ASCORBIC ACID FUNCTION AND METABOLISM DURING COLDS

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Investigations of the relationship between symptoms of the natural common cold and administration of supplementary vitamin C must record and take into account the following factors:

1. Definition of the cold syndrome in terms of incidence (number of colds per person in unit time, generally taken as duration of study); duration of syndrome (number of days during which a defined number of symptoms persist in individual patients); and severity of symptoms (recorded subjectively on a daily basis for individual symptoms on a graded scale of which the lowest value records absence of the symptom). Days of absence from work provide a general measure arising from cold disability.[1] Integrated morbidity[2] is defined as the product of incidence of colds, and severity of individual colds. The two values for incidence and integrated morbidity are not independent of one another. Integrated morbidity provides a general measure of intensity of the syndrome as opposed to the disability it produces. Total symptom intensity provides a similar measure to integrated morbidity. The Total Intensity Score is defined as the total severity of any symptoms of the cold syndrome present on each day of reporting, divided by the total number of days of reporting.[3] Variation in symptom quality can be evaluated by measuring the degree of association between symptom pairs.[4] If viral diagnostic tests, or antibody measurement, are not included in the investigation, a screening process is necessary to exclude upper respiratory allergic disease. Local respiratory symptoms of an allergic nature, those produced by mental stress in subjects prone to vasomotor rhinitis, and those arising from chronic infection of the nasal passages,[5] are eliminated from the data on the cold syndrome by excluding syndromes that exceeded 21 days in duration.[3]

2. Measurement of plasma and leukocyte ascorbic acid concentrations in the subjects. Correlation of these measurements with the cold symptomatology enables the relationship between tissue ascorbic acid and the cold syndrome to be measured directly. Administration of exogenous supplementary vitamin C during the cold syndrome only allows indirect assumptions to be made about the relationship between the cold syndrome and the effect associated with vitamin C administration.

3. The preliminary decision as to whether supplementary vitamin C will be administered on a prophylactic or a therapeutic basis. If it is decided that a prophylactic investigation will be carried out, it is essential that the period of administration of supplementary vitamin C is sufficiently long to ensure that tissue concentrations of ascorbic acid have been elevated in the subjects when they are included in the trial. Factors such as sex, age, dietary intake of vitamin C, and initial ascorbic acid status of the tissues can all have profound effects on

the outcome of the trial.[6] Administration of supplementary vitamin C during the cold syndrome alters tissue ascorbic acid levels, but the relationship between such therapeutic alterations and the prophylactic administration of supplementary vitamin C is unknown.

4. Control observations for comparison with the effect produced by supplementary vitamin C, whether administered on a prophylactic or a therapeutic basis. The control observations may compare the effect of the supplementary vitamin C with placebo tablets on the cold symptomatology, or examine the alterations produced by the vitamin C on blood and tissue concentrations of ascorbic acid and correlate these with the cold symptomatology. When the investigation consists of a therapeutic or prophylactic trial, it is essential to confirm that the medication is being taken by carrying out confirmatory measurement of blood ascorbic acid concentrations.

5. Information about the age, sex, dietary intake of vitamin C of the sample, and degree of tissue saturation with ascorbic acid at the beginning of the investigation, so that normal variations in ascorbic acid metabolism can be taken into account in evaluation of the results.

Evidence about the effect of vitamin C on the symptoms of the common cold has provided results of questionable significance because these factors have been inadequately defined and measured in the past. Incomplete definition of symptoms results in imprecise evaluation of the effect of placebo or vitamin C supplementation. The pathophysiological alterations in ascorbic acid which are associated with the presence of cold symptoms [7] may be of sufficient magnitude to mask effects produced by administration of the supplementary vitamin C, which would affect tissue ascorbic acid concentrations and metabolism under normal circumstances. Differences between individuals, and samples, in age, sex, vitamin C intake, tissue saturation, and other concurrent illnesses, may not only alter cold symptomatology, but also introduce into the trial design unrecognized variations in ascorbic acid metabolism. The way in which these factors can influence and be used to interpret results of investigations on the relationship between cold symptoms and administration of supplementary vitamin C, are discussed below.

PROPHYLACTIC TRIALS

The common cold is not a precisely defined disease. To both the professional and the layman the term "a cold" has a clearly understood meaning: a short mild illness in which the main local symptoms are found in the upper respiratory tract, and in which nasal symptoms predominate.[5] Its occurrence is shown by the appearance of these symptoms at varying intervals, and with different degrees of severity, during the progress of the disease. Several investigations [8-10] have indicated that associations tend to occur between the individual symptoms of the common cold. The degree and extent of these associations have, however, been analyzed only recently,[4] when it was found that the symptomatology of the cold syndrome can be assessed in terms of symptom complexes.

The cold syndrome comprises the following symptoms:

Toxic Complex	Catarrhal Complex
1. Sore throat	2. Cold in head
4. Headache	3. Cough
6. Feverish	7. Nasal obstruction
9. Out of Sorts	8. Nasal discharge

A score for the severity of each symptom when it occurs was obtained daily during prophylactic trials in which the effects of daily administration of vitamin C or placebo tablets were compared on the symptoms over a specified period in school children. The degree of association between pairs of symptoms was determined by the correlation values between individual pairs. These correlations measured the extent to which symptom pairs were reported together and extended over a range of 0 to 100%. All the symptoms were reported together to a varying degree in the whole common cold, the syndrome of the W-complex. This was demonstrated by the fact that there were no negative correlations among the symptom pairs. Toxic and catarrhal symptom complexes (T- and C-complexes) became separated at the value for the maximal correlation which linked a T-symptom and a C-symptom in the W-complex. Symptom-

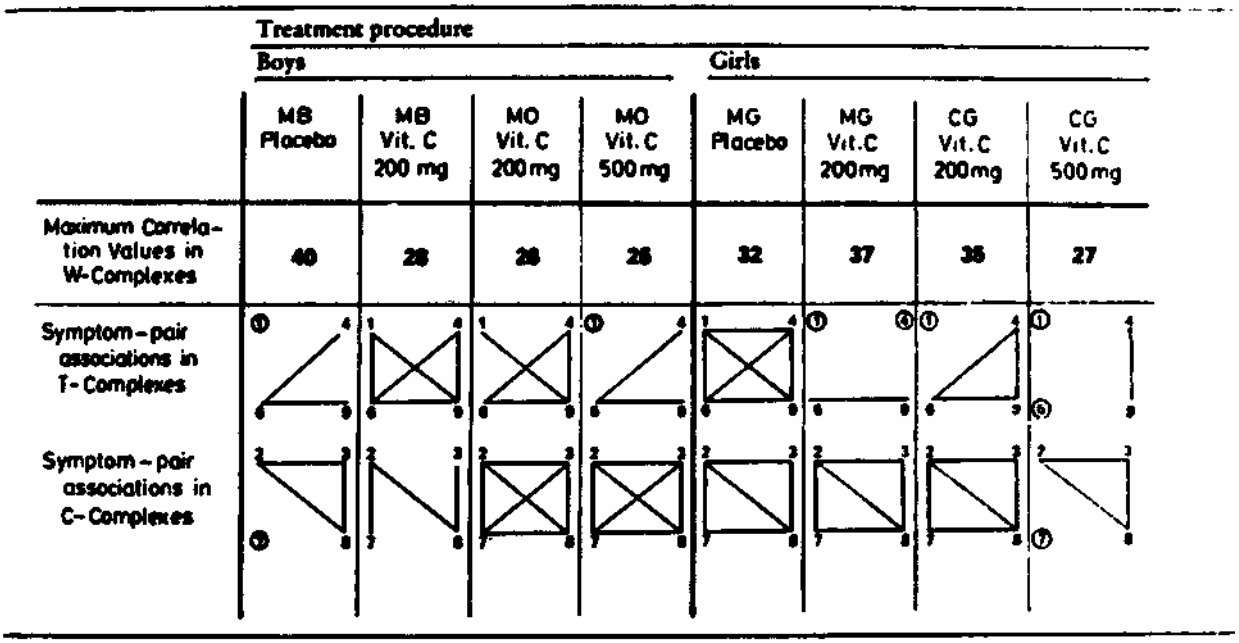

FIGURE 1. Maximal correlation values for the associations between symptom pairs making up the whole-cold complex (W-complex). Differentiation of the W-complex into linked association pairs at values higher than the maximal correlation values for the W-complex to form separate T- and C-complexes. Values and diagrams are shown for population samples of boys (B) and girls (G) receiving placebo tablets or different doses of vitamin C daily during 8½ months. (From Wilson *et al.*[4] By permission of the *European Journal of Clinical Pharmacology.*)

pairs in the T- or C-complexes were associated together at higher correlation values than the values that linked T- and C-symptoms in the W-complex. It was found that symptoms 1, 4, 6 and 9 were associated together. They were therefore defined as the toxic complex. Symptoms 2, 3, 7 and 8 were similarly associated. They were defined as the catarrhal complex. Symptom complexes were evaluated in terms of the average values for the correlation coefficients which made up the individual symptom-pairs. The correlations of individual complexes could be related to the characteristics of the groups being investigated, and to the treatment being administered, during the trial. The symptom complexes were analyzed qualitatively by diagrams. These illustrate how individual complexes were made up of symptom-pair associations which were interlinked to varying degrees (FIGURE 1).

Boys and girls treated with placebo tablets had high correlation values in their W-complexes in comparison to the boys and girls treated with 500 mg vitamin C daily. The average correlation within each of the complexes diminished when the dose of vitamin C was raised. Administration of 200 mg of vitamin C reduced the number of linking associations in the T-complexes in girls; 500 mg was required before the linking associations in the C-complexes in the girls became reduced. The average correlation for the W-complex in girls only became reduced when the numbers of linking associations in both T- and C-complexes had diminished. 500 Mg reduced the T-complex associations in boys in comparison with 200 mg, but this dose appeared to make their C-complexes more complicated. The average correlation for the W-complex became reduced with the smaller dose of vitamin C in boys.

Administration of prophylactic vitamin C altered the intensity and quality of the complexes in relation to the dose administered, and in relation to the response of the sexes participating in the trial. Toxic and catarrhal complexes become dissociated, and can develop and disappear independently of each other, during administration of vitamin C. This suggests that the general disturbance of cellular metabolism and the localized mucosal inflammation are independent pathophysiological processes. This method of analysis of symptoms that together make up a disease syndrome is more sensitive than, and the results obtained from it may be independent of, evaluation of severity or duration of the syndrome in its entirety. This analytical method can detect changes in the syndrome produced by prophylactic and therapeutic procedures.

The administration of prophylactic doses of 200 and 500 mg vitamin C to school children produced beneficial effects on the incidence, duration, severity and total intensity of symptoms when these are measured independently during the cold syndrome.[3] Supplementary vitamin C reduced catarrhal symptoms in the first instance. Larger doses were required in order to exert benefit on toxic symptoms. Vitamin C exerted more beneficial effect on total intensity and severity of catarrhal symptoms in girls than in boys. The larger dose of vitamin C appeared to enhance the severity of the catarrhal symptoms in boys in addition to maintaining the complexity of the C-syndrome. The whole-cold syndrome was reduced in intensity in girls. Its complexity was reduced only by the larger dose of vitamin C. The whole-cold syndrome was unaffected in total intensity in boys, although linking associations were reduced in their W-complexes.

Unless a beneficial effect is produced on toxic and catarrhal symptoms, the subject is not prepared to acknowledge that any beneficial effect is occurring on the cold syndrome. It has been reported that vitamin C reduces catarrhal more than toxic symptoms[11] and also that toxic symptoms are reduced more than catarrhal symptoms.[1] The total intensity of the C-complex influences the patient's subjective assessment of the T-complex when symptoms are being assessed throughout the duration of the syndrome. Analysis of total intensity and symptom-pair associations show that the T-complex is beneficially affected by a daily dose of 500 mg vitamin C in both sexes, and the C-complex is reduced in intensity in girls.

Measurement of incidence and total intensity of the cold syndrome[4] provides values for measurement of effects of prophylactic treatment. It has been stated that 8-10% of the population are immune to the common cold.[12] By making use of this information, and combining it with the results obtained from the cold trials in which the effects of 200 and 500 mg were compared with placebo

therapy on cold symptomatology, a log-dose response line has been drawn that shows the effect of these prophylactic doses on cold incidence[13] (FIGURE 2). The figure demonstrates that there is a difference between male and female response to prophylactic vitamin C therapy against the common cold. A daily dose of 500 mg provides protection against the common cold in 30-40% of the population of school girls, but has little protective effect in boys.

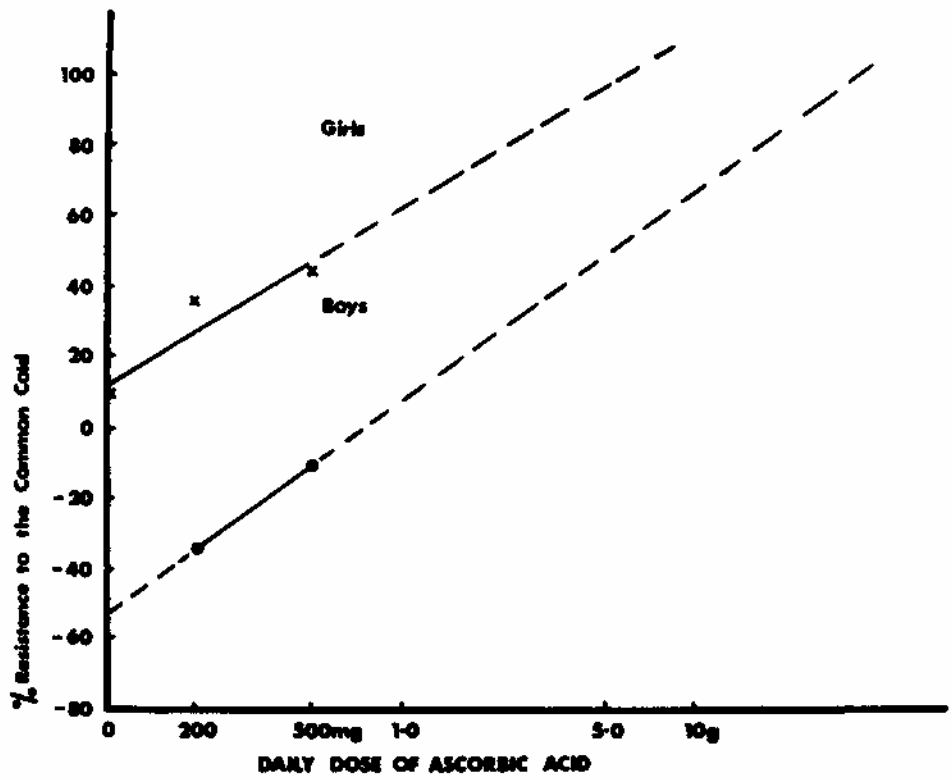

FIGURE 2. Relationship between percentage resistance to the common cold and administration of prophylactic daily doses of 200 and 500 mg supplementary vitamin C to male (●) and female (X) school children. (From Loh *et al.*[13] By permission of *Clinical Pharmacology and Therapeutics.)*

THERAPEUTIC TRIALS

The attainment of a beneficial therapeutic effect on the common cold can be defined as the production of a significant decrease in the duration and severity of some or all of the symptoms by administration of supplementary vitamin C during the progress of the syndrome. Its therapeutic action can be evaluated by comparing the effect of vitamin C or placebo administration from the time when the first symptoms appear until the last symptom disappears. Anderson et al.[1,14] have carried out long-term combined prophylactic and therapeutic trials in which vitamin C was found to exert beneficial effects in comparison with placebo therapy. Short-term prophylactic and therapeutic trials have been carried out [15,16] on natural and artificially induced colds in which doses of 1 g or more were administered daily to the subjects. Significant benefit was produced by vitamin C therapy in the naturally produced colds, whereas it was ineffective in the smaller number of subjects who were subjected to nasally instilled viral infection.

Another method for evaluating the therapeutic effectiveness of vitamin C in the common cold is to measure alterations in ascorbic acid metabolism, and relate such changes to the symptoms during the cold, and to ascorbic acid blood levels after disappearance of the cold symptoms. Such investigations must take account of the fact that plasma and leukocyte ascorbic acid concentrations are reduced in individual patients while cold symptoms are present.[17,18] Ascorbic acid shows great lability in its passage to and from the plasma into the tissues.[19] In consequence rapid changes in plasma levels may occur in response to metabolic demand from particular tissues which are not reflected by concurrent changes in leukocyte concentrations. Single loading doses of vitamin C in the range 500-2000 mg have been administered during colds, and plasma and leukocyte ascorbic acid concentrations measured during the following four hours. These ascorbic acid blood response curves (AABRC 500-2000) evaluate changes in ascorbic acid metabolism during the colds, and their determination is repeated after disappearance of the symptoms in the same subjects.[20,21] Changes in the plasma-leukocyte (P/L) correlations and regression angles between successive AABRC's enable the rate and direction of transfer of ascorbic acid between plasma and leukocytes to be evaluated.[22] The occurrence of positive correlation and regression coefficients, together with rising plasma and leukocyte concentrations, indicate a positive accumulation of vitamin C in the tissues for metabolism or storage. This is associated with uptake of ascorbic acid from the plasma into the leukocytes. Negative coefficients together with a rising plasma and low leukocyte ascorbic acid concentrations, indicate a negative metabolic balance. This is attributable to increased metabolic demand for, or inadequate dietary intake of, the vitamin.

During colds, and after recovery from cold symptoms, plasma levels were significantly raised four hours after administration of vitamin C. The rise was greater after 2,000 mg than after 500 mg of vitamin C (TABLE 1). The resting leukocyte values and levels after supplementation of ascorbic acid were lower in males than in females during colds. There was a considerable increase in leukocyte uptake of ascorbic acid in females, but the increase in male concentrations was slight after the loading dose of 2,000 mg. The loading dose of 500 mg was not associated with elevation of leukocyte ascorbic acid during colds in either sex, but was associated with raised ascorbic acid values in the postcold test. During colds the mean P/L regression coefficients were similar in males to their post-cold regressions. This indicates that there was a similar flow of ascorbic acid into the plasma after supplementation during cold and postcold periods. Inadequate ascorbic acid was available for storage in both tests. There were relatively high plasma and low leukocyte ascorbic acid concentrations. This suggests that male ascorbic acid metabolism had not been restored to normal at the time of the postcold test. In the females, the mean regression coefficient in the postcold test was higher than in the cold test. This indicates that the uptake of ascorbic acid into the leukocytes occurred simultaneously with the rise in plasma concentration after supplementation, and was associated with accumulation of ascorbic acid in the tissues. The following metabolic factors appear to determine leukocyte uptake of ascorbic acid during colds: (1) Metabolic requirements for ascorbic acid in the leukocytes may be greater during colds in females than in males. (2) Requirements in females for ascorbic acid in the inflamed and toxic tissues are less, and so more ascorbic acid is available for storage in the leukocytes during colds. In either case it appears that the level of ascorbic acid saturation in the leukocytes is raised

TABLE 1

MEANS AND STANDARD DEVIATIONS OF LEUKOCYTE AND PLASMA ASCORBIC ACID CONCENTRATIONS AT REST 2 AND 4 HOURS AFTER ORAL ADMINISTRATION OF VITAMIN C (AABRC 500 * AND 2000 †) TO THE SAME SUBJECTS DURING AND 3 WEEKS AFTER RECOVERY FROM SYMPTOMS OF THE COLD SYNDROME

Time after Loading Dose (hr)	Dose of Ascorbic Acid (mg)	Cold				Postcold			
		Leukocytes		Plasma		Leukocytes		Plasma	
		Males	Females	Males	Females	Males	Females	Males	Females
		Mean S.D.	Mean S.D.	Mean S.D.	Mean S.D.	Mean S.D.	Mean S.D.	Mean S.D.	Mean S.D.
0	500	25.0±5.8	34.0±6.5	0.8±0.3	0.9±0.4	31.1±8.3	31.9±9.5	0.9±0.4	0.9±0.3
	2,000	22.3±4.3	29.9±5.4	0.9±0.2	0.9±0.2	29.9±5.4	29.1±9.2	0.9±0.2	1.3±0.4
2	500	25.2±5.4	30.2±8.5	1.4±0.6	1.7±0.6	36.4±15.3	33.1±11.3	1.7±0.6	1.9±0.6
	2,000	29.1±9.2	49.6±12.7	2.0±0.6	2.9±0.9	32.5±4.1	37.0±11.7	2.4±0.5	2.4±0.4
4	500	24.7±4.7	34.0±6.5	1.6±0.5	1.7±0.5	32.9±8.6	34.1±11.6	1.8±0.6	2.0±0.4
	2,000	29.3±6.7	32.2±3.7	2.0±0.6	2.6±0.9	32.2±3.7	40.0±11.0	2.4±0.7	3.4±0.2

* AABRC 500:n, males, 11; females, 8.
† AABRC 1,000:n, males, 4; females, 5.

during colds in females and is lowered again after recovery from the colds. If the saturation level is raised in males during colds, it does not appear that a dose of 2,000 mg is adequate to increase the uptake in males (TABLE 1).

The relationship between plasma and leukocyte ascorbic acid concentrations, and the occurrence of toxic and catarrhal symptoms has been examined following loading doses of 500 and 2,000 mg vitamin C during colds.[20,21] To take into account concurrent changes in ascorbic acid metabolism and cold symptomatology, P/L regressions at the time of the AABRC were correlated with ratio of toxic and catarrhal symptoms (T/C ratio) during the progress of the cold. It was found that a significant positive correlation existed between these relationships with a loading dose of 500 mg vitamin C. This indicated that a flow of ascorbic acid from the plasma into the leukocytes was associated with colds predominantly of the toxic variety. C-colds were correlated with a reverse flow of ascorbic acid associated with high plasma and reduced leukocyte ascorbic acid concentrations. The correlation between total symptom score and P/L regression was not significant. When a loading dose of 2,000 mg was administered, a significant relationship was found to exist between severity of catarrhal symptoms and P/L regression particularly in females. This suggests that the larger dose was affecting predominantly the catarrhal symptoms, presumably because leukocyte and general tissue uptake was sufficient to meet metabolic requirements for dealing with toxic symptoms. During these colds the toxic symptoms were considerably less severe than catarrhal symptoms, and were less marked in males than in females.

Plasma concentrations of ascorbic acid have been compared with salivary and lingual concentrations in the same individuals during and after recovery from colds (FIGURE 3). During colds the concentrations were 60-70% of the values after recovery from the cold symptoms; the concentrations in plasma and tongue were significantly lower during the colds. The reduction in leukocyte ascorbic acid levels during colds are reflected by corresponding changes in ascorbic acid concentrations adjacent to the infected areas. It can be assumed that supplementary vitamin C passes into these tissues from the plasma during colds.

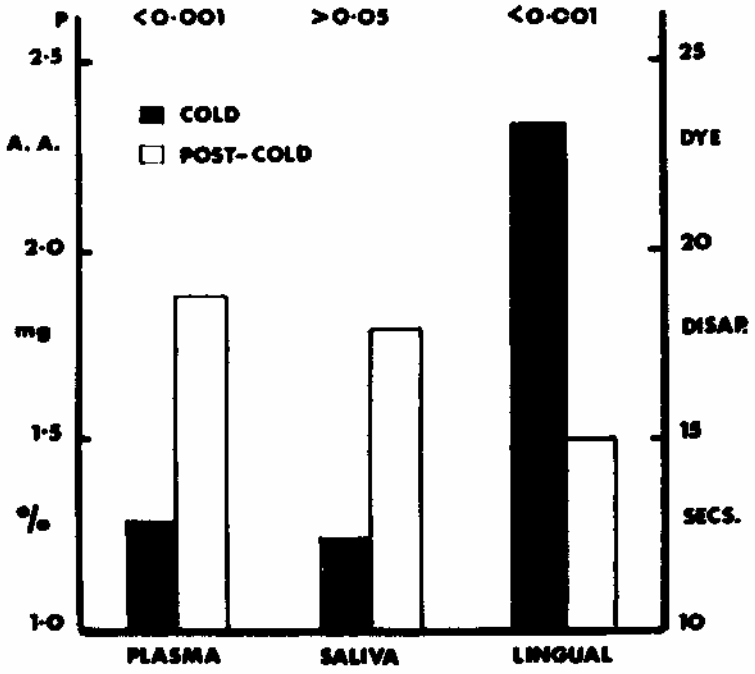

FIGURE 3. Comparison of plasma and salivary ascorbic acid concentrations during symptoms and after disappearance of symptoms of the common cold syndrome (mg/100 ml). Lingual ascorbic acid concentrations have been compared by measuring time in seconds required for disappearance of the blue dye chlorophenal indophenol on the tongue. A longer time indicates a lower concentration of ascorbic acid.

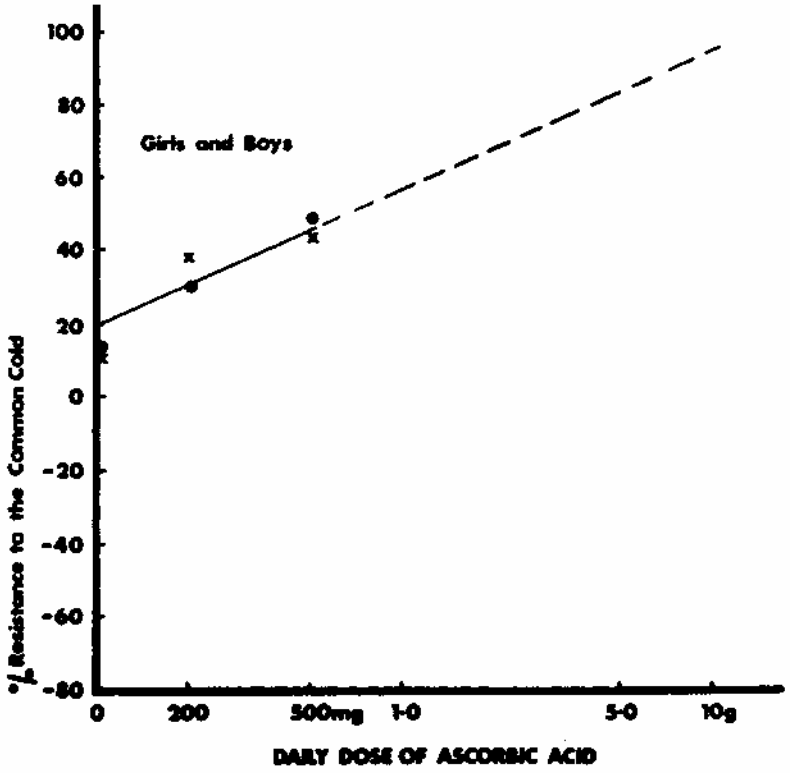

FIGURE 4. Relationship between percentage resistance to the common cold and administration of prophylactic daily doses of 200 and 500 mg supplementary vitamin C to male (●) and female (X) schoolchildren. Allowance has been made for the differences between male and female ascorbic acid metabolism as characterized by their plasma/leukocyte regression lines. (From Loh *et al.*[13] By permission of *Clinical Pharmacology and Therapeutics.)*

ASCORBIC ACID METABOLISM DURING COLDS

Changes in ascorbic acid metabolism during colds reflect not only pathophysiological alterations associated with the infection, but also indicate the continued existence of underlying physiological factors which interact with the pathologically induced changes. It is clear that the difference between the sexes with respect to ascorbic acid metabolism continues to operate and may become exaggerated during the period of pathophysiological desaturation. Allowance can be made for the differences between male and female ascorbic acid metabolism, as characterized by their P/L regression lines. If this is done, and the consequent values are correlated with the severity of cold symptoms while the school children are under prophylactic treatment with supplementary vitamin C, it is found that the male dose-response line becomes superimposed on the female line in the dose-response curve relating cold-incidence to dose of prophylactic vitamin C (FIGURE 4).

The effect produced by administration of supplementary vitamin C is always subject to variation depending upon the state of the individual's metabolism at any particular time. When pathophysiological demands are made for ascorbic acid, the effect of administration of supplementary vitamin C will be subject not only to normal physiological fluctuation, but also to the pathophysiological alterations induced by the cold or any other disease. To obtain accurate and sensitive results during investigations designed to evaluate the effect of supplementary vitamin C on the cold syndrome, cognizance must be taken of physio-

logical and pathophysiological factors that may influence the clinical pharmacological action of vitamin C in the tissues.

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DISCUSSION

DR. K. E. SCHAEFER: Do you know what infectious agents you were working with?

DR. WILSON: I used the definition of the common cold given by Tyrrell.

DR. R. E. HODGES: One comment: It has been well established that susceptibility to colds is a factor of age and that people who get along in years are virtually immune.

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SECOND CONFERENCE ON VITAMIN C *

Editors and Conference Chairmen

C. G. KING AND J. J. BURNS

CONTENTS

Part I. Metabolism of Ascorbic Acid

* This series of papers is the result of the Second Conference on Vitamin C, sponsored by The New York Academy of Sciences and the Institute of Human Nutrition, College of Physicians and Surgeons, Columbia University, and held on October 9-12, 1974 in New York, New York.

Part VI. Ascorbic Acid and Respiratory Illness