 1. Whole blood ascorbic acid concentrotions in control and supplemented mormosets.

TABLE 1

Mean concentrations of whole blood ascorbic acid (WBAA) in control and supplemented marmosets vs time

Days before (−) and after (+) inoculation	WBAA in μg % ($\bar{X}$ ± SD) [a]Control	[b]Supplemented	Percent increase (B−A)/A x 100
-74	2592 ± 324 N = 8	3656 ± 1060 N = 14	41
-61	2285 ± 424 N = 8	3274 ± 557 N = 14	43
-34	2863 ± 504 N = 8	3677 ± 611 N = 14	28
- 7	2700 ± 781 N = 7	3563 ± 891 N = 14	32
+14	2608 ± 622 N = 7	3340 ± 767 N = 14	28
+42	2000 ± 363 N = 6	2271 ± 343 N = 11	14
+56	2090 ± 234 N = 3	2327 ± 368 N = 9	11

WBAA baseline values on day -108; Control: 2266 ± 303 (N = 10): Group to be supplemented: 2570 ± 490(N = 14)

thesis that animals removed from vitamin C supplementation would become scorbutic under otherwise normal dietary conditions (10).

Parainfluenza type III virus was isolated from throat swabs taken from all of the animals. The virus was isolated as early as 3 days post-inoculation and as long as 14 days post-inoculation. No differences in virus isolation rates at 3, 7, 10, and 14 days post-inoculation were observed between supplemented and non-supplemented animals. No statistically significant differences in serum antibody response (geometric means) were detected between the supplemented and non-supplemented groups. HAd-I titers (30 days post-inoculation) in the supplemented group ranged from 1:16 to 1:1024, and in the control group they ranged from 1:16 to 1:128.

Marmosets were observed for morbidity signs daily. Signs of illness were nasal discharge, ocular discharge, transient fever up to 105°F (ca 41°C), and lethargy. All non-supplemented and 7 of 14 supplemented marmocets exhibited signs within 3 days; however. the other 7 supplemented marmosets did not exhibit signs until 7 days post-inoculation. Mortality was observed in 5 of 14 (36%) of the supplemented animals and 4 of 7 (57%) of the control animals. The mean duration of illness in supplemented survivors was 9 days compared with 20 days in the control group.

DISCUSSION

Marmosets supplemented with vitamin G were shown to be as susceptible to primary infection with parainfluenza virus as control marmosets not receiving the vitamin supplementation. However, there appeared to be some therapeutic value of vitamin C since supplemented animals had fewer mean days of illness and experienced less mortality post-inoculation than did non-supplemented marmosets. Two recent publications by Wilson *et al* (11) and Anderson *et al* (12) have also reported a correlation between vitamin C utilization and the duration of "cold symptomatology" in hurnans. It should be noted that after vitamin C was removed from the supplemented marmosets' diets, no signs of scurvy or subnormal concentrations of WBAA were found. This indicates that vitamin C

supplementation does not involve the risk of post-supplementation scurvy.

Large doses of the vitamin did not prevent virus infection, suggesting that the mechanism of ascorbic acid does not involve direct interaction with the virus. The results of our study with marmosets indicate that vitamin C exerts its effects on the host, possibly by maintaining cellular and tissue integrity. Further investigations should be continued using this biomedical model to answer current questions on the effects of ascorbic acid on respiratory disease.

REFERENCES

1. **Deinhardt F, Holmes AW. Devine J, *et al:* Marmosets as laboratory animals. IV. The microbiology of laboratory marmosets. *Lab Anim Care* 17: 48-70, 1967**
2. **Murphy BL, Maynard JE, Krushak DH, *et al:* Microbial flora of imported marmosets: Viruses and enteric bacteria. *Lab Anim Sci* 22: 339-343, 1972**
3. **Pauling L: *Vitamin C and the Common Cold.* San Francisco, WH Freeman, 1970**
4. **Murphy BL, Maynard JE, Krushak DH, *et at:* Occurrence of a carrier state for *Herpesvirus tamarinus* in marmosets. *Appl Microbiol* 21: 50-52, 1971**
5. **Hsiung GD, Henderson JR: *Diagnostic Virology.* New Haven, Yale University Press, 1964, p 124**
6. **Bradley DW, Emery G, Maynard JE: Vitamin C in plasma: A comparative study of the vitamin stabilized with trichloroacetic acid or metaphosphoric acid and the effects of storage at -70° C, -20° C, 4° C, and 25° C on the stabilized vitamin, *Clin Chim Acta* 44: 47-52, 1973**
7. **Bradley DW, Maynard JE, Emery G: Comparison of ascorbic acid concentrations in whole blood obtained by venipuncture or by finger prick. *Clin Chem* 18: 968-970, 1972**
8. **Roe JH, Kuether CA: Determination of ascorbic acid in whole blood and urine through 2, 4-dinitrophenylhydrazine derivative of dehydroascorbic acid. *J Biol Chem* 147: 399-407, 1943**
9. **Lowry OH, Lopez JA, Bessey OA: The determination of ascorbic acid in small amounts of blood serum. *J Biol Chem* 160: 609-615, 1945**
10. **Rhead WJ, Schrauzer GN: Risks of long term ascorbic acid overdosage. *Nutr Rev* 29: 262-263, 1971**
11. **Wilson CWM, Loh HS: Common cold and vitamin C. *Lancet 1:* 638-641, 1973**
12. **Anderson TW, Reid DB, Benton GH: Vitamin C and the common cold: A double-blind trial. *Can Med Assoc J* 107: 503-508, 1972**