Trial of Ascorbic Acid in Prevention of Colds

GEORGINA H. WALKER,* M.B., CH.B. ; M. L. BYNOE,* M.B., D.T.M.&H.; D. A. J. TYRRELL,* M.D., F.R.C.P.

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Ascorbic acid is widely used as a prophylactic and therapeutic agent against the common cold, and is in fact often recommended for this purpose by medical practitioners. It may be administered as a large dose as soon as possible after the first symptoms are felt, and this dosage is then continued for a few days in order to " abort" the cold. Though many people use this treatment on themselves and believe it is effective, it is impossible to assess its efficacy. Untreated subjects may experience symptoms such as sneezing or a mild sore throat, which then disappear without going on to a full cold, and patients' assessments of their colds are much influenced by any treatment owing to the placebo effect (Diehl *et* a/., 1938).

In these studies we attempted to show by in-vitro experiments that exposure of cells to ascorbic acid increased their resistance to infection with viruses. Though there was no activity in vitro, we also attempted to demonstrate a protective effect in ftnmy»1« or man, since it was thought that host resistance might be enhanced indirectly.

Materials and Methods

Virus Strains.—Strains of viruses selected from the biological groups known to cause common colds and related diseases were used (see Table I).

• From the Common Cold Research Unit, Salisbury, Wilts.

Tissue Cultures.—Roller-tube cultures were used, as follows: (1) " Diploid " human embryo lung fibroblast cells (HEL). A semicontinuous .line isolated in this laboratory was used and maintained in Bogle's medium with 2% OK serum and antibiotics. (2) Monkey kidney cells (MK) ; cultures of secondary trypsinized cells were washed three times and maintained in Eagle's medium. (3) Hela cells of the Bristol line were main-* tained in 2% rabbit serum in Eagle's medium.

Ascorbic Acid.—Ascorbic acid for injection was added to tissue culture medium immediately before use. Preliminary experiments showed that 10 mg. of ascorbic acid/100 mL of medium was not toxic in HEL, HeLa, or MK cells, and there was no crystal deposition. This concentration was therefore

TABUt I.—*Virus Used in Tissue-culture Experiment* with Ascorbic***

Family	Group	Strain	Tissue Used for Propagation
Picornavirus ..	Rhinovirus H	16/60	Human embryo lung
» ..	» H	FEB	» » »
» ..	» M	HGP	» » »
Myxovirus ..	Influenza B	B Eng/101/62	Monkey kidney
» ..	Parainfluenza 3	Prototype	» »
» ..	Respiratory syncytial	Randall	HeLa
Adenovirus ..	Adenovirus 5	Prototype	»
Enterovirus ..	Poliovirus 1	LSc 2ab	Human embryo lung
» ..	E.C.H.O. 11	U virus	» » »
» ..	Coxsackie A21	Local strain	» » »
Herpesvirus ..	Herpes simplex	Perrett*	» » »

* Albanese *et al* (1966).

used in all the tissue-culture worfc. The volunteers took 1 g. of ascorbic acid three times a day as effervescent tablets supplied by Roche Laboratories. These were given for three days before virus inoculation, and for six days after. No side-effects of the treatment were observed. Control subjects were given placebo tablets which were indistinguishable from the ascorbic acid tablets except by chemical analysis.

Volunteers.—These were housed and observed as described elsewhere (Tyrrell, 1965). They ranged in age from 18 to 50 (mean 30.2 years). They received a generous mixed diet which included fresh fruit and cooked vegetables. The dietary ascorbic acid was not measured, but the trials were conducted when the general dietary intake both before and during the trials was expected to be low, so that the effects of supplements would be expected to be maximal. Two serum specimens were taken from most volunteers ; the first before virus inoculation, and the second about a fortnight after inoculation. They were tested for antibodies against the virus with which the volunteer had been inoculated by neutralization in tissue-culture for rhinoviruses and by haemagglutination inhibition for influenza. Nasal washings were collected daily from the volunteers for five days, from the first day after virus inoculation. Phosphate buffered saline was used to take the washings, and the specimens were either inoculated immediately into tissue-culture tubes.or mixed with an equal volume of nutrient broth, stored at -70° C. and tested later. Representative viruses isolated from the washings were typed by neutralization tests with specific antiserum. Volunteers were inoculated with a virus which had been propagated by serial passage in volunteers. The virus was stored as nasal washing at —70° C and thawed and diluted 1/10 in Hanks's saline immediately before use.

Mice.—Porton strain white mice were used. They were given 4 mg. daily of a freshly prepared solution of ascorbic acid in saline or of plain saline by intraperitoneal injection for six days before and nine days after virus inoculation. Influenza A (Mel) virus was administered as intranasal drops under light ether anaesthesia.

Results

Experiments in Tissue Cultures

Table II shows the results of experiments in which cells in tissue-culture tubes were exposed to medium containing ascorbic acid for one to one and a half hours before being inoculated with viruses. After inoculation the culture tubes were rolled at 33º C. and observed daily for a cytopathic effect or, in the case of tissue infected with a myxovirus, tested several days later by haemadsorption. The minimum infectious dose for each virus was the same in treated and in untreated cultures. However, it was shown that there was a marked decrease in the ascorbic acid content of the medium within four hours of its addition to medium whether in contact with cultures or not. In one experiment with adenovirus type 5 the medium was therefore changed every 12 hours for four days, but there was still no apparent antiviral effect.

TABLE II.—*Minimal Infectious Dose of Virus for Tissue Culture* Maintained in Medium Containing Ascorbic Acid*

Virus	Infectious Dilution (TCD_{50}) in Titrations Performed in Cultures Treated with	
	Ascorbic Acid	No Ascorbic Acid
Rhino M	10^{-4}	10^{-3}
Rhino H (16/60)	10^{-3}	10^{-3}
Rhino H (FEB)	10^{-4}	10^{-4}
Polio 1	$10^{-5\cdot5}$	10^{-5}
E.C.H.O. 11	$10^{-5\cdot5}$	10^{-5}
Coxsackie A21	10^{-4}	10^{-4}
Influenza B	10^{-5}	10^{-4}
Parainfluenza 3	10^{-4}	$10^{-4\cdot5}$
Respiratory syncytial	10^{-3}	$10^{-1\cdot5}$
Adeno 5	10^{-3}	$10^{-1\cdot5}$
Herpes	10^{-5}	10^{-5}

Experiments on Mice

Mice were treated with intraperitoneal ascorbic acid at a dose of about 300 mg./kg. As shown in Table JII, neither the mortality rate nor the extent of the lung lesions in the survivors was reduced.

TABLE III.—*Results* m *Mice Treated with Ascorbic Add and Challenged with Influenza. A Virus*

Treatment		Dilution of Virus	
		10^{-3}	10^{-4}
Ascorbic acid	Lung lesion score*	12/16	3/16
	Deaths	2/4	0/4
Saline	Lung lesion score	6/16	3/16
	Deaths	1/4	0/4

* Slightly modified from the method of Horsfall (1939).

Experiments on Volunteers

The experiments took place in the first five months of 1966. Volunteers received 3 g. daily—that is, about 75 mg.Ag./day—of ascorbic acid by mouth for three days before being challenged with a small dose of virus which had been passed from man to man. Table IV shows the number of volunteers who developed colds, and Table V the results of virus isolation studies and of antibody titrations on acute and convalescent sera.

The viruses used were drawn from all those known to be frequent causes of colds. The rhinoviruses were represented by three strains, two of which belonged to the M and H types, the third, H.S., being a recently described type which can be cultivated only in organ cultures. The B814 virus is a recently cultivated virus which is now known to be morphologically similar to avian bronchitis and to at least one other virus causing human colds (Almeida and Tyrrell, 1967) ; the influenza B virus is a typical myxovirus, and influenza and parainfluenza viruses cause an appreciable proportion of colds, particularly in

TABLE IV.—*Clinical Response of Volunteers who received Ascorbic Acid Tablets and were then Inoculated with Viruses Capable of Causing Colds*

	Results in Volunteers Receiving							
	Rhinoviruses		Influenza B Virus		B814 Virus		All Viruses	
	Ascorbic Acid	Placebo	Ascorbic Acid	Placebo	Ascorbic Acid	Placebo	Ascorbic Acid	Placebo
Number inoculated	29	26	8	8	10	10	47	44
Number of colds	9	9	4	4	5	5	18	18
Mean incubation period (days)	3·1	2·4	3	2·8	3	3·8	3	2·9
Clinical severity of colds: Mild	7	7	1	1	3	3	11	11
Moderate	—	1	—	1	2	—	2	2
Severe	2	1	3	2	—	2	5	5
Mean duration of colds (days)	7·4	9·2	10·7	8·5	6·6	4·6	8	8
Mean score*	6·5	5·9	13·7	9·2	6·2	7·0	8	7
Paper handkerchiefs used daily at peak of cold: Range	4–32	3–56	6–82	10–23	8–25	7–38	4–82	3–56
Mean	10	14	35	17·5	14	19·8	16·5	16·5

* The score is calculated by allotting points for both the number of days on which individual symptoms and signs were recorded and the severity of each.

young children. If ascorbic acid had a general prophylactic effect on colds it should have had an effect on colds produced by rhinoviruses and probably also by the other viruses used.

Table IV shows the results obtained. It indicates that the number of colds and their severity were not affected by ascorbic acid. The length of the incubation period and the duration of symptoms were variable, but the mean values of both these periods were the same in treated and untreated volunteers. In parallel with these experiments 18 volunteers received non-infectious intranasal drops and two developed a cold. In the case of two rhinoviruses and the influenza B virus it was possible

TABLE V<—*Results of Laboratory Tests on Volunteers Inoculated with Three Different Viruses*

	Results in Volunteers Given									
	Ascorbic Acid					Placebo				
	Total Volunteers	Colds	Virus Isolated	Antibody Rise	Laboratory Evidence of Infection	Total Volunteers	Colds	Virus Isolated	Antibody Rise	Laboratory Evidence of Infection
M Rhinovirus PK	15	5	5	6	9	16	3	5	2/14	5
H „ DC	6	2	4	5	6	3	2	2	3	3
Total rhinoviruses	21	7	9	11	15	19	5	7	5/17	8
Influenza B ..	8	4	5	2	5	8	4	6	1	6

to test for virus infection, and Table V shows that virus infection was not reduced in frequency by the treatment. Naturally, the volunteers who were infected were drawn from the group who had low antibody titres and the number of these in each trial varied unpredictably, but there was no evidence that subjects with little or no antibody were protected from infection by ascorbic acid. We considered the possibility that the results described in the literature might be due to a rather specific effect of ascorbic acid on other the symptoms of sore throat or the occurrence of mucopurulent nasal discharge. Thirteen volunteers who developed colds while taking ascorbic acid complained of sore throat, while 15 who were on placebo tablets did so. The comparable figures for mucopurulent nasal discharge were 10 and 8. It was concluded that there was no evidence for a general, antiviral, or symptomatic prophylactic effect of ascorbic acid in these experiments.

In two trials the volunteers were told whether or not they had been given ascorbic acid before their colds had finished, and the colds in the treated groups were appreciably shorter than in the controls ; in the third and later trials they were not told which they had received, and the duration of the colds was similar in both groups. This could have been due to the effect of ascorbic acid or to coincidence, but may have been a result of therapeutic suggestion.

We wished to confirm that the vitamin was being taken and absorbed properly. Therefore, in the trial in which influenza B was used a urine sample was collected from each volunteer during the incubation period. This was titrated for its ascorbic acid content by the dichlorophenol-indophenol method. No ascorbic acid was detected in the urine of nine volunteers receiving placebo, and from 28 to 126 mg./100 ml. (mean 73 mg./100 ml.) was found in eight receiving the vitamin.

Discussion

In reading the literature it has been noted that conflicting claims have been made regarding the value of vitamin C either alone or in combination with bioflavonoids in the prevention add treatment of acute infections of the upper respiratory tract.

Uncontrolled observations are of course impossible to evaluate, but even where placebos are used as a basis for comparison there have been many important differences between the studies reported ; they have differed in the type of clinical illness occurring in the population, the day and method of clinical assessment, the age and nutritional state of the subjects, and the amount of vitamin given and whether it was administered prophylactically or therapeutically. One trial, for instance, suggested that there was a marked reduction in the duration of colds in baseball players given prophylactic vitamin C (Barnes, 1961), but the control group observed were not players.

In another controlled trial there was an apparent improvement two days after treatment in subjects given a mixture of bioflavonoids, ascorbic acid, and aspirin rather than another analgesic capsule (Macon, 1956); it is impossible, however, to decide which of the differences in medication accounted for the results. In another large trial (Tebrock *et d.*, 1956) a mixture of bioflavonoids and of 200 mg. of ascorbic acid daily was given after the onset of the cold, but the clinical observations, which were made only one and three days after treatment began, showed no evidence of improvement. Other smaller trials also showed no effect. Banks (1964, 1965) has drawn attention to the possibility that treatment with ascorbic acid may reduce the number of colds whkh are followed by long-continued purulent discharge. Table VI summarizes some facts from what seem to us to be among the most critically evaluated of the earlier trials ; this supports the view that small doses of ascorbic acid administered to those on a low intake or large doses administered to those on a normal intake may reduce the duration of pharyngitis or colds.

TABLE VI.—*Some Previous Controlled Studies on the Effect of Ascorbic Add on Length of Respiratory Infections*

Population Studied	Type of Illness	Dosage of Ascorbic Acid	Effect Observed
20 volunteers on scorbutogenic diet*	Colds	Continuous 10 or 70 mg. daily	Colds in deprived group lasted 6-4 days (G.M.), in non-deprived 2-3 days. Not statistically significant
Adolescents in institution with low vitamin C intake†	Tonsillitis (probably streptococcal) and colds	Continuous 200→50 mg. daily	No effect on incidence. Tonsillitis shorter in treated groups. Colds unaffected
Members of ski camp‡	Pharyngitis (colds apparently uncommon)	Continuous 1,000 mg. daily	Incidence reduced

* Hartley *r a/. (1953). t Glazcbrook and Thomson (1942>. * Ritzel (1961).

We thought it to be intrinsically unlikely that ascorbic add could influence the uptake or replication of viruses. Nevertheless, the " uncoating " of viruses and the damage they produce may be mediated by the release of enzymes from lysosomes (Mallucci and Allison, 1965), and these organdies may be stabilized by ascorbic acid—at least in photosensitized epithelial cells (T. F. Slater, personal communication, 1966). It was therefore possible that the vitamin might influence the multiplication and cytopathic effect of viruses. The results of the in-vitro experiments were negative, but were not entirely convincing because Of the rapid rate of inactivation of the vitamin in the cultures used. Since ascorbic acid can apparently improve the response to stress of the rat and influence the repair of skin, it might assist repair of the mucosa from the damage produced by viruses. It was therefore thought justifiable to undertake volunteer experiments with large doses of ascorbic acid administered before a known virus and a strict double-blind procedure to evaluate the clinical effects ; laboratory techniques were used to determine, when possible, which volunteers had antibodies and which were infected.

We conclude that there is no evidence that the administration of ascorbic acid has any value in the prevention or treatment of colds produced by five known viruses. The literature indicates that the administration of the vitamin to deficient subjects may reduce the incidence or severity of colds and possibly of pharyngitis due to streptococci, as well as the incidence of rheumatic fever and pneumonia (Glazebrook and Thomson, 1942). However, it is quite reasonable to conclude

that administering very large doses of vitamin to well-nourished people does not have the same effect.

It is sometimes said that the colds produced at this unit are not comparable to those which occur in people who are exposed to natural infection and who may develop hypothetical secondary bacterial infections. We, on the other hand, are impressed with the fact that the colds observed here experimentally are very similar clinically to those, caused by the same viruses, which are observed in clinical practice (Tyrrell, 1965), and we should be surprised if ascorbic acid had any prophylactic or therapeutic value for well-nourished subjects who caught colds at home or at work.

Summary

Preliminary experiments showed no evidence that ascorbic acid protected cultures against several respiratory viruses or white mice against influenza. In further experiments 91 volunteers were given either 3 g. of ascorbic acid daily or placebo tablets. Three days after the treatment began they received intranasal drops containing an M rhinovirus, an H rhinovirus, a "new" rhinovirus which grows only in organ cultures, influenza virus type B, or a " new" virus related to avian bronchitis. The number, duration, and severity of the resulting colds were the same in the treated and control groups. There was no evidence that colds produced by any single virus were favourably affected.

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