VOLUME 2

GRAS MONOGRAPH SERIES

ASCORBIC ACID

(COPIES OF ARTICLES CITED IN MONOGRAPH SUMMARY)

prepared for

THE FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION
AND WELFARE

OCTOBER 7, 1974

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Tracor Jitco, Inc.

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0116

The Effect of Ascorbic Acid Upon Bladder Uptake of β-Naphthylamine Metabolites[1] (36921)

BASSIMA S. ALAM, FRANCISCO E. JARAMILLO, J. U. SCHLEGEL, AND T. A. DEROUEN

Section of Urology, Department of Surgery, Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, Louisiana 70112; and Department of Epidemiology and Biostatistics, Tulane University School of Public Health and Tropical Medicine, 150 South Liberty Street, New Orleans, Louisiana 70112

The cause of bladder cancer is, in most instances, still a matter of speculation. More and more chemicals are added to the list of suspected carcinogens, in most instances on the basis of animal experiments. When cyclamate was found to be carcinogenic in mouse-bladder implantation studies (1), it was removed from the market. Since five naturally occurring tryptophan metabolites have been shown to be carcinogenic (2–8) by the same technique, it would follow logically that tryptophan should also be banned. This is obviously an absurdity, since tryptophan is an essential amino acid, and we must thus accept that carcinogenic substances are naturally occurring in the urine. Even though the concentration and types of carcinogens in the urine must be of obvious importance, results of work done in our laboratory have suggested that the redox potential of the urine is of equal, if not greater, importance (9). Several suspected carcinogens oxidize very readily (10), and we have previously shown that administration of ascorbic acid would prevent oxidation of 3-hydroxyanthranilic acid (11), as well as its carcinogenicity (8).

Since it is still controversial as to whether tryptophan metabolites can cause cancer of the uroepithelium in man, we felt it would be of interest to study the effect of ascorbic acid on the bladder reabsorption of Beta-naphthylamine metabolites. β-naphthylamine is a proven potent bladder carcinogen in man (12), dogs (13), and nonhuman primates (14) when ingested orally, although uncertainty exists as to which and how many of the urinary metabolites are the potent carcinogens. We consequently in this study gave a single dose of tritium-labelled β-naphthylamine to a dog, and extracted the urinary metabolites. The effect of ascorbic acid upon the reabsorption of the metabolites by the rabbit bladders was then investigated.

Materials and Methods. Female dogs weighing 5 kg were administered orally a capsule containing one millicurie of ^{3}H-β naphthylamine (125 μCi/μmole, New England Nuclear) dissolved in 1 ml ethanol. A Foley catheter (No. 14F) was inserted into the dog's bladder and the catheter connected to a drainage bag containing an excess of ascorbic acid (approximately 15 g) to prevent oxidation during collection. A brace was placed to keep supporting the bag, and to avoid any oxidation by light, and the urine collected for 16–18 hr.

Extraction of metabolites. The urine was divided into two equal portions (500–600 ml each) which sequentially were extracted with three solvents of increasing polarity; ether, ethyl acetate, and after saturation with ammonium sulfate, tert-butyl alcohol according to the method described by Deichmann and Radomski (15).

The solvents were evaporated using a rotary evaporator (under 30°) and the residues from ether, ethyl acetate and tert-butyl alcohol extracts were combined to obtain all the urinary metabolites of β-naphthylamine. The metabolites of each portion were dissolved in phosphate buffer (pH 5.6, 0.2 *M*) and the volume made up to 25 ml. To one sample, ascorbic acid was added (400 mg%) and nitrogen passed through for two hours, while

[1] Supported by a Grant from the Florida Citrus Commission.

TABLE I. Effect of Ascorbic Acid on the Recovery of Radioactivity from the Urinary Metabolites of ^{3}H-B-Naphthylamine by the Bladder Solution.

Replicate	With ascorbic acid (W AA)			Without ascorbic acid (W/O AA)			Comparison of means (W AA)—(W/O AA) (sign only)
	Radioactivity administered (DPM)	Average % recovered	Standard deviation (% recovery)	Radioactivity administered (DPM)	Average % recovered	Standard deviation (% recovery)	
1	22.21×10^{6}	76.6 (2)[a]	24.25	30.78×10^{6}	29.8 (2)[b]	11.88	+
2	28.07×10^{6}	73.2 (3)	1.37	28.32×10^{6}	67.7 (3)	9.44	+
3	28.61×10^{6}	33.9 (3)	9.54	25.09×10^{6}	30.2 (2)[b]	14.43	+
4	32.14×10^{6}	69.4 (2)[b]	13.22	30.84×10^{6}	50.5 (3)	4.24	+
5	35.32×10^{6}	58.2 (3)	2.85	35.06×10^{6}	53.1 (3)	0.95	+
6	40.99×10^{6}	68.4 (3)	8.61	40.99×10^{6}	63.2 (3)	5.00	+
7	23.51×10^{6}	39.8 (3)	3.71	18.81×10^{6}	32.5 (3)	5.28	+
8	22.42×10^{6}	35.0 (3)	7.40	20.70×10^{6}	27.9 (3)	7.37	+
Overall average	29.16×10^{6}	56.8 (22)		28.82×10^{6}	44.4 (22)		$p = 0.004$ for the Sign Test

[a] Number in parentheses is the number of rabbits used in each treatment.

[b] Indicates an observation missing due to the death of a rabbit before completion of the experiment from causes unrelated to the treatment administered.

TABLE II. Effect of Ascorbic Acid on the Uptake of Radioactivity from the Urinary Metabolites of ^{3}H-β-Naphthylamine by the Bladder Tissues.

Replicate[a]	With ascorbic acid (W AA)			Without ascorbic acid (W/O AA)			Comparison of average % (W AA)—(W/O AA)
	Radioactivity recovered[a]		Standard deviation (% recovery)	Radioactivity recovered		Standard deviation (% recovery)	
	Av DPM/g	% Adm dose[b]		Av DPM/g	% Adm dose		
3	133×10^3 (3)	0.466	0.173	129×10^3 (2)[d]	0.512	0.135	—
4	183×10^3 (2)[d]	0.568	0.107	539×10^3 (3)	1.74	1.15	—
5	273×10^3 (2)[d]	0.772	0.19	313×10^3 (3)	0.892	0.03	—
6	155×10^3 (3)	0.378	0.15	181×10^3 (3)	0.442	0.172	—
7	26×10^3 (2)[d]	0.11	0.03	51×10^3 (3)	0.271	0.11	—
8	30×10^3 (3)	0.13	0.085	55×10^3 (3)	0.267	0.086	—
Overall average	133×10^3 (15)	0.388		211×10^3 (17)	0.698		$p = 0.02$ For the Sign Test

[a] Administered doses are the same as those in Table I.
[b] DPM/g/Total adm. dose × 100.
[c] In replicates 1 and 2, no determination was made for radioactivity uptake.
[d] Indicates missing observation.

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oxygen was passed for two hours through the second sample.

Impregnation of the rabbit's bladder. Male rabbits weighing 2–3 kg were used. After anesthesia with sodium pentobarbital, the abdominal wall was incised and the ureters ligated in order to prevent the flow of urine from the kidneys into the bladder. The bladder was gently emptied by compression. A catheter was inserted into the bladder through the urethra, and 7–8 ml of the solution (with and without ascorbic acid) of the metabolites in buffer was introduced using a 10 ml syringe. The solution of the metabolites was incubated in the bladder for two hours. The bladder was then removed, and the contents emptied into a beaker. The bladder was rinsed gently with physiological saline and the washings collected. Portions of the bladder were next excised, weighed and solubilized in soluene. An aliquot of the solution was used for radioactivity measurements.

Liquid scintillation counting. For radioactivity measurement, aliquots (0.1 ml each) in duplicate were used for the following: (a) the original metabolites before inserting into the bladder; (b) bladder tissue solutions in soluene; and (c) the bladder solutions after recovery from the bladder. Fifteen ml of liquid scintillation fluid (aquasol) was added to each vial and the radioactivity measured in a Packard TriCarb 3200 liquid scintillation spectrometer. Quenching was corrected by the Automatic External Standard (AES) method.

The experiment was performed with 2–3 rabbits randomly allocated to each treatment, and repeated on several different days (six for the bladder tissue, eight for the bladder solution). Although an effort was made to standardize conditions from day to day, to avoid having any unaccountable day to day variability influence the results, comparisons between the two treatments are made only within the same day.

Results. The effect of ascorbic acid on the recovery of radioactivity in the bladder solutions and uptake by the bladder tissues of rabbits after *in vivo* instillation of urinary metabolites from ^{3}H-β-naphthylamine is shown in Table I.

The results of eight different replicates, each using 4–6 rabbits, show that there was greater recovery of radioactivity from the instilled bladder solutions in the presence of ascorbic acid than in its absence.

The average radioactivity (% of administered dose) varied from one replicate to another. However, in each set of replicates, there was more radioactivity recovered from the bladder solution of metabolites containing ascorbic acid. The average value of eight replicates conducted using 22 rabbits in each group (with and without ascorbic acid) was 56.8% recovery in the ascorbic acid-treated group versus 44.4% in the group without ascorbic acid.

Data on the radioactivity uptake by the bladder tissues are shown in Table II. As can be seen, in the presence of ascorbic acid there was less radioactivity taken up by the bladder.

Similar results were obtained in each of the six replicates in which bladder uptake was measured. Thus the presence of ascorbic acid upon the urinary β-naphthylamine metabolites affected the radioactivity recoveries measured in the bladder solutions and in the bladder tissues in opposite directions, *i.e.*, it decreased the bladder uptake, but increased the recovery from the bladder solutions.

The determination of the statistical significance of the two sets of data was done in two steps. The first step was merely a confirmation of what seems obvious at a glance. If the average percentages of radioactivity recovered in the bladder solution for the two treatments (with and without ascorbic acid) are compared for each day (the reason for making comparisons only within the same day having been previously stated), it is seen that the average percentage is always higher when ascorbic acid is present than when it is absent. On the other hand, for the percent of administered dose recovered in the bladder tissues, just the opposite is true; *i.e.*, the percentage is always higher when ascorbic acid is absent than when it is present. A simple way to determine the statistical significance of these trends is to employ the sign

test, a nonparametric statistical procedure (16). The sign test merely determines the probability of observing an average for one treatment that is less than the average for the other eight times in a row (for the bladder solution data; six times in a row for the bladder tissue data) if, in fact, there is no difference between the two treatments. As is indicated at the bottom of Tables I and II, the probabilities of observing these trends if there is no difference between the two treatments are 0.004 for the bladder solution data, and 0.02 for the bladder tissue data.

One criticism that can be levied against the use of only the sign test for these data is that it ignores variability within treatments, and compares only treatment means. For this reason, the data were also analyzed using analysis of variance procedures for data with unequal numbers of observations per cell and missing observations (17). Only the results of the statistical test for the difference between the two treatments in this analysis will be discussed here.

For the bladder solution data, the F-test for the difference between treatments had value 5.27. For an F-distribution with 8 and 28 degrees of freedom, this corresponds to a P-value of less than 0.001. Therefore the conclusion of this test is even stronger than for the sign test. For the bladder tissue data, the F-statistic had a value of 2.23 with 6 and 20 degrees of freedom, corresponding to a P-value between 0.05 and 0.10. Thus the analysis of variance test does not produce as strong a confirmation of the results of the sign test for the bladder tissue data as it does for the bladder solution data, but it still indicates a rather high level of significance. The slight discrepancy between the significance levels for the two tests on the bladder tissue data is probably due to the fact that there are several missing observations in this data, which is something that the sign test does not detect.

Discussion. Our studies indicate that the presence of ascorbic acid in the bladders of rabbits decreases the uptake of radioactivity from the urinary metabolites of ^{3}H-β-naphthylamine.

The variability from one replicate to another in the percentage recovery of the administered radioactivity in the bladder solution and in the radioactivity uptake by the bladder tissues may be due to variability among animals (rabbits) or more likely may be due to the different proportions of the various metabolic products of tritiated B-naphthylamine in the urine each time it was given to the rabbits. As β-naphthylamine is metabolized to many different compounds that are excreted in urine, it is possible that in an experimental situation, the urinary concentration of an individual potential carcinogenic metabolite excreted in dog's urine may vary from one replicate to another. Thus the proportion of different metabolites instilled in the rabbit bladders may therefore also vary. In spite of such variability, it is important to note, however, that in each replicate conducted under similar conditions, ascorbic acid has an effect, both on the bladder uptake and on the recovery from the bladder solution.

Previous studies from this laboratory (11) have shown that the addition of ascorbic acid to the urine containing 3-hydroxyanthranilic acid stabilized the latter, which is ordinarily very much susceptible to oxidation. Also, high levels of ascorbic acid in the urine afforded some protection to mice against the induction of bladder tumors as a result of implantation of 3-hydroxyanthranilic acid pellets in their bladders (8).

We have previously suggested that 3-hydroxyanthranilic acid *per se* may not be carcinogenic while oxidation may render it so. This would explain why ascorbic acid could prevent bladder tumor formation from 3-hydroxyanthranilic acid, since in competing for oxygen, it prevents the oxidation of the 3-hydroxyanthranilic acid. This same mechanism may exist as it relates to the carcinogenic metabolites of β-naphthylamine. If less bladder uptake occurs with the reduced metabolites and more with the oxidized ones, this would suggest that ascorbic acid could have a preventive effect on bladder carcinoma resulting from β-naphthylamine administration since a decreased uptake of a carcinogenic compound by the bladder would obviously render it less potent.

Pamukcu *et al.* (18) have recently shown that phenothiazine inhibits the intestinal and urinary bladder tumors induced in rats by bracken fern. Although the carcinogen in bracken fern has not yet been chemically characterized, it was shown by these investigators that phenothiazine acted as an inducer of microsomal benzopyrine (BP) hydroxylase and thus decreasing the carcinogenic effect of bracken fern.

It is interesting to note, however, that phenothiazine is also a reducing agent, like ascorbic acid, so it is quite possible that at least part of the inhibitory effect of phenothiazine on tumor production may be due to its reducing ability.

The current findings further substantiate our hypothesis that the urinary environment, especially its redox potential, may be an important factor which can modify the carcinogenicity of a particular compound. This may have important ramifications in the practical approach as a preventive measure in human carcinoma of the uroepithelium.

We are deeply indebted to Dr. R. L. Stjernholm, Department of Biochemistry, Tulane University School of Medicine, for the use of the liquid scintillation spectrometer. The technical assistance of Miss Heui-Mei Yeh is also greatly appreciated.

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ORIGINAL ARTICLES

0190

Vitamin C and the common cold: a double-blind trial

T. W. Anderson, B.M., PH.D., D. B. W. Reid, M.SC., and G. H. Beaton, PH.D., *Toronto*

Summary: A large scale double-blind trial was conducted to test the claim that the intake of one gram of vitamin C per day substantially reduces the frequency and duration of "colds". It was found that in terms of the average number of colds and days of sickness per subject the vitamin group experienced less illness than the placebo group, but the differences were smaller than have been claimed and were statistically not significant. However, there was a statistically significant difference ($P < 0.05$) between the two groups in the number of subjects who remained free of illness throughout the study period. Furthermore the subjects receiving the vitamin experienced approximately 30% fewer total days of disability (confined to the house or off work) than those receiving the placebo, and this difference was statistically highly significant ($P < 0.001$). The reduction in disability appeared to be due to a lower incidence of constitutional symptoms such as chills and severe malaise, and was seen in all types of acute illness, including those which did not involve the upper respiratory tract.

Résumé: *La vitamine C et le rhume banal: étude à double inconnue*

Il s'agit d'essais à double inconnue, effectués sur une vaste échelle et dans le but d'évaluer le bien fondé de la prétention que l'ingestion quotidienne d'un gramme de vitamine C diminue considérablement la fréquence et la durée des rhumes. Il a été constaté qu'au point de vue du nombre de sujets atteints et à celui des jours de maladie par sujet, les malades recevant la vitamine C ont été moins souvent touchés que ceux recevant le placébo. Ces différences ont été cependant plus faibles qu'on ne l'avait prétendu et, au point de vue statistique, n'avaient guère de signification. Néanmoins, toujours sur le plan statistique, on notait une différence significative ($P < 0.05$) entre les deux groupes quant au nombre de sujets qui sont restés indemnes de maladie pendant toute la durée de l'étude. De plus, chez les sujets traités par la vitamine, on a noté une moins longue période d'invalidité de près de 30% (nombre total de jours de confinement à la maison ou d'absence au travail) que chez ceux qui avaient reçu le placébo. Cette différence était très significative au point de vue statistique ($P < 0.001$). Cette réduction de la période d'invalidité a semblé attribuable à une moindre fréquence des symptômes généraux, frissons et malaises sévères notamment. Cette différence a été notée dans tous les types de pathologies aiguës, y compris celles qui ne touchaient pas les voies respiratoires supérieures.

This investigation was prompted by an article by Beaton and Whalen that appeared in this journal in August 1971[1] in which the authors reviewed the much-publicized book *Vitamin C and the Common Cold* by Linus Pauling.[2] While they were critical of the limited evidence upon which Pauling based his claims, Beaton and Whalen felt that properly controlled trials of the proposed therapy were justified in view of the scientific eminence of Linus Pauling (a Nobel prize-winner in biochemistry) and the large amount of public interest in the matter.

In the subsequent issue of the Journal a letter from Linus Pauling was published in which he specified that "the regular ingestion of 1000 mg. (of vitamin C) leads to the decreased incidence of colds by about 45% . . . (and) . . . to a decrease in total illness by about 60%."[3] Pauling based these estimates on the results of a double-blind study involving 279 students at a ski-school in Switzerland,[4] and although the differences observed in this study were statistically significant, the period of observation was only one week for each individual. We therefore decided to set up a double-blind study in which this quantity (1000 mg./day) would be given to a large group of subjects for a period of three or four months.

Since most of us involved in the study design were sceptical of Pauling's claims, we aimed to enrol a large number of subjects (1000) in the hope of avoiding an indecisive negative result. Furthermore, subjects were instructed to increase their intake to 4000 mg./day at the onset of a cold, in order that a negative result would not be open to the criticism that we had not followed all of Pauling's recommendations (which include raising the dosage at the first sign of a cold).[2] We recognized that this extra dosage feature might complicate the interpretation of the results, since we were adding a therapeutic

T. W. ANDERSON, Associate Professor, Department of Epidemiology and Biometrics.
D. B. W. REID, Professor, Department of Epidemiology and Biometrics.
G. H. BEATON, Professor and Head, Department of Nutrition.
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Reprint requests to: Dr. T. W. Anderson, Dept. of Epidemiology and Biometrics, School of Hygiene, University of Toronto, 150 College Street, Toronto, Ont.

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Summary: A large scale double-blind trial was conducted to test the claim that the intake of one gram of vitamin C per day substantially reduces the frequency and duration of "colds". It was found that in terms of the average number of colds and days of sickness per subject the vitamin group experienced less illness than the placebo group, but the differences were smaller than have been claimed and were statistically not significant. However, there was a statistically significant difference ($P < 0.05$) between the two groups in the number of subjects who remained free of illness throughout the study period. Furthermore the subjects receiving the vitamin experienced approximately 30% fewer total days of disability (confined to the house or off work) than those receiving the placebo, and this difference was statistically highly significant ($P < 0.001$). The reduction in disability appeared to be due to a lower incidence of constitutional symptoms such as chills and severe malaise, and was seen in all types of acute illness, including those which did not involve the upper respiratory tract.

Résumé: *La vitamine C et le rhume banal: étude à double inconnue*

Il s'agit d'essais à double inconnue, effectués sur une vaste échelle et dans le but d'évaluer le bien fondé de la prétention que l'ingestion quotidienne d'un gramme de vitamine C diminue considérablement la fréquence et la durée des rhumes. Il a été constaté qu'au point de vue du nombre de sujets atteints et à celui des jours de maladie par sujet, les malades recevant la vitamine C ont été moins souvent touchés que ceux recevant le placébo. Ces différences ont été cependant plus faibles qu'on ne l'avait prétendu et, au point de vue statistique, n'avaient guère de signification. Néanmoins, toujours sur le plan statistique, on notait une différence significative ($P < 0.05$) entre les deux groupes quant au nombre de sujets qui sont restés indemnes de maladie pendant toute la durée de l'étude. De plus, chez les sujets traités par la vitamine, on a noté une moins longue période d'invalidité de près de 30% (nombre total de jours de confinement à la maison ou d'absence au travail) que chez ceux qui avaient reçu le placébo. Cette différence était très significative au point de vue statistique ($P < 0.001$). Cette réduction de la période d'invalidité a semblé attribuable à une moindre fréquence des symptômes généraux, frissons et malaises sévères notamment. Cette différence a été notée dans tous les types de pathologies aiguës, y compris celles qui ne touchaient pas les voies respiratoires supérieures.

This investigation was prompted by an article by Beaton and Whalen that appeared in this journal in August 1971[1] in which the authors reviewed the much-publicized book *Vitamin C and the Common Cold* by Linus Pauling.[2] While they were critical of the limited evidence upon which Pauling based his claims, Beaton and Whalen felt that properly controlled trials of the proposed therapy were justified in view of the scientific eminence of Linus Pauling (a Nobel prize-winner in biochemistry) and the large amount of public interest in the matter.

In the subsequent issue of the Journal a letter from Linus Pauling was published in which he specified that "the regular ingestion of 1000 mg. (of vitamin C) leads to the decreased incidence of colds by about 45% . . . (and) . . . to a decrease in total illness by about 60%."[3] Pauling based these estimates on the results of a double-blind study involving 279 students at a ski-school in Switzerland,[4] and although the differences observed in this study were statistically significant, the period of observation was only one week for each individual. We therefore decided to set up a double-blind study in which this quantity (1000 mg./day) would be given to a large group of subjects for a period of three or four months.

Since most of us involved in the study design were sceptical of Pauling's claims, we aimed to enrol a large number of subjects (1000) in the hope of avoiding an indecisive negative result. Furthermore, subjects were instructed to increase their intake to 4000 mg./day at the onset of a cold, in order that a negative result would not be open to the criticism that we had not followed all of Pauling's recommendations (which include raising the dosage at the first sign of a cold).[2] We recognized that this extra dosage feature might complicate the interpretation of the results, since we were adding a therapeutic

T. W. ANDERSON, Associate Professor, Department of Epidemiology and Biometrics.
D. B. W. REID, Professor, Department of Epidemiology and Biometrics.
G. H. BEATON, Professor and Head, Department of Nutrition.
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Reprint requests to: Dr. T. W. Anderson, Dept. of Epidemiology and Biometrics, School of Hygiene, University of Toronto, 150 College Street, Toronto, Ont.

each day whether they were sick or well, and the total number of tablets taken during that day. On the reverse side of the sheet appeared a list of sites (nose, throat, etc.) against which, on each day of illness, was to be recorded 0 (not affected), + (mildly affected) or ++ (severely affected). The same coding was to be used to record the presence of malaise or chills, and in addition subjects were asked to record whether they had stayed indoors, stayed off work, seen a doctor, taken any prescription or non-prescription drug (and its nature) and, if possible, their highest oral temperature on that day. In addition to the monthly record-sheet, each subject completed an initial questionnaire and a final evaluation sheet.

At the end of each month the record-sheets were returned and the information coded and transferred to punch cards. On those occasions when two episodes of sickness were separated by only a few symptom-free days, the following rules were applied: if the symptom-free interval was two days or less, only a single episode was recorded; if the interval was three to six days, the episodes were counted separately unless the symptom patterns were essentially the same on the two occasions; if the interval was seven days or more, the episodes were counted separately, however similar the symptoms.

Subjects who were already sick on the day they entered the study were not considered to have started until their first symptom-free day.

A number of statistical analyses were carried out on the data. Where tests of significance were performed these were standard two-tailed tests.

Results

When the table code was broken it was found that of the 818 subjects whose experience was available for analysis 411 had been on the placebo and 407 on the vitamin. The randomization appeared to have been successful in producing two reasonably well-matched groups. The average age of the vitamin group was 28.8 years (range 10 to 64), and that of the placebo group 28.9 years (range 10 to 65). The total number of days in the study was 41,904 for the vitamin subjects (mean 103.2) and 41,864 for the placebo subjects (mean 101.9). There were no statistically significant differences in the frequency of any of the other recorded characteristics of the two groups (Table I). The relative distribution of recorded characteristics in the 182 subjects who dropped out of the study was essentially the same as that seen in the main group.

The 818 subjects recorded a total of 1170 episodes of illness. In the vitamin group 105 subjects (26%) remained free of illness throughout the study, while the corresponding figure for the placebo group was 76 (18%). This difference was statistically significant at the 0.05 level of probability ($X^2 = 5.92$).

Table II
Overall sickness experience of the subjects in the vitamin and placebo groups

	Episodes of illness		Days: Symptoms present		Days: Confined to house	
	V	P	V	P	V	P
Total number	561	609	2138	2474	531	769
Number per subject: Mean ± S. E.	1.38 ± 0.061	1.48 ± 0.056	5.25 ± 0.297	6.02 ± 0.284	1.30 ± 0.101	1.87 ± 0.138
t—value	1.21		1.87		3.33**	
V/P	93%		87%		70%	
†Mean number per episode: Mean ± S. E.	—		3.96 ± 0.162	4.18 ± 0.139	1.04 ± 0.074	1.32 ± 0.087
t—value	—		1.03		2.45*	
V/P	—		95%		79%	

V = vitamin group, P = placebo group
Approximate statistical probabilities: * < .05, ** < .001
†These figures are based on the average values for each subject. The corresponding mean values calculated direc ly from the over-all number of days and episodes were: (symptoms) 3.81 and 4.06, (house) .95 and 1.26.

Table III
The mean number of episodes of illness, days on which symptoms were present, and days confined to the house per subject in each group, classified according to site of predominant symptoms on day of onset

Site of predominant symptoms	Episodes per subject			Days per subject: Symptoms present			Days per subject: Confined to house		
	V	P	V/P (%)	V	P	V/P (%)	V	P	V/P (%)
Nose	0.79	0.84	94	2.92	3.07	95	0.64	0.78	82
Throat	0.36	0.43	84	1.67	2.02	83	0.47	0.73	64
†Other: a) Respiratory	0.10	0.10	100	0.39	0.45	87	0.13	0.21	62
b) Non-respiratory	0.12	0.11	109	0.32	0.31	103	0.10	0.15	67

†Episodes beginning mainly at sites other than nose or throat have been divided into those in which symptoms affecting either the nose, throat or chest appeared at some stage of the episode ("respiratory") and those in which there were no respiratory symptoms at any stage ("non-respiratory").

The overall sickness experience of the two groups is summarized in Table II in terms of frequency of episodes, duration of symptoms and duration of "disability" (as measured by days confined to the house). Although the mean number of episodes per subject was 7% lower in the vitamin group, this difference was not statistically significant. (In Table II the t-value is the ratio of the difference between means to its standard error; because of the very asymmetrical form of the distributions involved, the probability values are only approximate). Statistical evaluation of the average duration of the episodes was complicated by the fact that some individuals experienced more than one episode of illness and these episodes often varied considerably in length. The mean number of days per episode in each group has therefore been calculated from the average number of days per episode for each person. On this basis the mean duration (days of symptoms per episode) for the vitamin group was 5% lower than that for the placebo group, and the mean length of disability (days confined to the house) per episode was 21% lower for the vitamin compared to the placebo group. The latter difference was statistically significant ($P < 0.05$). (It should be noted that the percentage reductions in days per episode are underestimates, since an episode could not be less than one day in length.)

Owing to the combination of fewer episodes and fewer days per episode, the difference between the groups was marked in terms of days per subject, particularly days confined to the house, in which the mean figure for the vitamin group was 30% lower than that for the placebo group, a difference which was statistically significant ($P < 0.001$). There were 232 subjects in the vitamin group who did not report any days confined to the house, compared to 195 in the placebo group ($P < 0.01$.)

Other measures of severity or disability showed a similar pattern. The mean number of days off work was 0.88 per subject in the vitamin group, compared to 1.31 in the placebo group ($V/P = 67\%$, $P < 0.01$). (The number of days off work was lower than the number of days confined to the house because some of the latter occurred on weekends and holidays.) The number of subjects who did not report any days off work was 275 in the vitamin group and 243 in the placebo group ($P < 0.05$). Similarly, while 40 of the vitamin subjects saw a doctor on a total of 60 occasions, 56 of the placebo subjects saw a doctor on a total of 97 occasions. The intake of antibiotics and other prescription drugs showed a similar distribution.

In Table III episodes of illness have been classified according to the site of the predominant symptoms on the first day of the episode. It is interesting, in view of the hypothesis being tested, that those episodes beginning with predominantly nasal symptoms showed the least difference between the two groups in the days of sickness and disability per subject. Indeed, the most striking feature of the figures shown in Table III is the similarity of the vitamin/placebo differences regardless of the type of onset. The only exception is in the episode-per-subject rate in those episodes with predominantly throat onset, in which there was a lower but statistically not significantly different rate in the vitamin group. Of particular interest, in spite of the small numbers involved, was the similarity of the pattern in the "Other: non-respiratory" group, made up of those episodes which at no time involved the nose, throat or chest. (About half of these appeared to be typical attacks of gastroenteritis, while others were characterized by malaise or chills without any localizing symptoms.)

A large number of cross-tabulations were prepared to see if the difference in the disability experience between the vitamin and placebo groups could be explained on the basis of mismatching of the two groups, or different disease patterns at different times during the trial. This line of enquiry was essentially negative, since although there were differences in the size of the "vitamin effect", the vitamin subjects always showed less disability, whatever their other characteristics. These cross-tabulations are summarized in Tables IV and V. For brevity, only the figures for days confined to the house per subject are given, since these show some of the largest differences between the groups, and are based on reasonably large numbers. They are also likely to be of more medical and economic importance than the actual number of episodes or the number of days on which symptoms were present.

In Table IV it can be seen that there was a greater reduction in disability experience for the subjects who reported that they usually had two or more colds, who were in contact with young children, or who were frequently in crowds. Since each of these characteristics might be associated with an increased vulnerability to infection, the subjects were reclassified according to the number of episodes they experienced, to see if the higher average duration of disability in the placebo group was due to the experience of a few particularly vulnerable individuals. This was found not to be the case, although there was evidence of a trend in the size of the reduction of average disability per episode, from 17% in persons with one episode to 27% in those with three or more (Table VI).

Table IV

The mean number of days confined to the house per subject in each group, subdivided according to certain other characteristics of the subjects

		Days confined to house per subject		
		V	P	V/P (%)
Age:	<25	1.40	2.00	70
	25+	1.20	1.74	69
Sex:	Male	1.15	1.79	64
	Female	1.43	1.94	74
Occupation:	Student	1.46	1.78	82
	Other	1.18	1.94	61
Usual colds:	<2	1.30	1.50	87
	2+	1.31	2.29	57
Contact with young children?	Yes	1.31	2.43	54
	No	1.30	1.56	83
Frequently in crowds?	Yes	1.27	1.92	66
	No	1.41	1.69	83
Daily juice (ounces)	0–3	0.98	1.87	52
	4+	1.45	1.87	78
Vitamin supplements?	Yes	1.36	2.39	57
	No	1.29	1.79	72
Smoker?	Yes	1.27	1.82	70
	No	1.32	1.90	69

Two other characteristics that showed substantial differences in the size of the "vitamin effect" were the daily intake of fruit or vegetable juice and the use of vitamin supplements (Table IV). The possible significance of these differences will be considered in the Discussion.

The data were also analyzed to see if there was any particular type of symptom that appeared less frequently in the vitamin group. A summary of the findings is given in Table VII, and it will be noted that the difference between the two groups was most marked in the generalized, constitutional symptoms—severe malaise, chills (mild or severe) and fever (oral temperature 100° F. or higher). The local symptoms (referable to nose, throat and chest) showed less striking differences. With the exception of severe malaise and fever, the reduction was mainly in the number of days per episode, rather than in the number of episodes per subject. In the case of fever the reduction was almost entirely in the frequency of episodes (with the majority of episodes containing only one day of fever), while in the case of severe malaise there was an almost equal reduction in both the mean number of episodes per subject and in the mean number of days per episode.

The apparent similarity between the effectiveness of the vitamin on the limitation of constitutional symptoms and on the reduction of days confined to the house is explained by the fact that it was the constitutional rather than the local symptoms which were most frequently associated with disability.

Other findings: It has been claimed that in addition to a specific effect on "colds", the intake of large doses of vitamin C produces an increased sense of well-being.[2] When questioned at the end of the study only 77 (19%) of the vitamin group said that they had felt an increased sense of well-being during the study, and 78 (19%) of the placebo group answered in the same way. This claim therefore seems to have little justification.

Of the 182 subjects who dropped out of the study 28 did so because of suspected side effects, distributed almost equally between the vitamin (15) and placebo groups (13). Gastrointestinal symptoms such as nausea, abdominal cramps or diarrhea were the symptoms in nine (vitamin: five, placebo: four), skin rash in five (vitamin: two, placebo: three) and genito-urinary in three (vitamin: none, placebo: three). The occurrence of less severe side effects was not monitored in any detail, but at the end of the study, in answer to the question "Did you have any unusual symptoms while you were taking the tablets?", the proportion answering yes was almost identical in the two groups (vitamin 12%, placebo 11%).

Table V

The mean number of days confined to the house per subject in each group during each month of the study, according to the subjects' starting date and total days in the study

	Number of subjects		Days confined to house per subject		
	V	P	V	P	V/P (%)
Month: December	212	213	0.29	0.61	48
January	403	404	0.52	0.66	79
February	407	408	0.42	0.59	71
March	391	385	0.23	0.34	68
Starting Date: December 5	160	160	1.34	1.91	70
January 2	133	123	1.43	1.78	80
Other	128	114	1.11	1.91	58
Total Days: 118 (Dec. 5—March 31)	122	111	0.95	1.72	55
90 (Jan. 2—March 31)	133	120	1.43	1.80	79
Other	152	180	1.48	2.01	74

Table VI

The mean number of days confined to the house (per episode) for subjects in each group, according to the total number of episodes of illness experienced during the study

Number of episodes	Number of subjects		Mean days confined to house (per episode)		
	V	P	V	P	V/P (%)
0	105	76	—	—	—
1	138	155	1.18	1.43	83
2	104	114	1.00	1.31	76
3+	60	66	0.81	1.11	73

Table VII

The mean number of days per subject in each group on which certain symptoms were recorded, (++ = severe, + = mild)

	Days symptom recorded		
	V	P	V/P (%)
Nose ++	1.59	1.67	95
+	2.28	2.53	90
Throat ++	0.88	1.03	85
+	1.97	2.18	90
Chest ++	0.40	0.45	89
+	1.08	1.20	90
Malaise ++	0.65	0.95	68
+	1.96	2.13	92
Chills ++	0.17	0.22	77
+	0.78	0.99	79
Temperature >100°F.	0.09	0.20	45

Table VIII

The mean number of episodes and days sick per subject in each group, for episodes during which there were symptoms affecting the nose or throat.

Symptoms	Episodes per subject			Days of symptoms		
	V	P	V/P (%)	V	P	V/P (%)
Predominant on day of onset: Nose	0.79	0.84	94	2.92	3.07	95
Nose or throat	1.16	1.27	91	4.49	5.20	86
Present at any time: Nose	1.08	1.19	91	4.40	5.05	87
Nose or throat	1.14	1.24	92	4.61	5.36	86

Discussion

The primary objective of this investigation was to test the claim that persons taking 1 g. of vitamin C per day would experience a substantial reduction in both the frequency and the total duration of "colds".[3]

We found it difficult to arrive at a single, generally acceptable definition of a "cold", other than that the episode of illness should at some time be marked by symptoms in either nose or throat. The average frequency and total duration of "colds" per subject were therefore calculated according to four different definitions of a "cold" (Table VIII). The difference between the vitamin and the placebo groups was essentially the same according to each of these definitions, the largest differences being a 9% reduction in frequency and a 14% reduction in days sick (using the definition of nose or throat symptoms predominating on day of onset). Neither these differences nor any of the others shown in Table VIII achieved statistical significance, but there was a statistically significant difference in the number of subjects who did not experience any nose or throat symptoms at any time (V: 131, P: 101, $P < 0.05$).

Our estimates are therefore considerably lower than the 45% reduction in frequency of "colds" and the 60% reduction in total days of illness that were observed in Ritzel's study of ski students,[4] on which Pauling based his claims.[3] However, both Ritzel's study and the present one involved limited numbers of subjects and therefore provide estimates that are subject to error. While it is difficult to determine exact limits for the percentage reductions observed by Ritzel, the approximate 95% confidence interval for the 45% reduction in frequency is 34% to 91%. The estimates from the two studies are therefore not necessarily in conflict.

Our finding that disability was substantially less in the vitamin group was entirely unexpected, and may have important theoretical and practical implications. Further studies will, of course, be required to confirm this finding and to establish its magnitude more precisely, but the high level of statistical significance associated with it encourages us to believe that it is likely to be a real effect rather than a statistical artefact. (The theoretical possibility that the placebo tablets were causing a prolongation of disability was considered but rejected. Citric acid is not known to be harmful at these dosages, and even at the maximum intake of 16 tablets per day, the amount of citric acid ingested was barely one tenth the quantity contained in an average-sized grapefruit.) We have therefore examined our data to see if they provide any clues as to the way in which these large doses of ascorbic acid might be exerting their effect.

One of the possibilities considered was that the reduction in days of disability in the vitamin group was due to some of our subjects being on diets that were, by conventional standards, deficient in vitamin C. (If this were the case the massive doses would be merely ensuring that the basic requirement of approximately 30 mg. a day was being received, and a much lower dose would have achieved the same effect.) While this possibility could not be ruled out, since we made no attempt to measure dietary intake or body stores of vitamin C, it would seem to be an unlikely explanation, since not only are foods containing vitamin C readily available in Canada, but over two-thirds of our subjects reported an intake of at least four ounces of fruit or vegetable juice each day.

Another question that was examined was whether the reduction in disability in the vitamin group was due to a lower incidence of complications. The data provided little support for this belief, since in both groups approximately 60% of the spells of disability began on the first day of the episode—too early to be ascribed to secondary complications. Furthermore, the number of episodes beginning with nose or throat symptoms in which chest symptoms first appeared on the second day or later was approximately the same in both groups (67 in the vitamin group, compared to 69 in the placebo group).

A third question that is of considerable theoretical interest is whether the large intake of ascorbic acid was exerting a specific anti-viral (or anti-bacterial) effect, or whether the mechanism involved was a non-specific one responding to any type of acute illness, or indeed to any acute stress. Our data cannot provide a clear answer to this question, but the fact that general rather than local symptoms were the most strongly influenced, and that different types of illness appeared to be more or less equally affected, would seem to favour a relatively non-specific mechanism. The high concentration of ascorbic acid normally found in the adrenal cortex (and its depletion at times of stress) may be relevant to this question.

Whatever the final answers may be to these and other questions, it would seem that further research in this area is well justified. In economic terms alone the rewards might be substantial, since the disability from acute (mainly respiratory) illness in Canada amounts to approximately 1.5 days per person each year.[5] In terms of total personal income (approximately 66 billion dollars in 1970[6]) this is equivalent to a loss of approximately 270 million dollars annually; even a small reduction in total disability would represent a very large saving to the national economy. However, before these potential economic benefits can be realized, further studies are required to establish the most appropriate dosage levels, the relative importance of the prophylactic and therapeutic features, and the safety of prolonged ingestion of large doses of ascorbic acid or its salts. Until more information is available on these questions we do not feel that any firm recommendations can be made concerning the place of large doses of ascorbic acid in the prevention and treatment of "colds" or other acute infections.

Many individuals assisted in the planning and execution of this investigation, and while it is impossible to name them all, we would particularly like to thank E. Van Sickle, R.N., for her valuable contribution, and S. Whalen, H. Moghadam, K. L. Weldon, A. Csima, J. Hatcher and M. Fears for their help in the design of the trial and the analysis of the results. We would also like to thank A. Harris and C. Retchford of Empire Laboratories for their assistance in preparing a convincing placebo tablet at very short notice, and, last but not least, we thank the large number of subjects who participated in the study.

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THE EFFECT OF GRADED DOSES OF VITAMIN C UPON THE REGENERATION OF BONE IN GUINEA-PIGS ON A SCORBUTIC DIET

By GEOFFREY BOURNE,[1] *From the University Laboratory of Physiology, Oxford*

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Both experimental and clinical observations [Höjer, 1923; Aschoff & Koch, 1919] have indicated that in scurvy it was the bony tissue which showed the most constant and characteristic lesions. Typical changes were the loosening of the attachments of the periosteum to the bone, the appearance of subperiosteal haemorrhages which often extended considerable distances along the shaft, and a thinning of the cortices of the long bones and of the bony trabeculae. In addition, the marrow was found to lose its red colour and to become pale and gelatinous in appearance.

The earliest worker to investigate experimentally the effect of scurvy on the regeneration of bone was Shinya [1922], who showed that a graft of bone from a scorbutic to a normal guinea-pig would not 'take' and that conversely the transplantation of a sound bone into a scorbutic animal was ineffective; although the early death of the animals from scurvy made prolonged investigation of the latter experiment impossible. Subsequent investigators found in scorbutic guinea-pigs delay in the formation of new bone in fracture calluses [Ferraris & Lewi, 1923; Israel & Frankel, 1926; Hanke, 1935; Hertz, 1936; Lexer, 1939] and in saw cuts in the cranial bones [Watanabe, 1924; Schilozew, 1928].

Halàsz & Marx [1932] have demonstrated that if vitamin C is given to guinea-pigs in amounts exceeding the normal requirement there is no acceleration of callus formation. On the other hand, Hanke [1935] and Giangrasso [1939] claimed that in rabbits, extra vitamin C did accelerate the healing of fractures. Bourne [1942], using what was probably a more accurate technique, was unable to show that extra vitamin C had any effect in accelerating the regeneration of bone in rats (which like rabbits synthesize their own vitamin C and can be presumed therefore to be saturated with it).

[1] Mackenzie Mackinnon Research Fellow of the Royal College of Physicians of London and the Royal College of Surgeons of England.

Although some of the workers mentioned have used mild or severe scurvy in their experiments none appear to have made any attempt to find the critical value for the amount of vitamin C required to promote optimum regeneration of bone. This is the object of the present work.

Methods

Twenty-eight young male guinea-pigs of approximately the same age and weight were placed on a scorbutic diet. The diet was composed of a daily mash of crushed oats, wholemeal flour, cod-liver oil and water, and of a hard cake which was provided in excess. The cake contained 'weatings', ground wheat, ground oats, ground barley, ground maize, meat and bone meal, dried skim milk, salt, dried yeast, fish meal and cod-liver oil. The animals were given varied doses of vitamin C by subcutaneous injection each day of the amounts shown below:

5 received 0·25 mg.
4 received 0·50 mg.
5 received 1·00 mg.
5 received 2·00 mg.
4 received 4·00 mg.

At the end of 1 week on this diet a hole, 1 mm. in diameter, was bored aseptically in both femora of each guinea-pig (for details see Bourne, 1942). Standard holes of this size provided exactly comparable areas for regeneration in each animal. After 7 days the animals were killed and the femora fixed, decalcified, sectioned and stained with haematoxylin and van Gieson.

Results

Animals receiving no vitamin C

There is considerable variation in the histological picture between the femora of various animals in this group and in some cases between the right and left femora of the one animal. This latter finding indicates that local factors may play as important a part, under certain circumstances, as systemic factors. One of the first normal responses to bone injury is usually a multiplication of cells in what is called the 'cambial layer' of the periosteum, that is, the inner layer of the periosteum near the bone. Even this reaction was absent in some of the femora of this group. Occurring more or less simultaneously with the cambial reaction is the aggregation of fibroblasts in the clot and the beginnings of organization, i.e. the production of fibres, presumably by the activity of the fibroblasts, which, according to Danielli [1942], may spin such fibres from a layer of protein adsorbed on to their surfaces. Fibroblasts are present in the clot in most of the femora but in some they appear to have produced no fibres. The clot was brittle and in some sections had crumbled, presumably because

one femur in which there was no reaction at all to the injury. The marrow had oozed through the hole and there was no accumulation of fibroblasts and fibres in the region of the hole.

Animals receiving 0·5 *mg. vitamin C*

In all the femora examined in this group there was a distinct reaction on the part of the cambial layer. Although there was multiplication of the cells in this region there was no formation of trabeculae between the bone and the periosteum. Four out of seven femora examined showed a considerable reaction on the part of the periosteum, which was particularly intense near the hole.

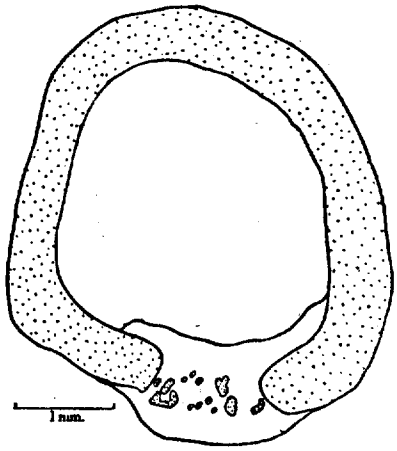

Fig. 3.

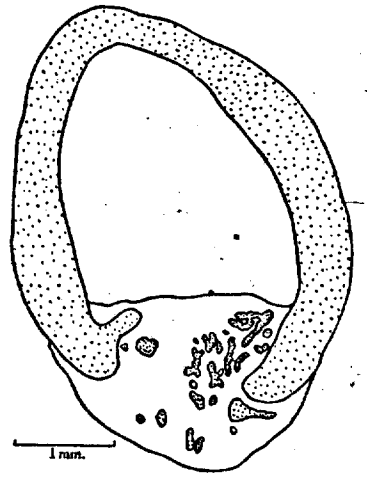

Fig. 4.

Fig. 3. Projection drawing of section through the hole in the femur of a guinea-pig on a scorbutic diet with 0·5 mg. vitamin C per day.

Fig. 4. Projection drawing of section through the hole in the femur of a guinea-pig on a scorbutic diet with 1·0 mg. vitamin C per day.

For lettering see Fig. 6.

In four femora there were the beginnings of an endosteal reaction, i.e. a few small trabeculae which appeared to be formed from the endosteum near the margin of the hole. Most femora contained a number of fibres in the hole, but while in one femur of one animal there was a mass of dense fibres, in the other femur the hole contained a number of trabeculae. A number of osteoblast-like cells were present in the hole in most femora and some capillaries were present.

Animals receiving 1 *mg. vitamin C*

In all the eight femora examined in this group there was a considerable reaction on the part of the periosteum and the endosteum. The former showed not only cellular multiplication of the cambial layer but in most cases the

formation of bony trabeculae in this layer between the periosteum and the surface of the bone, in other words the osteogenetic power of the periosteum had been retarded up to this point. The endosteum had produced bony trabeculae in most of the femora in the immediate precincts of the hole. The hole itself was in all cases densely packed with fibres which did not stain with van Gieson. In a few of the femora some of these fibres, although not arranged in trabeculae, stained with varying intensity with van Gieson. There were many capillaries in the hole and numerous osteoblasts were present, particularly in regions where trabeculae were forming.

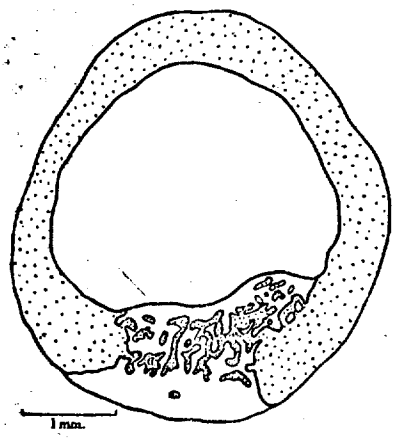

Fig 5.

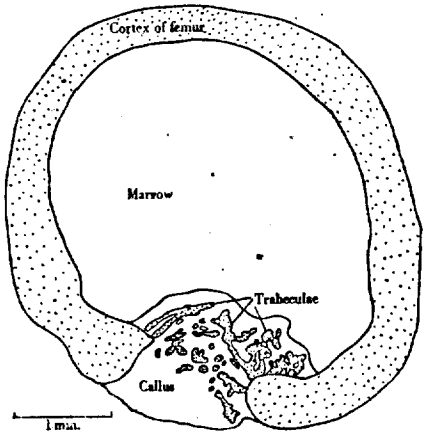

Fig. 6.

Fig. 5. Projection drawing of section through the hole in the femur of a guinea-pig on a scorbutic diet with 2·0 mg. vitamin C per day.

Fig. 6. Projection drawing of section through the hole in the femur of a guinea-pig on a scorbutic diet with 4·0 mg. vitamin C per day.

Animals receiving 2 mg. vitamin C

In most of the femora of this group the inner part of the hole (near the endosteum) was filled with bony trabeculae which linked with those formed by the endosteum. The outer part of the hole was packed with fibres many of which stained with van Gieson. The periosteum in all members of this group appeared to have attained its full osteogenetic power and to have produced large numbers of thick trabeculae in association with the uninjured bone, but it had not formed many trabeculae in the outer part of the hole. Possibly this stage does not appear in the guinea-pig until later in the healing process. In a rat in most cases such a hole is completely filled with trabeculae at the end of a week. There were many osteoblasts and capillary blood vessels present in the holes of all animals.

Animals receiving 4 mg. vitamin C

In the femora of this group too there was considerable periosteal and endosteal reaction with the formation of bony trabeculae by both. Most of the holes contained numerous fibres, but generally speaking there appeared rather less van Gieson staining material than in the preceding group. This fact is illustrated in figures given later. There were numerous osteoblasts and capillary blood vessels present in the tissue filling the holes in all animals.

Summary of changes

Dose of vitamin C mg.	Periosteal reaction	Endosteal reaction	Fibres in hole	Trabeculae
None	Negative	Negative	A few	None
0·25	Slight multiplication of cells in cambial layer	Negative	Fairly numerous	None
0·50	Considerable multiplication of cells in the cambial layer	Slight reaction. Beginnings of formation of trabeculae	Numerous	None
1·00	Cambial layer forming trabeculae	Small number of trabeculae forming	Very large numbers	A few
2·00	Very large reaction. Enormous multiplication of cambial layer cells. Many trabeculae formed	Extensive formation of trabeculae	Very large numbers	Numerous, particularly in inner part of hole
4·00	Ditto	Ditto	Ditto	Ditto

An attempt was also made to obtain figures which would indicate the degree to which the formation of new bone has occurred in various groups of animals. A technique for this purpose has been described by Bourne [1942]. Briefly it is as follows.

The trabeculae in five random sections taken through the region of the middle of the hole in each femur were projected on to paper and drawn. The weight of a standard rectangle of paper which fitted into the hole was obtained and the trabeculae contained in this rectangle were drawn and subsequently cut out and weighed. The division of the second weight by the first gave a figure called the 'trabecular index'. Since some of the indices obtained in this work were so small it was found necessary for convenience to multiply all indices by 100.

The results obtained by the application of this technique were as follows:

		Trabecular index times 100
5 animals on a scorbutic diet.	No supplement of vitamin C	7·73 ± 2·25
5 " " "	Supplement 0·25 mg. vitamin C	6·70 ± 2·30
4 " " "	" 0·50 mg. "	9·74 ± 2·48
5 " " "	" 1·00 mg. "	19·41 ± 3·53
5 " " "	" 2·00 mg. "	23·73 ± 6·75
4 " " "	" 4·00 mg. "	18·09 ± 4·48

The apparent decrease in the amount of trabeculae formed in the group receiving 0·25 mg. vitamin C compared with that of the animals receiving no vitamin C supplement, is probably spurious. In these two groups, and also in the next group (that receiving 0·50 mg. of vitamin C per animal per day), there was no formation of true trabeculae. Small spots of van Gieson staining material were found and occasionally relatively large areas which appeared to have been formed by the clumping together and fusing of pre-collagen fibres were seen. These masses stained very faintly, and it was difficult to decide whether to include them as trabeculae or not. However, they were included and since there were rather more of them in the completely scorbutic group than in the group receiving 0·25 mg. vitamin C, their inclusion as trabeculae weighted the results towards the former group.

There was a slight increase in the amount of van Gieson staining material present in the next group (0·25 mg. vitamin C).

In the 1 mg. group there was a considerable increase in the van Gieson staining material, and in this group for the first time the isolated islands of van Gieson staining material linked up to form trabeculae. In the 2 mg. group this process was carried a stage further. The drop in the amount of van Gieson staining material in the 4 mg. group may be due to the retarding effect of the higher dose of vitamin C, but it seems more likely to be due to experimental error. In fact it is very difficult satisfactorily to apply the technique of estimation of trabeculae to this work owing to the difficulty in the scorbutic and partially scorbutic animals of deciding what material to include as trabeculae and what not to include; under the circumstances the application of Fisher's 't' test for significance to the figures would be of little value.

Discussion

These results show that without adequate vitamin C there is practically a complete inhibition of the reparative processes in a damaged bone. As the dose of vitamin C increases, one reparative function after another comes to life, until, with a daily supplement of 2 mg., regeneration appears to be taking a normal course. The critical dose lies somewhere between 1 and 0·5 mg. per day as can be seen from the list of trabecular indices. There is still further improvement in the histological picture when 2 mg. vitamin C are given per day but no apparent further improvement at the 4 mg. level.

The effects of complete deficiency of vitamin C obtained in this work are similar to those obtained by other workers [e.g. Wolbach & Howe, 1926; Hertz, 1936]. Hertz points out that in a completely scorbutic animal, following bone injury, there is no periosteal hyperaemia, and that in general it is difficult for a scorbutic animal to produce an inflammatory reaction in response to injury, and that, in the absence of such a reaction, the normal processes of healing cannot be introduced. The enlargement of the cambial

layer of the periosteum, Hertz believes to be an essential stage of this inflammatory reaction.

Hertz [1936], like Watanabe [1924], noticed a delayed absorption of the haematoma at the site of the fracture in scorbutic animals. The same phenomenon has been observed in this work. In the animals receiving no vitamin C and in those receiving 0·25 mg. of the vitamin, at the end of the experimental period (7 days after injury) the whole of the connective tissue supporting the muscles associated with the femur was permeated with blood which had not been absorbed. The impression given was that the blood had not clotted very readily and that it had continued to ooze from the hole for some time after the operation. Finally, it extended through practically the whole of the soft tissues of the upper part of the leg. The connective tissue was (as has been found by other workers) obviously oedematous and had a soft jelly-like appearance. The animals receiving 0·5 mg. of vitamin C showed a small haematoma at the site of injury, but in the 1·00 mg. group and the 2·0 and the 4·0 mg. group it had disappeared by the time the animals were killed.

This work confirms the results of other workers that there is a delay in the development of collagen fibres and delay in their maturation [e.g. Hunt, 1941] in scurvy.

In view of the present war situation it would be of interest to attempt to interpret the results with guinea-pigs in terms of human requirements. This, however, is no easy task.

Göthlin [1934] has stated that the stage of scurvy in guinea-pigs which is recognizable only by means of microscopic alterations in the teeth is equivalent to that prescorbutic stage in man which is indicated by fragility of the capillaries. He found that such teeth changes could be prevented in the guinea-pig by a minimum dose of 1·33 mg. a day of vitamin C and that 19–27 mg. a day were required to prevent capillary changes in man. Therefore the factor for converting guinea-pig doses of vitamin C to human doses should be approximately 17, and anything less than 17 mg. of vitamin C a day for a human being with a fractured bone would be likely to retard seriously the healing of the fracture and 34 mg. a day would be required to secure the maximum formation of callus.

On the other hand, Kellie & Zilva [1939] state that the daily dose necessary to maintain the condition of saturation in a human being is 30–40 mg., and that a guinea-pig requires for the same purposes about 20 mg. [Zilva, 1936]. These results suggest that a human being needs only twice as much vitamin C as a guinea-pig, and that therefore 4 mg. of the vitamin per day in a human being with an injured bone would be sufficient to induce regenerative changes equivalent to those obtained in the guinea-pig with 2 mg.

But a guinea-pig requires 0·5–1 mg. of vitamin C a day to protect it from macroscopic signs of scurvy, and Fox, Dangerfield & Gottlich [1940] found

that a daily intake of 12–25 mg. of vitamin C resulted in the appearance of scurvy in twelve out of 950 mine labourers studied. Apparently then an intake of this order is approaching the scorbutic danger level. The vitamin C demands of mine labourers may be greater than those of persons lying in bed in hospital with a fracture, but even so it seems that anything less than 10 mg. a day in such a patient would be undesirable. If we accept 10 mg. of vitamin C as the possible minimum amount required to prevent macroscopic scurvy in such patients, then we see that it is necessary to multiply a guinea-pig dose by 15–20 in order to obtain the human equivalent. Therefore to secure the same degree of bone healing present in a guinea-pig with 2 mg. of vitamin C a day, human beings should receive about 40 mg., and anything less than 20 mg. would seriously retard the healing process. Fox *et al.* [1940], however, found no evidence that fractures or wounds healed more rapidly in mine labourers on 40 mg. of vitamin C than on those receiving 12–25 mg. per day. But they admit that comparison was difficult owing to the difference in situation and severity of the lesions.

There are many other complications, for example Crandon [see Crandon, Lund & Dill, 1940] found that it took him 169 days to develop macroscopic scurvy on a vitamin C-free diet, Wolbach & Howe [1926] found that when the bones of scorbutic guinea-pigs were injured there was a temporary healing process initiated in the incisor teeth, and Lauber, Nafziger & Bersin [1937] found that a complete fracture of the humerus in rabbits caused an increase in the excretion of vitamin C.

Nevertheless, if we assume that 40 mg. vitamin C a day is needed to secure maximum regeneration of injured bone then the results are in keeping with recent observations on the vitamin C requirement of man [see Smith, 1938; Harris, 1941]. In any case it is desirable that patients with fractured bones should be given at least 40 mg. vitamin C a day as long as there is the slightest doubt about the efficacy of smaller doses.

Summary

1. The effect of the injection of graded doses of vitamin C on the regeneration of bone in guinea-pigs has been investigated by measuring the amount of trabeculae formed in a hole bored in the femur at the end of one week.

2. It has been found that guinea-pigs require 2 mg. of vitamin C a day by injection to secure adequate regeneration of bone and that less than 1 mg. seriously retards regeneration.

3. It is suggested that the corresponding doses to produce the same results in human beings may be 40 mg. and 20 mg. vitamin C.

4. Pure synthetic vitamin C is alone able to promote regeneration of bone in scorbutic guinea-pigs.

I am indebted to Roche Products for a supply of vitamin C for these experiments.

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Vitamin C and Immunity

By G. H. BOURNE, *London Hospital Medical College, Whitechapel, London, E. 1*

The term immunity means complete insusceptibility to infection, but we are not perhaps as much interested in this absolute condition as in relative immunity: in other words, a variable which may be anywhere between zero and infinity. The word immunity is better replaced by the word resistance, so that what I should talk about in this paper is whether vitamin C plays any part in improving the relative resistance of the body to infective agents.

The resistance of the body to infection depends upon a series of factors which are summarized by Wilson & Miles (1946) as follows:

(1) Innate immunity.
(2) Acquired immunity.
  (*a*) Active.
    (i) Naturally acquired.
    (ii) Artificially induced.
  (*b*) Passive.
    (i) Naturally acquired (congenital).
    (ii) Artificially induced.

It is not possible in this paper to deal with all the literature on the relationship of vitamin C to this problem and I propose to refer only to some aspects of it.

Relation of vitamin C to complement

One of the important factors in innate immunity is 'complement'. This is a non-specific, anti-bacterial activity which is present in all normal serums and is not increased by immunization. It is probably due to interaction of a number of separate components which, according to some authors, are four in number.

A number of papers has been published on the relationship of vitamin C to complement. Simola & Brunius (1933) claimed that in vitamin C deficiency in guinea-pigs complement titre was reduced. This claim was supported by Marsh (1936). Other authors, however, e.g. Zilva (1936) and Chakraborty (1937), could find no significant alteration in the complement titre of scorbutic guinea-pigs. Ecker, Pillemer, Wertheimer & Gradis (1938) and Ecker & Pillemer (1939) claimed that if guinea-pigs on a scorbutic diet were given graded doses of vitamin C the larger doses of the vitamin were paralleled by increases in the complement titre. The apparent conclusiveness of these two papers was challenged by Chu & Chow (1938) and Maccolini (1939), who found that guinea-pig complement titre was not influenced by a scorbutogenic diet and that the addition of vitamin C to such a diet also had no effect on the complement titre. The observations of Crandon, Lund & Dill (1940) showed that in human scurvy the complement titre did not change, and Spink, Michelson & Agnew (1941) and Natvig (1941) came to a similar conclusion. Feller, Roberts, Ralli & Francis (1942) studied immunological phenomena in relation to intake of both vitamin C and vitamin A. They tested the capacity of nasal secretion to inactivate influenza virus, the titre in blood serum of neutralizing antibodies for influenza virus, the activity of lysozyme in the nasal secretion, the phagocytic activity of leucocytes and complement titre. None of these tests was significantly influenced by marked changes in the plasma levels of vitamin C induced by a period of severe vitamin C deficiency followed by a large excess of the vitamin. The authors argued that, because of the large number of factors involved in immunological reactions, it is difficult to generalize from their results, and it may be that the number of variables is responsible for the very diverse results that have been obtained in such studies. Kodicek & Traub (1943) tried to eliminate as many of these variables as possible but still found no significant change in complement in

guinea-pigs partly or completely deprived of vitamin C, and finally Deeny, Murdock & Rogan (1943) found that in eighty patients in Northern Ireland with acute infections there was no relation between the vitamin C level of the blood and complement titre.

It is difficult in the light of these contradictory results to say whether vitamin C does, in fact, play any part in maintaining the complement titre of the blood, although the weight of evidence seems to be against it.

Vitamin C and acquired immunity

Having dealt with one of the measures concerned in natural immunity, we may turn now to consider for a moment whether vitamin C is concerned with acquired immunity. Is it, for example, concerned with antibody production? Jusatz (1936*a*, *b*) found that if 100 mg. of vitamin C were added to an immunizing dose of horse protein there was a 500% increase in the specific precipitation reaction in rabbits. Jusatz's work was confirmed by Mutsuro (1939), Izutumi & Sowai (1939), Madison & Manwaring (1937–8) and Madison, Fish & Frick (1938). Cameron (1938) found that if guinea-pigs were injected with diphtheria toxoid, those receiving 3·6 mg. of vitamin C daily had more antitoxin in their serum that those receiving 0·9 mg. Here we seem to have something more definite. What still needs to be found out is at what level of vitamin C intake in animals and man maximum antibody production can be secured for selected organisms. At this point mention may perhaps be made of the work of Birkhaug (1939), who found that if he injected guinea-pigs with tubercle bacilli and administered 10 mg. of vitamin C a day to the animals, his controls receiving an adequate diet but no special supplement of the vitamin, there was a significant inhibition of the tuberculin reaction in the animals receiving extra vitamin C. The tuberculin reaction is of course an allergic reaction. Further, he found that the inhibition of the tuberculin reaction was definitely correlated with urinary excretion of the vitamin and the amount of vitamin C in the animal's adrenal glands. Unfortunately, Heise & Steenken (1939) were not able to confirm Birkhaug's work, but Steinbach & Klein (1941) found that the administration of vitamin C to tuberculous guinea-pigs increased the tolerance to repeated large doses of tuberculin. In chronic tuberculosis, fibrosis of infected areas in the lung occurred more rapidly and caseation was less well marked in guinea-pigs receiving the vitamin. In rabbits, which synthesize their own vitamin C, the administration of the vitamin, as might be expected, has no effect. This point is of interest in connexion with the work of Osborn & Gear (1940), who suggested that animals which are able to synthesize their own vitamin are naturally more resistant to the tubercle bacillus.

There is a large number of references in the literature concerning the clinical use of vitamin C in tuberculosis, but there is not time to consider them here. Since vitamin C is concerned with collagen production and the work of Abbasy, Harris & Ellman (1937) showed that excretion of vitamin C is reduced in tuberculosis, it seems that the vitamin might be beneficial in the clinical treatment of tuberculosis if only because it would facilitate the production of scar tissue.

Vitamin C and inactivation of toxins

There seems to be evidence that certain toxins are inactivated in vitro by vitamin C. For example, Jungeblut (1939) and Kligler, Guggenheim & Warburg (1938) showed that tetanus toxin can be neutralized in vitro by crystalline vitamin C. Souto & Lima (1938) and Catz (1939) claimed that vitamin C also neutralizes the toxin of *Clostridium oedematiens*, and Otani (1939) claimed that if vitamin C is added to the toxin of whooping cough bacteria (*Haemophilus pertussis*), its effect, when it is injected intracutaneously into rabbits or guinea-pigs, is much diminished. Toxicity is less as the quantity of the vitamin increases.

There is also evidence that vitamin C prevents the growth of some bacteria. Büsing (1939*a*, *b*) found that it had this effect on cultures of pneumococci, streptococci and staphylococci. Otani (1939) found that a concentration of vitamin C of 2·5 mg./ml. in the media on which the bacillus of whooping cough was grown had a marked retarding action on its growth and that even concentrations of 1·2–1·8 mg./ml. greatly reduced the virulence of the bacilli.

One should note, however, that in normal human beings the blood plasma level of vitamin C is only 0·6–2·5 mg./100 ml. and that the concentration used by Otani in the medium in which he grew his bacteria was a hundred times as great. In such concentrations the reducing action of vitamin C might well be enough to exert a retarding effect on bacterial growth and to inactivate toxins; the amount used thus appears to be above physiological levels.

Since vitamin C appears to have these antitoxic and antibacterial effects, one would expect that blood having a high concentration of vitamin C would be relatively more bactericidal or at least bacteristatic than blood having a low concentration of vitamin C. However, Pfannenstiel & Dotzer (1940) found that the bactericidal titre of blood was independent of its vitamin C level. Further, Dotzer & Schuller (1942) and Spink, Agnew, Michelson & Dahl (1942) found that if vitamin C was given intravenously to human subjects with low vitamin C levels, it did not result in an increase in the bactericidal action of the blood against staphylococci, *Bacterium coli* and *Salmonella typhosum*. They then oxidized the vitamin C of human blood with copper and found no decrease of the bactericidal action against the same organisms or against *Shigella paradysenteriae* or *S. flexneri*.

A number of papers deal with the effect of vitamin C on *Corynebacterium diphtheriae* and diphtheria toxin. This toxin particularly affects the adrenal glands, causing loss of vitamin C (Bourne, 1935) and destruction of adrenal tissue. Jungeblut & Zwemer (1934–5) found that cortico-adrenal extracts had an inhibiting effect on diphtheria toxin. These observations are of interest in view of the idea that vitamin C may act as a side chain to corticosterones, making them water soluble, and suggest that the hormones of the adrenal and vitamin C may play an important part in the anti-infective processes of the body. It is known, for example, that one of the cortical hormones maintains the water and salt equilibrium of the body, which depends on the integrity and permeability of cell membranes. Some toxins affect the permeability of these membranes; hence there may be an antagonism between a toxin and the hormone.

The effective mobilization of the hormone presumably depends upon adequate vitamin C reserves, apart from the possible direct effect of the vitamin. In addition to this relation between vitamin C and diphtheria there is the more direct work of Jungeblut (1941), who found that diphtheria toxin is inactivated in vitro by vitamin C, particularly in the presence of cupric ions. Willison (1943) confirmed this and found that two minimum lethal doses of the toxin were inactivated by 1 mg. of vitamin C at body temperature.

Greenwald & Harde (1934–5) and others found that increased vitamin C intake reduced the severity of a diphtheria infection.

Against these positive results must be set the negative experiments of Zilva (1937), who found that when guinea-pigs were injected with vitamin C they had no more resistance to diphtheria toxin than guinea-pigs which were depleted of vitamin C, and of Torrance (1938), who found that diphtheria toxin was not inactivated by vitamin C and that there was no loss of vitamin C from the adrenals of test animals injected with diphtheria toxin. On the other hand, Willison (1943) claims that failure to demonstrate the inhibitory effect of the vitamin on diphtheria toxin could be due to the use of the wrong pH, to too short an incubation of the vitamin and toxin, or to an unfavourable ratio of the two. There appears, therefore, to be a sufficient number of variables to account for the disparity of some of the results.

Vitamin C and inactivation of viruses

Vitamin C is also said to inactivate some viruses. Klein (1945) has claimed this for influenza virus and Jungeblut (1939) for poliomyelitis virus. In experiments in vivo they found that in rhesus monkeys vitamin C had a protective effect if the animals were treated with minimum infective doses of the virus. Post-mortem investigation showed fewer pathological changes in animals that had received vitamin C than in those without the vitamin. Sabin (1939), however, was not able to confirm this work. Lyman, Schultze & King (1937) state that when vitamin C oxidizes in air, hydrogen peroxide is formed and that it is the peroxide that inactivates viruses when the vitamin is applied directly to them. This may also explain the effect of the vitamin on bacterial toxins.

Vitamin C deficiency affects phagocytosis of bacteria by leucocytes (Cottingham & Mills, 1943) and delays leucocytic migration (Bourne, 1948). Leucocytes are known to absorb large amounts of vitamin C particularly when they migrate into an injured (Bourne, 1944) or infected (Tonutti & Matzner, 1938) area. A scorbutic diet also causes a fall in white blood-cells (Crandon *et al.* 1940) and subsequent administration of vitamin C causes leucocytosis.

Vitamin C requirements

In this paper I have not attempted to discuss clinical experiments to test the value of vitamin C in infections. In acute infections, metabolism is increased and presumably the demand for all vitamins is increased, possibly much above normal. There seems to be a good case there for increasing total vitamin intake. Whether the vitamin C level

in the tissues and blood of the normal person affects his susceptibility to infection or the virulence of an attack, has yet, I think, to be satisfactorily answered. Man's nearest zoological relations are the great apes. The gorilla weighs more than man and its average energy requirement is probably something like 4000 or 5000 Cal. daily. The chief food of these creatures in the wild is grass or leaves, which have a low energy value. In fact, to obtain 4000 Cal. it would be necessary for a gorilla to eat at least 20 lb. of greenstuff a day. Twenty pounds of such green feed would provide about 4·5 g. of vitamin C daily. Before the development of agriculture and the large-scale growing of cereals which made the development of civilization possible, it is likely that man existed largely on greenstuff supplemented with what meat on the hoof he could catch. It may be possible, therefore, that when we are arguing whether 7 or 30 mg. of vitamin C a day is an adequate intake we may be very wide of the mark. Perhaps we should be arguing whether 1 or 2 g. a day is the correct amount. Perhaps it is normal for our blood and tissues always to be saturated with the vitamin and for large quantities to be flushing constantly through our urinary system and excreted in our sweat. We may find that continuous doses of vitamin C at this level over a considerable period of time may have a pronounced and unequivocal anti-infective action.

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VITAMIN C AND COLDS

SIR,—Professor Wilson and Dr Loh (March 24, p. 638) reported that girls with colds respond to an oral load of vitamin C by larger increases in leucocyte ascorbate than boys. They found relief of symptoms by the vitamin to be significantly better in girls than boys. In a previous study,[1] they reported leucocyte ascorbate concentrations in unsupplemented men and women to be similar, though plasma ascorbate was significantly higher in the women. No sex differences in ascorbate concentrations were seen in geriatric patients.

These findings suggest that sex hormones may influence the metabolism and tissue distribution of ascorbate. We have reported [2] significantly reduced platelet and leucocyte ascorbate in women taking oral contraceptives. We have now examined the effects of vitamin-C supplementation on leucocyte and plasma ascorbate in untreated healthy women, geriatric patients, and women under treatment with various steroid hormones. Our results are summarised in the accompanying table. All blood specimens were analysed by the 2,4-dinitrophenylhydrazine method [3] within 60 minutes of collection.

It is clear that leucocyte and plasma ascorbate are both low in geriatric patients, menopausal women taking œstrogen, and young women taking combined-type oral contraceptives. In contrast, young women taking a progestagen-only product, or receiving long-acting progestagen injections, had ascorbate concentrations not significantly different from untreated controls.

Vitamin-C supplements increased leucocyte and plasma ascorbate in all groups, but to different degrees. The rise in leucocyte ascorbate was 25% to 45% in controls and young women receiving progestagens, but was 85% to 135% in geriatric patients and women receiving œstrogen-containing products. Similar, though larger, changes were seen in plasma ascorbate (75% to 90% increases in controls and progestagen-treated women; 125% to 235% in geriatric patients and œstrogen-treated women).

It seems likely that œstrogens increase ascorbate breakdown and lower tissue levels, perhaps by stimulating release of ascorbate from cells such as leucocytes into plasma. Vitamin-C requirements of œstrogen-treated women are probably raised, while they may well be more susceptible to virus infections, such as the common cold.

Biochemistry Department,
Alfred Hospital,
Prahran, Victoria 3181,
Australia.

MICHAEL BRIGGS.

Kingston Centre,
Cheltenham, Victoria 3193,
Australia.

MAXINE BRIGGS.

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LEUCOCYTE AND PLASMA ASCORBATE IN WOMEN

Group	No.	Mean age (yr.)	Condition	Ascorbate concentration (mean ± S.D.)			
				Leucocytes (μg./10⁸)		Plasma (mg./100 ml.)	
				Before	After (a)	Before	After (a)
A	16	23	Healthy, untreated	35 ± 12	48 ± 15	0·9 ± 0·19	1·6 ± 0·36
B	19	25	Healthy, combined oral contraceptives (b)	24 ± 16	46 ± 19	0·4 ± 0·30	0·9 ± 0·41
C	16	22	Healthy, progestagen-only oral contraceptive (c)	36 ± 11	51 ± 17	0·9 ± 0·22	1·7 ± 0·39
D	12	30	Healthy, depot progestagen (d)	38 ± 17	48 ± 16	0·8 ± 0·21	1·5 ± 0·42
E	10	68	Geriatric patients	23 ± 11	51 ± 14	0·5 ± 0·16	1·5 ± 0·30
F	10	56	Menopausal, œstrogen therapy (e)	20 ± 9	47 ± 16	0·3 ± 0·32	1·0 ± 0·40

(a) After 500 mg. daily vitamin C for 14 days.
(b) 50 μg. daily ethinylœstradiol plus either 1·0 mg. daily norethisterone acetate or 0·25 mg. daily *d*(−)-norgestrel.
(c) 0·35 mg. daily norethisterone.
(d) 150 mg. 3-monthly medroxyprogesterone acetate i.m.
(e) 625 mg. daily conjugated œstrogens.

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CHAPTER 7

Ascorbic Acid

J. J. Burns

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I. INTRODUCTION

The importance of L-ascorbic acid in the prevention of scurvy has been known for many years, but definitive information on its mode of action at the molecular level is not available. L-Ascorbic acid is synthesized in a great variety of plants and in all known mammals, except primates and the guinea pig. Mechanisms for the biosynthesis of L-ascorbic acid in plants and aminals, along with those involved in its catabolism, have been presented in recent years. These studies have disclosed new enzymic reactions for such compounds as D-glucuronic acid, D-galacturonic acid, L-gulonic acid, and L-xylulose. As a consequence of this work a new route of glucose metabolism, the glucuronic acid pathway, has been demonstrated in mammalian tissues. This chapter is concerned primarily with the metabolic pathways involved in the formation and degradation of L-ascorbic acid in animals and plants. Comprehensive reviews on the interaction of L-ascorbic acid with various enzymes, as well as information on the occurrence, chemistry, and physiological functions of the vitamin, are available (*1–4*).

II. BIOSYNTHESIS OF L-ASCORBIC ACID IN ANIMALS

Studies carried out in recent years have shown that glucose is utilized for synthesis of L-ascorbic acid in the rat (*5–7*). In these experiments, both C-1- and C-6-labeled glucose were administered to rats and the incorporation of ^{14}C into urinary L-ascorbic acid was determined (Table I). Glucose-1-^{14}C was converted to L-ascorbic acid labeled

TABLE I

CONVERSION OF VARIOUS LABELED COMPOUNDS TO L-ASCORBIC ACID IN RATS

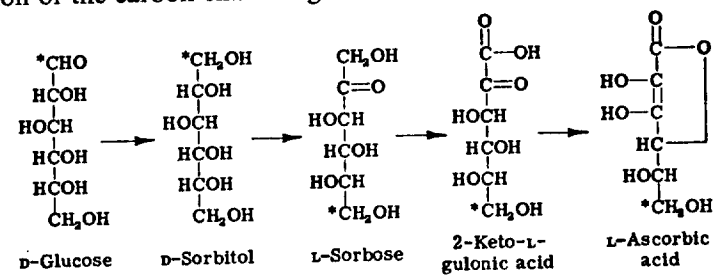
Compound	Percent Conversion to L-ascorbic acid	Percent Total L-ascorbic acid ^{14}C in		References
		C-1	C-6	
D-Glucose-U-^{14}C	0.40	—	—	(*5*)
D-Glucose-1-^{14}C	0.55	11	56	(*6*)
D-Glucose-6-^{14}C	0.50	61	15	(*7*)
L-Sorbose-U-^{14}C	0.40	—	—	(*9*)
L-Sorbose-6-^{14}C	0.30	27	27	(*9*)
D-Glucuronolactone-U-^{14}C	2.2	—	—	(*12*)
D-Glucuronolactone-6-^{14}C	1.2	90	—	(*13*)
L-Gulonolactone-1-^{14}C	3.6	85	—	(*13*)
D-Galactose-1-^{14}C	0.43[a]	—	92	(*25*)
D-Glucose-1-^{14}C	0.045[a]	10	60	(*8,25*)

[a] In these experiments the percent conversion was estimated from the amount of ^{14}C in body L-ascorbic acid at 24 hours by a technique based on the unusually long half-life of L-ascorbic acid in the rat (*13*). In the other experiments, the percent conversion was estimated from the amount of ^{14}C excreted as urinary L-ascorbic acid during a 24-hour period after administration of the labeled compounds to Chloretone-treated rats (*5*).

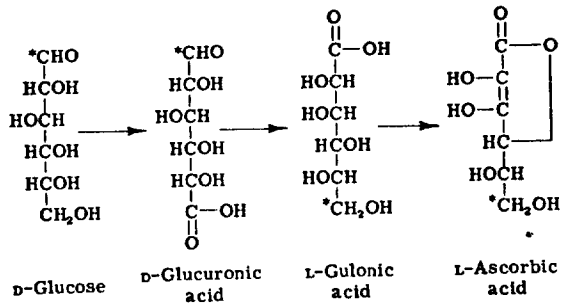
chiefly in C-6, and glucose-6-^{14}C was converted to L-ascorbic acid labeled chiefly in C-1. These results indicate that the carbon chain of glucose is converted intact to L-ascorbic acid by a mechanism in which it undergoes an inversion of configuration. In these studies the synthesis of L-ascorbic acid was stimulated by administration of Chloretone, but a similar precursor role of glucose for L-ascorbic acid has been observed in rats not receiving the drug (*8*).

Two possible pathways, reactions (1) and (2), have been considered

for the biosynthesis of L-ascorbic acid that would account for an inversion of the carbon chain of glucose:



(1)



(2)

According to reactions (1) and (2), C-1 of glucose would become C-6 of L-ascorbic acid as indicated by the asterisks.

Reaction (*1*). This pathway was ruled out by results of experiments (*9*) employing L-sorbose-6-^{14}C. If L-sorbose is an intermediate, it would be expected that L-sorbose-6-^{14}C would yield C-6-labeled L-ascorbic acid. However, this was found not to be the case since administration of L-sorbose-6-^{14}C to rats resulted in L-ascorbic acid labeled about equally in C-1 and C-6. In fact, any ^{14}C incorporated into L-ascorbic acid following administration of the labeled L-sorbose may be accounted for by the intermediate formation of glucose. In addition, experiments employing uniformly ^{14}C-labeled 2-keto-L-gulonic acid showed no detectable conversion of this compound to L-ascorbic acid in the rat (*10*).

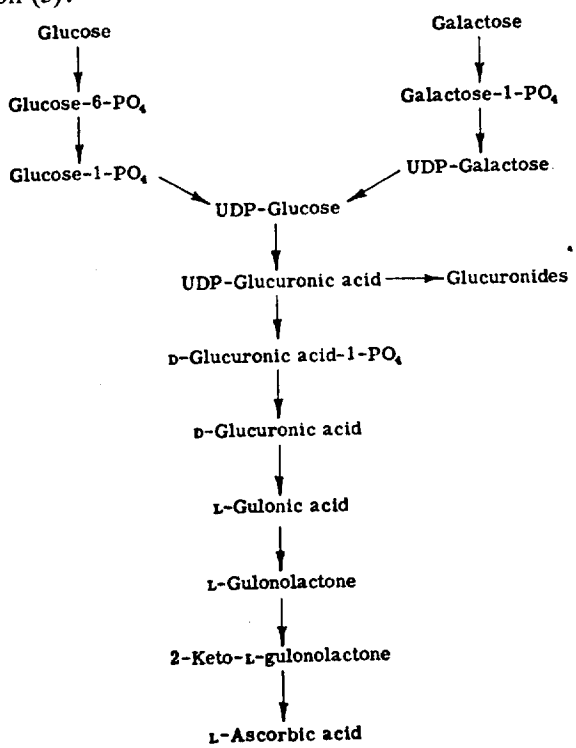
Reaction (*2*). Considerable evidence is now available for the conversion of glucose to L-ascorbic acid by this pathway in animals. Isherwood and co-workers (*11*) reported that the administration of D-glucuronolactone and L-gulonolactone to rats produced an increase in the urinary excretion of L-ascorbic acid. Definitive evidence for conversion of the intact carbon chain of D-glucuronolactone and L-gulonolactone to

L-ascorbic acid in rats came from isotopic experiments (Table I). Administration of uniformly labeled D-glucuronolactone to Chloretone-treated rats resulted in the excretion of uniformly labeled L-ascorbic acid (*12*). Carboxyl-labeled D-glucuronolactone and L-gulonolactone yielded carboxyl-labeled L-ascorbic acid in both normal and Chloretone-treated rats (*13*). In addition, the conversion of D-glucose-1-^{14}C to urinary D-glucuronic acid and L-gulonic acid has been shown to occur in rats receiving barbital and Chloretone, drugs known to stimulate the synthesis of L-ascorbic acid (*14,15*).

Further evidence for reaction (2) has come from the finding that enzymes exist in liver that convert D-glucuronic acid and L-gulonic acid to L-ascorbic acid (*16,17*). An NADP-linked dehydrogenase present in the soluble fraction reversibly catalyzes the reduction of D-glucuronic acid to L-gulonic acid (*16,18,19*). A lactonase has also been demonstrated in the soluble fraction that carried out the interconversion of L-gulonic acid with L-gulonolactone (*20,20a*). In addition, enzymes are present in liver microsomes that convert L-gulonolactone to L-ascorbic acid (*16,21*). This microsomal system has been solubilized, and evidence was obtained for 2-keto-L-gulonolactone as the most likely intermediate in this reaction (*22,22a*).

The only known, mechanism for conversion of glucose to D-glucuronic acid is via the oxidation of uridine diphosphoglucose to uridine diphosphoglucuronic acid (UDPGA), a reaction catalyzed by an NAD-linked enzyme in the soluble fraction of liver (*23,24*). An enzyme has been demonstrated in liver microsomes that forms glucuronides by transferring D-glucuronic acid from UDPGA to a receptor (*23,24*). Although the mechanisms involved in the formation of glucuronides through uridine nucleotides have been known for several years, the relevance of these reactions to the biosynthesis of L-ascorbic acid was suggested by Evans *et al.* (*25*). In this study, galactose-1-^{14}C was found to be a considerably better precursor of L-ascorbic acid in rats than is glucose-1-^{14}C (Table I). The L-ascorbic acid formed from galactose-1-^{14}C had about 92% of its total activity in C-6 compared to 60% for that from glucose-1-^{14}C, indicating that galactose is converted to L-ascorbic acid without intermediate formation of glucose. In addition, rat liver was shown to have the enzymes required for conversion of D-galactose to L-ascorbic acid through D-glucuronic acid. It is of particular importance that an enzyme system been found in rat liver microsomes that converts UDP-glucuronic acid to glucuronic acid 1-PO_4 (*25a*), and studies by Touster (*25b*) have demonstrated the presence of an enzyme in liver that hydrolyzes D-glucuronic acid 1-PO_4 to D-glucuronic acid. Earlier studies by

Ginsburg *et al.* (*26*) pointed out a similar system in the particulate fraction of rat kidney, capable of converting UDP-glucuronic acid to D-glucuronic acid, presumably via D-glucuronic acid 1-PO_4. However, this kidney system is not required for L-ascorbic acid formation, since D-glucose-1-^{14}C is converted to labeled D-glucuronic acid and L-ascorbic acid in nephrectomized rats to about the same extent as in normal rats (*25*). Based upon currently available evidence, the most likely overall scheme for biosynthesis of L-ascorbic acid from glucose or galactose is reaction (3):



(3)

III. INABILITY OF PRIMATES AND GUINEA PIGS TO SYNTHESIZE L-ASCORBIC ACID

Man, other primates, and the guinea pig are the only mammals known to be unable to synthesize L-ascorbic acid, and consequently require vitamin C in the diet for prevention of scurvy. An explanation for this

has come from recent studies showing that these species are unable to convert L-gulonolactone to L-ascorbic acid. For instance, it has been demonstrated *in vivo* that guinea pigs could not convert D-glucuronolactone-6-^{14}C and L-gulonolactone-1-^{14}C to L-ascorbic acid; in contrast, rats converted appreciable amounts of both compounds (*13*). Results that appeared almost simultaneously from two laboratories further pointed out the nature of this missing step. Lehninger and co-workers (*16,27*) found no net synthesis of L-ascorbic acid from L-gulonolactone in human, monkey, and guinea pig liver homogenates, as measured colorimetrically (*28*). Under these conditions there was an appreciable synthesis of the vitamin from L-gulonolactone in rat, mouse, rabbit, and dog liver homogenates. Burns and co-workers (*21,29*) reported no detectable conversion of L-gulonolactone-1-^{14}C to labeled L-ascorbic acid in human, monkey, and guinea pig liver homogenates, conversion being less than 1/100 that obtained in the rat (Table II).

TABLE II

CONVERSION OF L-GULONOLACTONE-1-^{14}C TO L-ASCORBIC ACID IN RAT, GUINEA PIG, MONKEY, AND HUMAN LIVER[a]

Species	Percent Conversion	
	Homogenate	Microsomes
Rat	8.0	10.0
Guinea pig	<0.05	<0.05
Monkey	<0.07	—
Man	<0.07	—

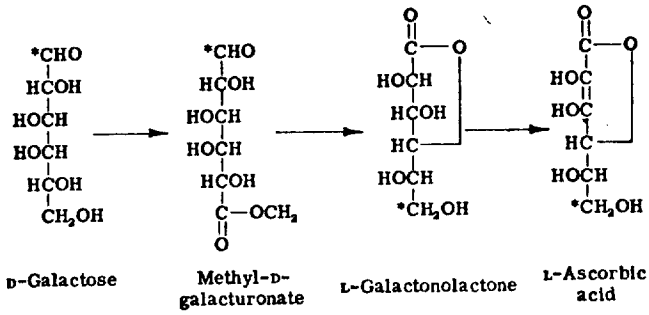
[a] In these experiments 5.0 mg L-gulonolactone was incubated for 90 minutes at 37° under air in 5 ml 10% homogenate (or microsomes from an equivalent amount of liver) in 0.15 *M* phosphate buffer (pH 7.20) containing 0.13 *M* sucrose. L-Ascorbic acid was isolated after addition of carrier (150 mg) from a 5% trichloroacetic acid extract of the incubation mixture as its 2,4-dinitrophenylosazone derivative.

Man, monkey, and guinea pig can carry out the various steps required in reaction (3) for biosynthesis of the vitamin, except the conversion of L-gulonolactone to L-ascorbic acid. The actual missing step in these species is most likely the initial oxidation of L-gulonolactone to 2-keto-L-gulonolactone. This keto derivative is a tautomeric form of

L-ascorbic acid, and presumably would be transformed spontaneously into the vitamin.

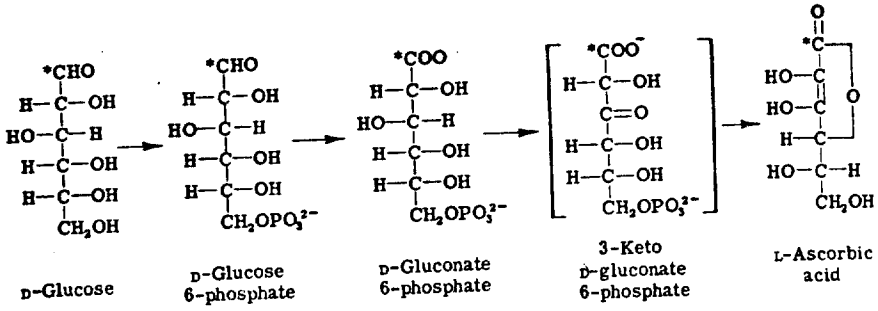
IV. BIOSYNTHESIS OF L-ASCORBIC ACID IN PLANTS

Isherwood and co-workers (*11,30,31*) demonstrated that L-gulonolactone, L-galactonolactone, D-glucuronolactone, and methyl-D-galacturonate were converted to L-ascorbic acid in cress seedlings. From their studies, they proposed two pathways for synthesis of L-ascorbic acid in plants: reaction (2), the same that they postulated for animals, and reaction (4):



(4)

Recent studies by Loewus *et al.* (*32–34*) in the detached ripening strawberry and in the germinating cress seedling do not support these mechanisms as major pathways for biosynthesis of the vitamin in plants, since no evidence for an inversion between D-glucose and L-ascorbic acid was found. For instance, D-glucose-1-^{14}C yielded L-ascorbic acid containing 65–70% of its activity in C-1 and 14–19% in C-6. With D-glucose-6-^{14}C, 73% of the total activity in L-ascorbic acid was present in C-6 and 24% in C-1. When D-galactose-1-^{14}C was used as the substrate, the L-ascorbic acid had about the same labeling in C-1 and C-6. These findings are in marked contrast to the data obtained in rats that showed that both C-1 of glucose and C-1 of galactose became C-6 of L-ascorbic acid (Table I). Thus, different pathways must exist in plants and animals for biosynthesis of L-ascorbic acid. Loewus and co-workers have postulated the scheme in reaction (5) as a possible mechanism for conversion of glucose to L-ascorbic acid that would be in accord with their isotropic data in plants. According to this scheme,



C-1 of glucose would become C-1 of L-ascorbic acid as indicated by the asterisks. 3-Keto-D-gluconate-6-PO_4 has been postulated to be the intermediate involved in the formation of D-ribulose 5-PO_4 according to the reactions of the hexose monophosphate shunt (*35*). For conversion of this keto acid to L-ascorbic acid, an epimerization reaction at C-5 would be required, followed by loss of phosphate from the resulting compound and its subsequent lactonization and enolization. Conclusions on the importance of reaction (5) in the biosynthesis of L-ascorbic acid must await information on the enzymic steps involved.

V. CATABOLISM OF L-ASCORBIC ACID IN ANIMALS

Studies with L-ascorbic acid, labeled in various positions with ^{14}C, have shown that the vitamin is extensively oxidized to respiratory CO_2 in rats and guinea pigs (Table III). In contrast, no conversion of L-ascorbic acid-1-^{14}C to respiratory CO_2 was detected in man. L-Ascorbic acid disappears slowly in man, with a half-life of about 16 days (*41*) compared to a half-life of about 3 days for the guinea pig (*37,42*). This is correlated with the much longer time it takes human subjects to show signs of scurvy. It requires about 4 months for a man on a vitamin C-free diet to develop scurvy, while the guinea pig is scorbutic in about 3 weeks.

On the basis of available evidence, the most likely scheme for catabolism of the vitamin in animals is given in Fig. 1. The formation of dehydroascorbic acid and 2,3-diketogulonic acid from L-ascorbic acid was postulated initially by Penny and Zilva (*43*), and evidence for these intermediates has come from numerous studies. The conversion of L-ascorbic acid to dehydroascorbic acid is reversible, but the formation of 2,3-diketogulonic acid occurs irreversibly.

TABLE III

^{14}C IN RESPIRATORY CO_2 AND URINE AFTER ADMINISTRATION OF LABELED L-ASCORBIC ACID TO VARIOUS ANIMAL SPECIES

Compound[a]	Animal	Collection period (days)	Percent of administered ^{14}C found in		Reference
			CO_2	Urine	
L-Ascorbic acid-1-^{14}C	Guinea pig	1	20	7.2	(*36*)
		10	70	20	(*36*)
L-Ascorbic acid -U-^{14}C	Guinea pig	1	22	11	(*37*)
L-Ascorbic acid-6-^{14}C	Guinea pig	1	18	6.0	(*38*)
L-Ascorbic acid-2,3,4,5,6-^{14}C	Guinea pig	1	28	7.5	(*39*)
L-Ascorbic acid-1-^{14}C	Rat	1	14	25	(*40*)
	Man	10	< 5.0	42	(*41*)

[a] The labeled compounds were administered intraperitoneally to guinea pigs and rats and intravenously to man.

L-Ascorbic acid is converted to urinary oxalate in man (*41,43a*), guinea pig (*36*), and rat (*40*), presumably through intermediate formation of dehydroascorbic acid and diketogulonic acid. However, the mechanism by which oxalate is formed is unknown, since attempts to demonstrate significant conversion of L-ascorbic acid to oxalate in rat and guinea pig tissues have not been successful (*44,45*). It should be noted that the conversion of L-ascorbic acid to oxalate and L-threonic acid can be readily demonstrated under nonbiological conditions (*46,47*).

Chan *et al.* (*44*) have shown that dehydroascorbic acid is decarboxylated in guinea pig liver homogenates with the formation of L-xylose as a product. In addition, a system has been described in rat kidney that decarboxylates L-ascorbic acid through intermediate formation of dehydroascorbic acid and diketogulonic acid (*45*). L-Lyxonic and L-xylonic acids were identified as products of this reaction in rat kidney (*48,48a*).

Ashwell *et al.* (*18*) have described an interesting new reaction in which L-xylonic acid is converted by rat liver enzymes to L-erythroascorbic acid (Fig. 2). The significance of this reaction in respect to the action of vitamin C remains to be determined.

In order to obtain further information on the pathways of metabolism of the vitamin in animals, the conversion of labeled L-ascorbic acid, dehydroascorbic acid, and diketogulonic acid to liver glycogen was measured (Table IV). Carbon-6-labelled L-ascorbic acid, dehydroascorbic

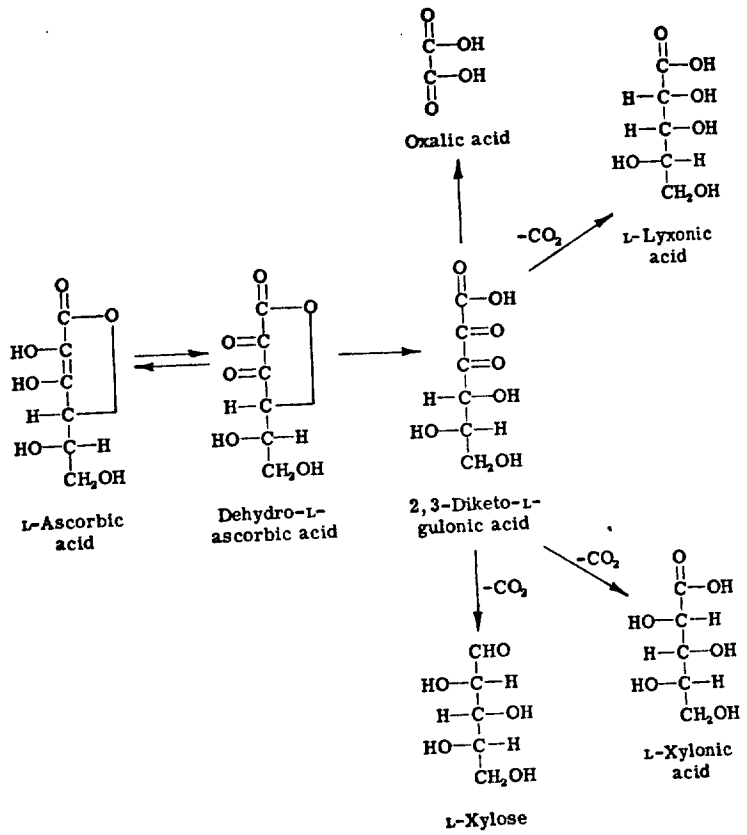


FIG. 1. Metabolism of L-ascorbic acid.

acid, and diketogulonic acid were converted to glycogen that contained the major fraction of the total ^{14}C in its glucose residues in C-1 and C-6. This isotopic pattern is markedly different from that obtained in similar experiments with L-gulonolactone-6-^{13}C. According to the data in Table IV, L-gulonolactone-6-^{13}C yields glycogen labeled predominantly in C-1 and C-3. These findings argue against appreciable conversion of L-ascorbic acid to glycogen via L-xylulose as postulated for its precursor, L-gulonolactone (*49*). The appearance of isotope predominantly in C-1 and C-6 of glucose derived from C-6-labeled L-ascorbic acid, dehydroascorbic acid, and diketogulonic acid suggests the intermediate formation of trioses. Since these precursors are of the L configuration, one triose form each should be of that configuration. The ability of L-triose to participate in glucose synthesis has been suggested

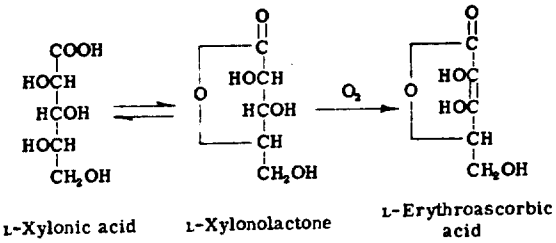


FIG. 2. Biosynthesis of L-erythroascorbic acid.

TABLE IV

INCORPORATION OF ISOTOPE INTO GLUCOSE FOLLOWING ADMINISTRATION OF VARIOUS LABELED COMPOUNDS

Compound	Percent of administered isotope in glucose	Percent of total glucose isotope in			Reference
		C-1	C-3	C-6	
Animal[a]					
L-Ascorbic acid-6-^{14}C	0.28	38	6.5	20	(*38*)
Dehydroascorbic acid-6-^{14}C	1.4	49	6.5	27	(*38*)
Diketogulonic acid-6-^{14}C	2.1	41	5.6	37	(*38*)
L-Ascorbic acid-3,4,5,6-^{14}C	—	15	—	7	(*44*)
L-Ascorbic acid-2,3,4,5,6-^{14}C	0.52	—	—	—	(*39*)
L-Gulonolactone-6-^{13}C	22	55	17	3.0	(*49*)
L-Sorbose-6-^{14}C	12	44	—	34	(*9*)
Plant[b]					
L-Ascorbic acid-6-^{14}C	—	51	5	34	(*34*)

[a] The conversion of labeled compounds to liver glycogen was measured either in guinea pigs or rats.

[b] The conversion of L-ascorbic acid-6-^{14}C to sucrose-derived glucose was measured in a ripening strawberry.

from experiments (*9*) in which L-sorbose-6-^{14}C yields glucose labeled mainly in C-1 and C-6.

It is of interest that a similar isotopic pattern was found, in sucrose-derived glucose from a ripening strawberry stem-fed with L-ascorbic acid-6-^{14}C to that obtained in glycogen isolated from rats injected with diketogulonic acid-6-^{14}C (Table IV). These findings suggest the possibility of similar pathways for L-ascorbic acid catabolism in plants and animals.

VI. L-ASCORBIC ACID, AN INTERMEDIATE IN CARBOHYDRATE METABOLISM

Evidence has been presented for conversion of glucose to L-ascorbic acid (Section II), as well as for its transformation back to glucose (Section V). Thus it now appears that L-ascorbic acid may be considered an intermediate in carbohydrate metabolism, as shown by the cyclic pathway in Fig. 3. Man, monkey, and guinea pig are unable to carry out the

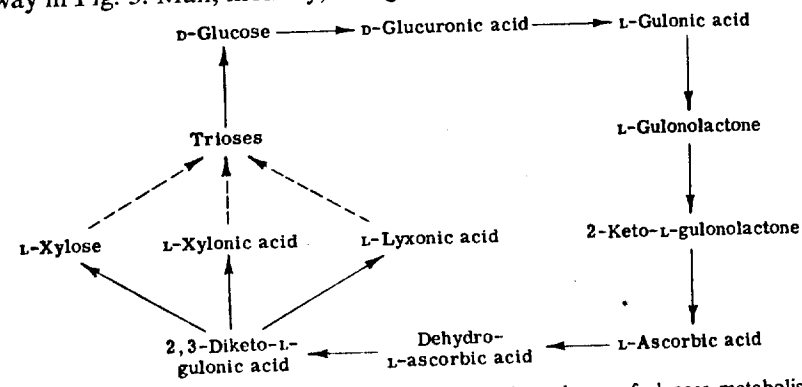


FIG. 3. L-Ascorbic acid as an intermediate in a cyclic pathway of glucose metabolism in animals.

conversion of L-gulonolactone to L-ascorbic acid (Section III), which is presumably the result of a gene-controlled enzyme deficiency (*50*). Consequently, scurvy in these species may be considered to arise from a defect in carbohydrate metabolism (*51*).

Loewus (*34*) and co-workers have recently postulated a role for L-ascorbic acid as an intermediate in carbohydrate metabolism in plants. This was pointed out by the rapid conversion of ^{14}C from L-ascorbic acid-6-^{14}C into the carbohydrate pool of the young grape leaf. Mature tissues such as the strawberry fruit continue to form L-ascorbic acid, but are no longer able to metabolize the compound rapidly, resulting in its accumulation. It should be recalled that glucose is converted to L-ascorbic acid in plants by a pathway different from that given in Fig. 2.

VII. GLUCURONIC ACID PATHWAY OF GLUCOSE METABOLISM

In recent years a new route of glucose metabolism, the glucuronic acid pathway, has been proposed, based on studies (*15,52,53*) of the biosyntheses of L-ascorbic acid and L-xylulose (Fig. 4). According to this

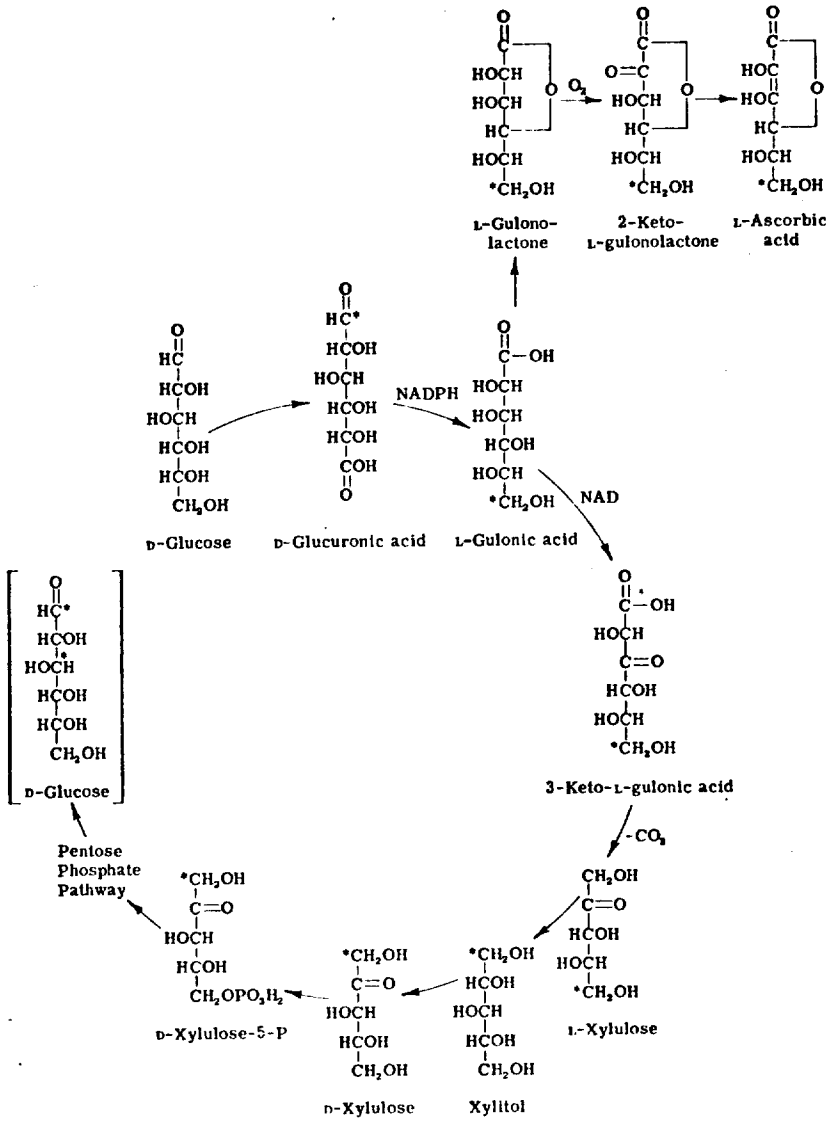
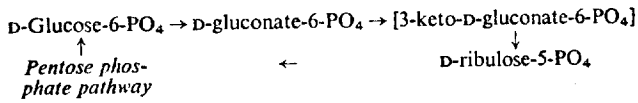


FIG. 4. The glucuronic acid pathway of glucose metabolism in aminals. The asterisks denote the fate of C-1 of glucose in the scheme.

scheme, L-gulonic acid serves as a precursor for L-xylulose (*19,27,53*) as well as for L-ascorbic acid. All the animals studied can metabolize L-gulonic acid via L-xylulose, but only primates and the guinea pig lack the ability to convert L-gulonic acid to L-ascorbic acid (Section III).

Recent studies have provided evidence for participation of 3-keto-L-gulonic acid as an intermediate in the formation of L-xylulose by an NAD-dependent enzyme in the soluble fraction of kidney (*54*). Touster *et al.* (*55,56*) have demonstrated an enzyme system in liver capable of reversibly reducing both L-xylulose and D-xylulose to a common intermediate, xylitol, thereby providing a mechanism for interconversion of the stereoisomers of this ketopentose. The subsequent findings by Hickman and Ashwell (*57*) of a specific liver kinase, capable of forming D-xylulose-5-PO_4 from the free sugar, indicated that mammalian tissues possessed the complete enzymic structure necessary to carry out the conversion of L-xylulose to glucose via the pentose cycle (*58,59*). Evidence for the occurrence of this cyclic pathway in the intact rat and guinea pig has come from the recent findings that labeled D-glucuronolactone (*60*), L-gulonolactone (*49,60*), and xylitol (*52*) are converted to liver glycogen in accordance with this scheme.

The glucuronic acid pathway represents a new oxidative pathway of glucose metabolism in animals. It differs from the hexose monophosphate shunt (*35*), reaction (6), in that glucose is oxidized at C-6 to form D-glucuronic acid instead of at C-1 to form D-gluconic acid. However, both pathways channel glucose into the same pentose pool.



(6)

The glucuronic acid pathway is of importance for synthesis of L-ascorbic acid in animals other than primates and the guinea pig. It is of considerable interest that Loewus (*34*) and co-workers have recently proposed that the hexose monophosphate shunt may be involved in the formation of L-ascorbic acid in plants, as shown in reaction (5). Although there is considerable evidence that the hexose monophosphate shunt is involved in the synthesis of D-ribose, recent studies by Hiatt and Lareau (*61*) indicate that the glucuronic acid pathway plays little or no role in D-ribose production from glucose. The glucuronic acid pathway also accounts for the origin of L-xylulose, the sugar excreted by human subjects with essential pentosuria.

Various drugs markedly increase the rate at which glucose is metabolized via the glucuronic acid pathway. For example, administration of barbital and Chloretone to rats leads to a marked increase in the conversion of glucose to free D-glucuronic acid (*15*), L-gulonic acid (*14*), and L-ascorbic acid (*15*). This effect on L-ascorbic acid biosynthesis in the rat is shown by many drugs, including various barbiturates, aminopyrine, and antipyrine (*62*), but the mechanisms involved are not known. It is of considerable interest that in 1935 Enklewitz and Lasker (*63*)

found that two of these drugs, aminopyrine and antipyrine, markedly increased the urinary excretion of L-xylulose in patients with essential pentosuria. It is now possible to explain their observation in terms of the scheme in Fig. 3. Administration of these drugs would be expected to increase the formation of L-xylulose from D-glucose. Since the pentosuric patient is not able to metabolize L-xylulose (*64*), the pentose would consequently be excreted in urine.

Recent studies by Marsh and Reid (*65,66*) have pointed out an additional pathway for D-glucuronic acid metabolism in mammalian tissues. D-Glucuronic acid can be oxidized to D-glucuric acid, and this reaction is catalyzed by a NAD-requiring enzyme in the soluble fraction of liver. Interestingly, drugs such as barbital and Chloretone, which stimulate ascorbic acid synthesis in rats, also enhance markedly the urinary excretion of D-glucaric acid (*66*).

VIII. METABOLISM OF D-ASCORBIC ACID

Dayton and Burns (*67*) have compared the metabolism of D-ascorbic acid-1-^{14}C and L-ascorbic acid-1-^{14}C in guinea pigs and rats. D-Ascorbic acid was oxidized appreciably to CO_2, and was distributed in tissues very much like L-ascorbic acid. However, D-ascorbic acid was excreted by the kidney at a considerably more rapid rate than L-ascorbic acid, thereby suggesting an explanation for the reported lack of vitamin C activity of the D-isomer in guinea pigs (*68*).

In order to test this hypothesis, bioassay experiments were carried out in guinea pigs under conditions in which essentially comparable tissue concentrations of D-ascorbic acid and L-ascorbic acid were maintained (*69,70*). The results obtained showed that D-ascorbic acid was able to replace some of the activities of L-ascorbic acid when administered in relatively small doses under the conditions of these experiments. For instance, D-ascorbic acid could maintain the weight and prolong the survival time of scorbutic guinea pigs, but was unable to prevent joint hemorrhages. The most striking effect of D-ascorbic acid was revealed by histochemical studies of the teeth. The dentin produced in the animals receiving D-ascorbic acid was normal in morphology and staining properties. However, the vitamin C effect of D-ascorbic acid was not complete, in that the predentin formed in these animals was still abnormal in its staining properties. A recent report (*71*) indicates that D-ascorbic acid has the ability to promote collagen synthesis in the scorbutic carrageenan granuloma in guinea pigs when administered in large doses twice daily.

The results of these experiments furnish further evidence for multiple actions of vitamin C: those that are specific for only L-ascorbic acid, and those that are nonspecific, in which D-ascorbic acid and presumably other compounds with the same redox properties can substitute. The separation of the biological activity of vitamin C has also been indicated by studies with D-araboascorbic acid in guinea pigs (*72*) and with D-ascorbic acid in monkeys (*73*). One of the best examples of a nonspecific role for the vitamin has come from studies of tyrosine metabolism, in which L-ascorbic acid can be replaced by various other structurally unrelated compounds that are readily susceptible to oxidation and reduction (*74*). It is possible that further work with D-ascorbic acid or other analogs of the vitamin in guinea pigs may throw light on the nature of these nonspecific functions.

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09025

Experimental Arteriosclerosis: Inhibitory Effects of Ascorbic Acid and Inositol. (19850)

Oscar Davis and Y. T. Oester. (Introduced by A. G. Mulder.)

From the Department of Pharmacology and Graduate School, Loyola University, Chicago, Ill.

The term arteriosclerosis has been variously defined. Too commonly, lipid, and in particular cholesterol infiltration (atheroma), has been identified as the essential lesion in this syndrome. For purposes of this paper we have defined arteriosclerosis as any thickening, or increase in density, of the arterial wall. Atherosclerosis may be defined as a degenerative metabolic subdivision of arteriosclerosis, characterized by hyaline degeneration, lipid infiltration and finally, calcinotic changes of the intima and or media.

Our immediate purpose in investigating this problem, was 2-fold: 1. To produce experimentally, in the rabbit, arteriosclerotic lesions of the aorta. 2. To inhibit or reverse the formation of these lesions. Josue(1) observed experimentally, that repeated injections of epinephrine were capable of producing sclerosis of the aorta. Numerous investigators(2,3) have, since that time, reaffirmed this experimental fact, usually describing the resultant plaques as erosions or necroses of the tunica media. Oester and Mikulicich(4) have recently observed that combined injections of epinephrine and thyroxine result in an apparent potentiation in the production of this experimental aortic sclerosis. The present communication is a report on the results of experiments with inositol and ascorbic acid, used separately, in an endeavor to influence the epinephrine-thyroxine induced arteriosclerosis in rabbits.

Methods. Rabbits totalling 111, were used. The method for production of the sclerosis was as follows. Twenty-five μg of epinephrine hydrochloride per kilo of body weight were very slowly injected intravenously by ear, on 2 consecutive days. On the third day the dose of epinephrine injected was increased to 40 μg per kilo. On the fourth day it was increased to 50 μg per kilo. This regimen of 50 μg of epinephrine per kilo of body weight was maintained for 11 more days, a total of 15 days of epinephrine administration. Thyroxine was injected subcutaneously, in the region of the middle of the back, daily, for the 15 days, at a constant dose level of 0.15 mg/kg body weight. Post-mortem examinations were routinely made. Our primary observations were concerned with the frequency and severity of sclerosis in the aorta from the region of the heart into the iliac bifurcation. Final conclusion as to whether sclerosis was actually present was based on histological study. It is virtually impossible to compare plaques accurately, but for purposes of differentiation we undertook their gradation on the following basis: zero, no plaques; 1, mild single patches (plaques); 2, mild, scattered patches; 3, severe, extensive patches; 4, widespread, confluent and extensive patches. The control group of 25 animals received only epinephrine and thyroxine injections. Identical regimens of epinephrine and thyroxine injections were also administered to the groups of animals which received inositol, and to those which received ascorbic acid. Thirty-six animals were treated with ascorbic acid, 18 received 100 mg per day, 9 received 500 mg per day, and 9 received 50 mg per day. All ascorbic acid was administered subcutaneously in the region of the middle of the back. The ascorbic acid treatment was begun 3 days before epinephrine and thyroxine. All injections were continued for 15 more days, thereby totalling 18. Thirty animals were treated with inositol, the subcutaneous injections being given, as with Vit. C, before and during the epinephrine and thyroxine for a total 18 day program. The animals were divided by dose of inositol into 2 groups of 15 each, the first receiving 3 g per kilo per day, the second 6 g per kilo per day. Table I provides a summary of the protocol from these experiments. Table II is an analysis of the data from Table I.

Results and discussion. Investigation with massive doses of inositol indicates it has no significant value in the treatment or prophylaxis of the experimental epinephrine-

TABLE I. Influence of Inositol and Ascorbic Acid on Epinephrine-Thyroxine Sclerosis.

Treatment*	No. of rabbits	Deaths before 6 days	Deaths 6 to 15 days	No. animals sacrificed on 16th day	Degree of sclerosis 0	1	2	3	4
E + T (C)	25	5	12	8	2	4	4	3	7
C + I, 3 g/kg	17	2	12	3	3	5	3	3	1
" 6 "	19	4	11	4	4	4	3	4	0
C + A, 50 mg/kg	12	3	5	4	3	2	1	1	2
" 100 "	22	4	11	7	7	2	4	3	2
" 500 "	10	1	6	3	4	1	2	2	0

* E = Epinephrine; T = Thyroxine; C = Controls; I = Inositol; A = Ascorbic acid.

TABLE II.
Significance of Influence of Inositol and Ascorbic Acid on Epinephrine-Thyroxine Sclerosis.

	No. with sclerosis /Total No. animals	% incidence	Probability*	Severity factor (% incidence × avg degree of sclerosis)
E + T (C)†	18/20	90		90 × 2.45 = 220
C + I, 3 g/kg	12/15	80	>.1	80 × 1.6 = 128
" 6 "	11/15	73	>.1	73 × 1.46 = 106.5
C + A, 50 mg/kg	6/9	66	>.2	66 × 1.66 = 109.5
" 100 "	11/18	61	<.01	61 × 1.5 = 91.5
" 500 "	5/9	55	>.1	55 × 1.22 = 67.1

* Probability compared to controls, a value of <.05 is assumed significant. Probability: (1) for total inositol group = >.1; (2) for total ascorbic acid group = <.01. Animals whose death occurred before 6th day of treatment were not included in biometrical analysis.
† E = Epinephrine; T = Thyroxine; C = Controls; I = Inositol; A = Ascorbic acid.

thyroxine arteriosclerosis. Ascorbic acid proved valuable in the inhibition of these lesions, especially in daily doses of 100 and 500 mg. The tables indicate the results which were obtained. The data obtained with the use of inositol, although it demonstrates a lower incidence of sclerosis, is not of statistical significance. The results with ascorbic acid are of statistical significance. In addition, the severity of the sclerosis developed under the influence of ascorbic acid was markedly less than either the controls or when inositol was used. This is shown by the lessened number of cases which were recorded as four plus sclerosis. In addition, a factor which we call severity factor also expresses this relationship. This number is obtained by multiplying the percentage incidence of sclerosis in any group by the average degree of sclerosis, expressed in our plus units. The higher this number, the higher is the total gross degree of sclerotic severity. It is seen that this value is markedly lower with each of the ascorbic acid groups and there is a progressive decrease with increasing dose of ascorbic acid.

The severe median and often intimal sclerosis induced in the aorta of rabbits by combined injections of epinephrine and thyroxine indicates the possibility that such a combination of hormonal influences may be active in the production of arteriosclerosis in other animals.

Epinephrine scleroisis, potentiated by thyroxine and perhaps by other hormonal imbalances, may exert sufficient injury to the arterial wall so that later, lipid deposition is favored. The experimentally demonstrable influence of epinephrine and thyroxine might be considered as part of the pre-lipid phase of atherosclerosis. It is our feeling that some shock phase precedes active lipid-infiltration. We suggest that a dysfunction of lipid metabolism may be a factor which is secondary to the lesions resulting from these shocking stresses, hormonal or otherwise.

The role of thyroxine in the production of arteriosclerosis is paradoxical and, as yet, not well defined. It has been used successfully to produce remission of the experimental cholesterol sclerosis(5) and yet as stated it adds significantly to the degree and incidence of the sclerosis produced by epinephrine in the rabbit. This last fact is supported somewhat

by the findings of Lortat and Sabareaunu(6) who inhibited the epinephrine-induced sclerosis by extirpation of the thyroid gland.

Summary. 1. Combined injections of epinephrine and thyroxine were used to produce, in rabbits, aortic sclerosis which was characterized by lack of atheroma, and the presence of severe medial and intimal sclerosis. 2. 90% of all the control animals developed aortic sclerosis when treated with epinephrine and thyroxine. 3. Massive doses of inositol appeared to inhibit the influence and severity of these lesions, to only a slight degree. 4. Daily concomitant administration of 50 mg of ascorbic acid lowered the incidence of scleroses to 66%; 100 mg lowered the incidence to 61%; 500 mg lowered it to 55%. The severity of the lesions produced was also considerably lessened. The inhibition due to ascorbic acid was statistically significant.

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Effect of Dietary Copper, Iron and Ascorbic Acid Levels on Hematology, Blood and Tissue Copper, Iron and Zinc Concentrations and ^{64}Cu and ^{59}Fe Metabolism in Young Pigs

W. F. GIPP,[1] W. G. POND,[2] F. A. KALLFELZ,[3] J. B. TASKER,[4] D. R. VAN CAMPEN,[5] L. KROOK[6] AND W. J. VISEK[2]

Department of Animal Science, New York State College of Agriculture and Life Sciences, New York State Veterinary College, and U. S. Plant, Soil and Nutrition Laboratory, Cornell University, Ithaca, New York 14850

ABSTRACT Two levels each of Cu, Fe and ascorbic acid (AA) were used in a factorial arrangement in diets fed to 3-week-old pigs in an attempt to determine the influence of high levels of dietary Fe and AA on the deleterious effects of 250 ppm dietary Cu, to ascertain the mode of action of high Cu in inducing anemia and to delineate the effects of high dietary AA on Fe and Cu metabolism. Pigs fed high Cu had decreased plasma and liver Fe concentrations, increased latent and total Fe binding capacity, decreased saturation of transferrin, increased rate of removal of intravenously administered ^{59}Fe from the plasma and increased utilization of ^{59}Fe by RBC, all manifestations of Fe deficiency. The rapid uptake of ^{59}Fe by RBC, low levels of tissue Fe and low degree of transferrin saturation indicate no block in Fe release from the reticuloendothelial system. Liver Cu concentration of pigs fed high Cu was greatly reduced by addition of 0.5% AA to the diet. AA had no effect on altering the level of Fe retained in the liver or intestine, but did increase the plasma Fe level, the degree of saturation of plasma transferrin, and the rate of removal from plasma and uptake by RBC of ^{59}Fe. It is concluded that the Fe deficiency induced by high dietary Cu is due to impairment in Fe absorption from the gastrointestinal tract and that this impairment is ameliorated by AA. J. Nutr. *104:* 532–541, 1974.

INDEXING KEY WORDS dietary Cu · dietary Fe · dietary ascorbic acid · Fe utilization

Previous work (1, 2) has shown that a microcytic hypochromic anemia is produced in pigs by the addition of 250 ppm of Cu to the diet. The anemia is accompanied by decreased concentrations of Fe in the plasma and liver, indicating that Cu interferes with normal utilization of Fe. Ascorbic acid (AA) increases the absorption of Fe, possibly by its reducing action and by the formation of soluble ascorbate–Fe chelates (3, 4), and decreases the absorption and/or retention of Cu (5–8). The present study was undertaken to determine the influence of high levels of dietary Fe and AA on the deleterious effects of 250 ppm dietary Cu, to ascertain the mode of action of high Cu in inducing anemia and to delineate the effects of high dietary AA on Fe and Cu metabolism.

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[1] Present address: Cargill Research Farm, Elk River, Minn. 55330.

[2] Department of Animal Science, New York State College of Agriculture and Life Sciences.

[3] Department of Physical Biology, New York State Veterinary College.

[4] Clinical Pathology Section, New York State Veterinary College.

[5] U. S. Plant, Soil and Nutrition Laboratory, U. S. D. A., Ithaca, N. Y. 14850.

[6] Department of Pathology, New York State Veterinary College.

MATERIALS AND METHODS

Thirty-two 3-week-old Yorkshire pigs weighing an average of 5.8 kg were randomly assigned from outcome groups based on weight and sex to eight diet groups of four pigs each in a factorial arrangement of treatments as follows: 1) basal diet containing 100 ppm Fe, 10 ppm Cu and 0.011% ascorbic acid (B); 2) B + 240 ppm Cu as $CuSO_4 \cdot 5H_2O$; 3) B + 200 ppm Fe as $FeSO_4 \cdot 7H_2O$; 4) B + 240 ppm Cu + 200 ppm Fe; 5) B + 0.5% ascorbic acid (AA); 6) B + 240 ppm Cu + 0.5% AA; 7) B + 200 ppm Fe + 0.5% AA; 8) B + 240 ppm Cu + 200 ppm Fe + 0.5% AA. The copper supplemental diets thus contained a total level of 250 ppm Cu, the iron supplemental diets contained a total level of 300 ppm of iron and the ascorbic acid supplemental diets contained a total level of 0.511% of ascorbic acid. The composition of the basal diet is shown in table 1.

Animals were housed two per pen indoors in concrete-floor pens equipped with automatic waterers and wooden self-feeders. Body weight was recorded weekly and blood was obtained weekly from the anterior vena cava of each pig for determination of hemoglobin (9), packed cell volume (PCV), ceruloplasmin (10, 11), red blood cell count (RBC), mean corpuscular volume (MCV),[7] mean corpuscular hemoglobin (MCH),[8] mean corpuscular hemoglobin concentration (MCHC),[9] latent iron binding capacity (LIBC),[10] total iron binding capacity (TIBC),[10] percentage transferrin saturation and plasma Fe, Cu and Zn concentrations. After 5 weeks each animal was placed in a metal metabolism cage and given an oral dose of 100 μCi of ^{64}Cu (as $Cu(NO_3)_2$ contained in glucose in a small gelatin capsule which was swallowed when placed at the base of the tongue). The animals were fasted for at least 3 hours (replicate 2) or 12 hours (replicate 2) prior to administration of the isotope. The fast continued until after the 10-hour blood sample was taken. The animals were then given 3 kg of feed/day in three feedings, with the first feeding being immediately after the 10-hour blood sample was taken. Blood samples were taken at 1, 2, 3, 4, 6, 8, 10, 21, 28 and 46 hours after isotope administration. Five milliliters of whole blood from each sample obtained from each pig were counted for ^{64}Cu with a gamma counter[11] immediately after collection. The appearance of the orally administered ^{64}Cu in the blood was plotted against time for each diet group. The ^{64}Cu counted in the blood was counted for radioactive decay and expressed as a percentage of the dose administered. A ^{64}Cu counting standard was prepared which contained 0.02 × the concentration of ^{64}Cu as the ^{64}Cu dosing solution. This counting standard was counted each time the blood samples were counted and the counts per minute obtained were multiplied by 50 to determine the cpm of the dosing solution at the time the blood samples were counted. (Decay during the time elapsed between counting the sample from individual animals within a group of samples was counted to the time the standard was counted by use of the published decay constant for ^{64}Cu and the time elapsed during counting.) The cpm/ml of blood was multiplied by the blood volume of the pig (assumed to be 66.5 ml blood/1 kg body weight) (13) and this was divided by the cpm of the standard (times 50) to determine the percentage of the ^{64}Cu dose present in the blood at that time.

TABLE 1

Composition of basal diet

	Percent
Glucose monohydrate[1]	13.0
Cornstarch[2]	13.5
Dried skimmilk	60.0
Corn oil[3]	5.0
Cellulose[4]	5.0
Iodized salt	0.5
Mineral mixture[5]	2.0
Vitamin mixture[6]	1.0
Total	100.0

[1] Cerelose, Corn Products Co., Argo, Ill. [2] Corn Products Co., Argo, Ill. [3] Mazola, Corn Products Co., Argo, Ill. [4] Solka-Floc. [5] Supplied per kg diet: 10 mg F from NaF; 4.4 g Ca from $CaHPO_4 \cdot 2H_2O$ and $CaCO_3$; 2.0 g P from $CaHPO_4 \cdot 2H_2O$; 40 mg Mn from $MnSO_4 \cdot H_2O$; 100 mg Zn from ZnO; 100 mg Fe from $FeSO_4 \cdot 7H_2O$; 10 mg Cu from $CuSO_4 \cdot 5H_2O$.

[7] $$\text{MCV (cubic micra)} = \frac{\text{Hematocrit} \times 10}{\text{RBC (million/cu mm)}}.$$

[8] $$\text{MCH (picograms)} = \frac{\text{Hemoglobin (g/100 ml)} \times 10}{\text{RBC (million, cu mm)}}.$$

[9] $$\text{MCHC (\%)} = \frac{\text{Hemoglobin (g/100 ml)} \times 100}{\text{Hematocrit}}.$$

[10] IRONSORB B-59, Diagnostic Kit, Abbott Labs., N. Chicago, Ill. 60064.

[11] Nuclear Chicago.

TABLE 2

Daily weight gain, feed consumed, hemoglobin, PCV, ceruloplasmin, MCV, MCH, MCHC, RBC, LIBC, TIBC and percentage transferrin saturation in pigs[1]

Diet	Basal	Cu[2]	Fe[3]	Cu + Fe	AA[4]	AA + Cu	AA + Fe	AA + Cu + Fe	Significant differences
Daily gain, kg	0.45	0.46	0.48	0.47	0.42	0.44	0.48	0.45	NS[5]
Feed/day, kg	0.86	0.87	0.93	0.95	0.81	0.90	0.85	0.89	NS
Hemoglobin, g/100 ml	10.8	8.9	12.6	9.8	11.8	11.1	11.4	11.4	AA, Fe, Cu, AA × Cu, $P < 0.01$ AA × Fe, $P < 0.05$
PCV	37.8	29.8	42.8	36.0	40.3	39.0	39.5	39.0	AA, Fe, Cu, AA × Fe AA × Cu, $P < 0.01$
Ceruloplasmin, IU/ml	75	71	69	69	75	70	71	81	Fe × Cu, $P < 0.01$
MCV	58.6	46.6	63.1	54.9	60.5	61.2	59.7	64.2	AA, Cu, AA × Cu, $P < 0.01$
MCH	16.7	12.5	18.6	14.9	17.6	17.4	17.3	18.7	AA, Cu, AA × Cu, $P < 0.01$
MCHC	28.5	26.7	29.4	27.2	29.1	28.4	28.9	29.2	AA, Cu, $P < 0.05$
RBC (× 10^6)	6.46	6.39	6.78	6.57	6.65	6.46	6.62	6.08	NS
LIBC, μg/100 ml	471	628	346	598	366	457	340	392	AA, Cu, $P < 0.05$
TIBC, μg/100 ml	737	829	607	807	812	848	806	817	AA, $P < 0.01$, AA × Cu, $P < 0.05$
Transferrin saturation, %	36.5	24.3	43.1	25.5	54.6	45.1	58.0	51.2	AA, Cu, $P < 0.01$

[1] Four pigs per treatment, 35-day test period, 5.8 kg initial wt, 21-day initial age. [2] Two hundred fifty ppm total dietary Cu. [3] Three hundred ppm total dietary Fe. [4] 0.5% total dietary ascorbic acid. [5] No significant differences.

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Four days after isotope administration animals were returned from the metabolism cages to their original pens. Three days later each animal was given 30 μCi of ^{59}Fe (as $FeSO_4$) by injection into the anterior vena cava. Blood samples were taken from the same vein immediately before and 10, 30, 60, 120 and 240 minutes after ^{59}Fe administration. The plasma from each blood sample was counted for ^{59}Fe using a gamma counter[11] and the values were plotted against time on semilogarithmic paper for determination of the half-time of plasma ^{59}Fe removal. Additional blood samples were obtained daily for 9 days and 2 ml of whole blood from these samples were counted for ^{59}Fe. These values were expressed on the basis of cpm per ml of RBC [cpm/ml RBC = (cpm/ml blood)/PCV expressed as a decimal] and plotted against time to obtain the pattern of ^{59}Fe incorporation into RBC hemoglobin. The animals were fed their respective diets ad libitum throughout this ^{59}Fe study. After collection of the 9-day blood animals were necropsied and liver, kidneys, spleen and a section of the duodenum were removed. Each organ was homogenized in a Waring blender and subsamples of the fresh homogenate were used for radiochemical and Cu, Fe and Zn analyses. Plasma and tissue samples were wet-ashed using nitric and perchloric acids prior to determination of Cu, Fe and Zn by atomic absorption spectrophotometry.[12]

All data were subjected to analysis of variance using a randomized complete block design with a factorial arrangement of treatments. The variables were dietary AA level, dietary Fe level and dietary Cu level.

RESULTS

Daily weight gain, feed consumption, and hematological measurements at the end of week 5 of the experiment are summarized in table 2. Plasma and tissue concentrations of Fe, Cu and Zn are summarized in table 3. In contrast to previous experiments of longer duration (1), gain and feed consumption were not significantly affected by diet. However, a marked pattern of change was observed in hematological parameters by week 5 on test.

Reductions in hemoglobin ($P < 0.01$), PCV ($P < 0.01$), MCV ($P < 0.01$), MCH ($P < 0.01$), MCHC ($P < 0.05$), plasma Fe, and percentage saturation of plasma transferrin ($P < 0.01$) occurred when 250 ppm of Cu were present in the diet. The LIBC and TIBC were increased ($P < 0.05$) by this addition.

The presence of 300 ppm of Fe in the diet produced increases in hemoglobin ($P < 0.01$), PCV ($P < 0.01$) and MCH ($P < 0.05$) and plasma Fe ($P < 0.05$). Three hundred parts per million Fe in the presence of 250 ppm Cu reduced the degree of change in hematological parameters due to the high level of Cu but did not return any of the parameters to basal levels. Significant Fe × AA interactions were obtained for hemoglobin ($P < 0.05$) and PCV ($P < 0.01$) but were not obtained for other hematological parameters.

The presence of 0.5% of AA in the diet increased hemoglobin ($P < 0.01$), PCV ($P < 0.01$), MCV ($P < 0.01$), MCH ($P < 0.01$), MCHC ($P < 0.05$), plasma Fe ($P < 0.01$), TIBC ($P < 0.01$), and percentage saturation of plasma transferrin ($P < 0.01$). The LIBC was reduced ($P < 0.05$) by the presence of AA in the diet.

The improvements in most hematological parameters due to AA additions occurred even at the low Fe and Cu levels.

However, significant AA × Cu interactions for hemoglobin ($P < 0.01$), PCV ($P < 0.01$), MCV ($P < 0.01$), MCH ($P < 0.01$), plasma Fe ($P < 0.05$) and TIBC ($P < 0.05$) demonstrate that the greatest response to AA occurred when a high level of Cu was present in the diet. The reductions in hematological parameters which occurred when 250 ppm of Cu alone was present in the diet were completely overcome when AA was also present in the diet.

RBC count was not significantly affected by diet, although the lowest mean was in the high Cu–low Fe–low AA group. Ceruloplasmin activity was not significantly affected by diet. Plasma Cu was influenced only by the high Cu addition ($P < 0.01$).

Liver Fe concentration was not influenced by any of the dietary treatments, but liver Cu levels were dramatically increased

[12] Perkin-Elmer Model 303, Atomic Absorption Spectrophotometer.

TABLE 3

Plasma and tissue concentrations of Fe, Cu and Zn in pigs[1,2]

Diet[3]	Basal	Cu[3]	Fe[4]	Cu + Fe	AA[5]	AA + Cu	AA + Fe	AA + Cu + Fe	Significant differences
Plasma Fe, μg/100 ml	266	201	262	209	447	391	466	425	AA, $P < 0.01$, Fe, AA × Cu, $P < 0.05$
Plasma, Cu, μg/100 ml	181	240	170	218	171	213	185	194	Cu, $P < 0.01$
Plasma Zn, μg/100 ml	96	91	96	74	99	125	115	88	Cu, Fe × Cu, $P < 0.01$
Liver Fe, ppm dry	124	69	100	93	104	79	169	141	NS[6]
Liver Cu, ppm dry	34	6080	197	6271	32	3335	30	1949	AA, Cu, AA × Cu, $P < 0.01$
Liver Zn, ppm dry	201	432	204	414	255	302	310	241	Cu, $P < 0.05$, AA × Cu, $P < 0.01$
Spleen Fe, ppm dry	337	182	299	205	254	178	222	234	NS
Spleen Cu, ppm dry	11	18	8	19	18	16	21	24	NS
Spleen Zn, ppm dry	95	110	113	95	119	114	98	109	NS
Kidney Fe, ppm dry	50	26	38	32	41	38	50	39	Cu, $P < 0.05$
Kidney Cu, ppm dry	42	130	67	117	27	56	28	48	AA, Cu, AA × Cu, $P < 0.01$
Kidney Zn, ppm dry	109	117	109	93	86	118	95	76	NS
Intestine Fe, ppm dry	138	64	138	98	146	93	227	243	AA, Fe, AA × Fe, Cu, $P < 0.01$
Intestine Cu, ppm dry	19	102	45	175	14	51	11	89	Cu, $P < 0.05$
Intestine Zn, ppm dry	89	78	76	98	91	89	85	124	NS

[1] Five weeks for plasma, slaughter at approximately 7 weeks. [2] Four animals on each diet. [3] Two hundred fifty ppm total dietary Cu. [4] Three hundred ppm total dietary Fe. [5] 0.5% total dietary ascorbic acid. [6] No significant differences.

by high dietary Cu ($P < 0.01$). AA added to the basal diet had no influence on liver Cu, but AA in the presence of high dietary Cu partially prevented the sharp rise in liver Cu associated with high dietary Cu. The Cu × AA interaction was significant ($P < 0.01$). High dietary Fe did not prevent the high liver Cu levels obtained with high dietary Cu.

The level of Fe in a small section of duodenum was increased ($P < 0.01$) by high dietary AA or Fe and still further increased when both AA and Fe were present in the diet resulting in a Fe × AA interaction ($P < 0.01$). High dietary Cu increased ($P < 0.05$) intestinal Cu, but depressed ($P < 0.01$) intestinal Fe. This depression was overcome only when high levels of AA and Fe were also present in the diet.

Kidney Fe concentration was decreased by high dietary Cu ($P < 0.05$) but was not influenced by any other treatment. Kidney Cu concentration was increased ($P < 0.01$) by high dietary Cu and decreased ($P < 0.01$) by dietary AA. The Cu × AA interaction was significant ($P < 0.01$) indicating that AA was effective in ameliorating the influence of dietary Cu on kidney Cu concentration. The level of Fe or Cu in the spleen was not influenced by dietary treatment.

The addition of 240 ppm of Cu to the basal diet decreased plasma Zn concentration ($P < 0.01$). The reduction in plasma Zn was greater when both high dietary Cu and Fe were present constituting a significant ($P < 0.01$) Cu × Fe interaction. Liver Zn was increased ($P < 0.05$) when high Cu was present, but the presence of AA in the diet reduced the degree of increase due to high Cu as indicated by a significant ($P < 0.01$) Cu × AA interaction. Spleen, kidney and intestinal Zn concentrations were not affected by diet.

The effect of diet on the pattern of appearance of ^{64}Cu in the blood (% of dose)[13] following oral administration is summarized in figure 1. The concentration rose slowly for all diet groups during the first 21 hours (12% of dose), then rose rapidly during the next 25 hours. The highest concentration of ^{64}Cu in the blood at 46 hours was

[13] Blood volume was assumed to equal 6.65 ml blood/100 g body wt (13).

found in pigs fed the basal diet. High Fe reduced ($P < 0.01$) ^{64}Cu appearance in the blood and there was a Fe × Cu interaction ($P < 0.05$). High Cu in the presence of high Fe partially prevented the interference with ^{64}Cu uptake associated with

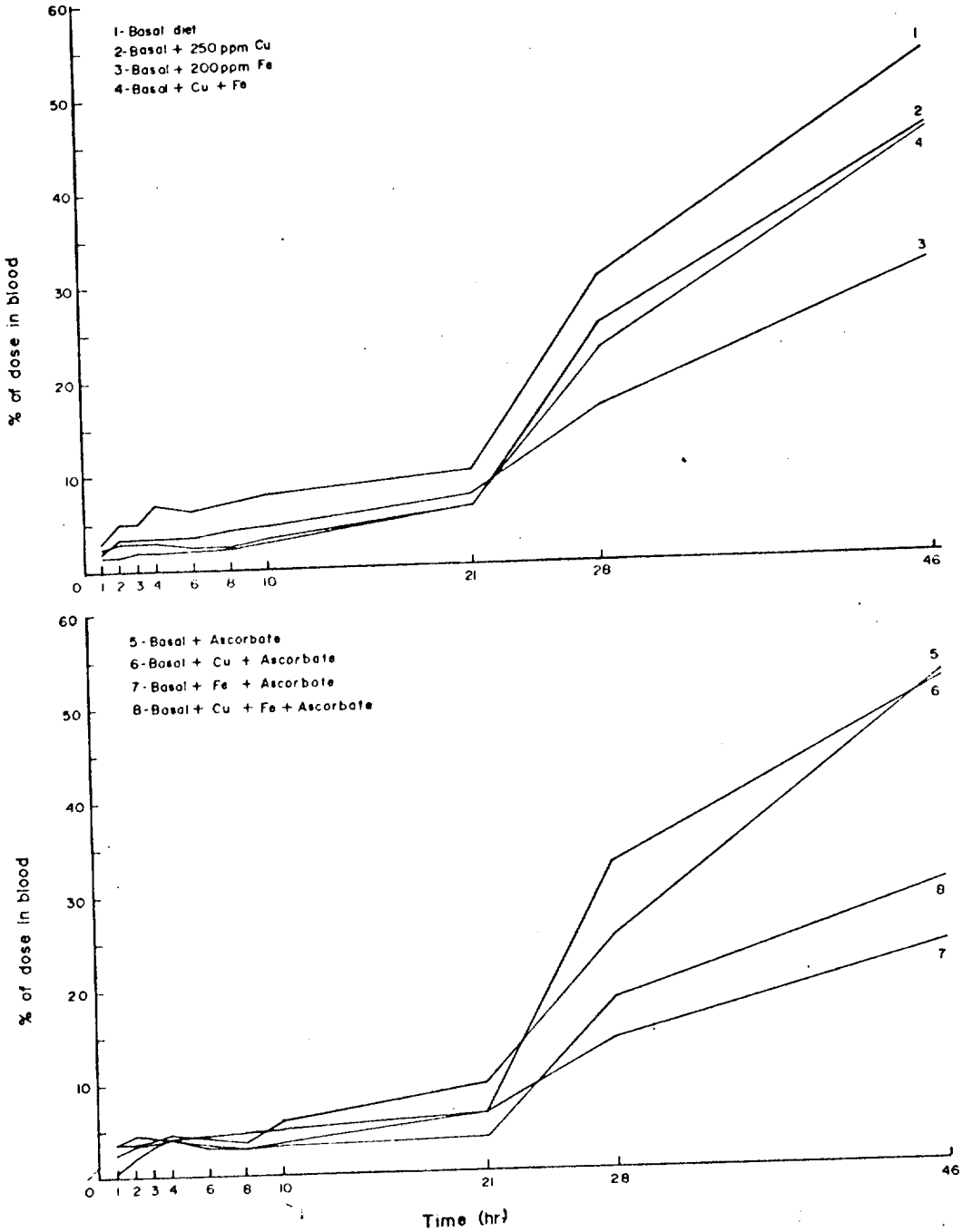

Fig. 1 The appearance of an oral dose of ^{64}Cu in the blood (% of dose).

TABLE 4

Effect of dietary Cu, Fe and ascorbic acid level on plasma clearance of intravenously administered ^{59}Fe in pigs[1]

Diet	$T_{0.5}$[2,3]
	min
Basal	53[4]
Cu	26
Fe	41
Cu + Fe	31[5]
Ascorbic acid (AA)	30
AA + Cu	32
AA + Fe	48
AA + Cu + Fe	43[5]

[1] Four animals per group; blood obtained at 10, 30, 60, 120 and 240 minutes postinjection. [2] Time for one-half of injected dose of ^{59}Fe to disappear from plasma. [3] Significant differences: Cu, AA × Fe, $P < 0.01$; Cu × AA, $P < 0.05$. [4] Two pigs only (one animal injured, one animal isotope apparently not given intravenously). [5] Three pigs only; some isotope apparently not given intravenously.

high Fe alone. When both Fe and AA were added to the basal diet the level of ^{64}Cu uptake was less than one-half of that obtained with the basal diet regardless of dietary Cu level. The reduction when both Fe and AA were present tended to be greater than the reduction with either added alone, although the effect was not statistically significant.

The half-times of removal of intravenously administered ^{59}Fe from the plasma are summarized in table 4. Disappearance of ^{59}Fe from plasma was faster in pigs fed the high Cu diet than in those fed the basal diet (26 vs. 53 minutes). The addition of both Fe and AA to the high Cu diet restored half-time to a value approaching that obtained on the basal diet (43 vs. 53 minutes), although the addition of either alone did not restore the values to basal levels. The addition of AA to the basal diet appeared to increase the ability to utilize iron as evidenced by the more rapid rate of removal of ^{59}Fe in this treatment.

The patterns of reappearance of ^{59}Fe in the blood at nine daily intervals after intravenous administration are presented in figure 2. The ^{59}Fe activity of the blood increased steadily in pigs in all diet groups until day 3 and then tended to plateau. Activity rose most rapidly and reached the highest level in pigs fed the high Cu diet. High dietary Fe produced ^{59}Fe activity

TABLE 5

Effect of dietary Cu, Fe and ascorbic acid level on retention of intravenously administered ^{59}Fe in pig liver, spleen, kidney and intestine

Diet	Liver ^{59}Fe		Spleen ^{59}Fe		Kidney ^{59}Fe		Intestine[1]
	cpm/g[2]	% dose	cpm/g[2]	% dose	cpm/g[2]	% dose	cpm/g[2]
Basal	293	4.92	394	0.39	109	0.22	79
Cu[3]	212	3.88	732	0.74	97	0.22	36
Fe[4]	221	3.91	338	0.37	85	0.19	46
Cu + Fe	168	2.91	292	0.30	83	0.19	41
Ascorbic acid (AA)[5]	274	4.65	423	0.42	96	0.20	59
AA + Cu	151	2.47	298	0.28	118	0.25	41
AA + Fe	315	5.20	349	0.34	172	0.37	42
AA + Cu + Fe	186	3.27	245	0.24	99	0.20	50
Significant differences	Cu $P < 0.01$	Cu $P < 0.01$, AA × Fe, $P < 0.05$	NS	NS	NS	NS	NS

[1] Impossible to calculate % of dose as entire intestine was not sampled. [2] Expressed as counts per minute per gram wet tissue. [3] Two hundred fifty ppm total dietary Cu. [4] Three hundred ppm total dietary Fe. [5] 0.5% total dietary ascorbic acid.

similar to that of pigs fed the basal diet except when fed in combination with high Cu which resulted in an increase in activity midway between the level achieved on the basal diet and that of the low Fe–high Cu diet. High AA resulted in ^{59}Fe activity intermediate between basal and high Cu groups, except when high Fe was fed which resulted in ^{59}Fe activity lower than that obtained with the basal diet.

The retention of intravenously administered ^{59}Fe in liver, spleen, kidney and intestine is summarized in table 5. The retention of ^{59}Fe in liver and intestine tended to be decreased by high dietary Cu or Fe. High AA either alone or in the presence of high Fe was not associated with a change in ^{59}Fe retention from that obtained on the basal diet; high AA also failed to prevent the reduced ^{59}Fe retention obtained with high Cu.

DISCUSSION

Pigs fed high Cu had decreased plasma and liver Fe concentrations, increased LIBC and TIBC, decreased saturation of TIBC, increased rate of removal of intravenously administered ^{59}Fe from the plasma and increased utilization of ^{59}Fe by RBC, all manifestations of Fe deficiency. The results are similar to those obtained in copper deficiency (14, 15) and indicate that once Fe enters the blood from the diet or from other tissues it is readily utilized for incorporation into hemoglobin. The low level of tissue Fe and the degree of transferrin saturation would indicate that there was no block in Fe release from the reticuloendothelial system.

Bush et al. (15) and Jensen et al. (16) found that both plasma removal rates of ^{59}Fe and RBC uptake of ^{59}Fe were lower in normal than in Cu-deficient pigs. They observed two rates of removal in some animals and suggested that the more rapid initial rate of removal was due to the combined effects of removal of Fe by liver and bone marrow while the slower second rate indicated rate of removal by bone marrow alone. In the present study, control animals had a half-time for ^{59}Fe disappearance from the plasma of approximately 50 minutes. There was some indication of an initial rapid removal and a later slower removal but insufficient samples were available to isolate two rates of removal. The short half-time (26 minutes) for disappearance of ^{59}Fe from the plasma in pigs fed 250 ppm Cu compares favorably with values reported by Bush et al. (15) in Cu-deficient pigs. In the work of Bush et al. (15) and Jensen et al. (16) Cu-deficient pigs had depressed uptake of ^{59}Fe by RBC, while in the present study, pigs fed 250 ppm Cu had a marked increase in ^{59}Fe uptake by RBC. In our work (2), plasma ceruloplasmin levels were decreased in Cu-deficiency, but not in animals fed 250 ppm Cu. It appears that both Cu-deficiency (15, 16) and Cu toxicity are associated with depressed utilization of Fe, but that different mechanisms exist for the development of the anemia which is common to both.

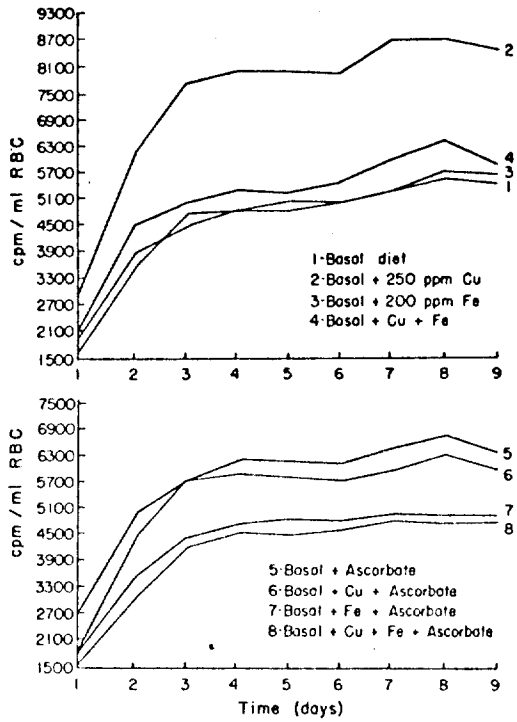

Fig. 2 Red cell utilization of ^{59}Fe.

AA is believed to influence both Cu and Fe utilization. It reduces absorption and/or retention of Cu (5–8). In the present

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work liver Cu concentration in pigs fed high Cu was greatly reduced by the addition of 0.5% AA to the diet. AA increases Fe absorption from the gastrointestinal tract by its reducing action and by formation of soluble Fe–ascorbate chelates (3, 4). Ferrous Fe is more readily absorbed, incorporated into heme and bound to transferrin than is ferric Fe (17). In the present work AA had no effect on the level of Fe retained in blood of other tissues. However, increased AA did result in increased plasma TIBC, a decrease in LIBC and, hence, an increase in degree of transferrin saturation. AA also resulted in an increased rate of removal of ^{59}Fe from plasma and an increased uptake by RBC. These changes may indicate that AA overcomes the detrimental effects of high dietary Cu by increasing the absorption and utilization of Fe. Alternatively, the reduction in the amount of an oral dose of ^{64}Cu that appears in the blood when AA is present in the diet suggests the possibility that the detrimental effects of high dietary Cu are overcome by interfering with Cu absorption.

The results of these studies do not precisely define the site of the primary lesion(s) produced by a high level of dietary Cu. Hemoglobin production was greatly curtailed. Decreased levels of plasma and liver Fe as well as an increased LIBC, suggest a reduced level of iron in the body. Limited Fe availability for hemoglobin synthesis could be due to: 1) a block in release of Fe from the reticuloendothelial system; 2) defective Fe transport; 3) defective Fe uptake by and transport in the immature erythroid cell; or 4) a decrease in total body Fe due to blood loss or impaired Fe absorption (18). The lack of a build-up of elemental or radioactive iron in the liver or spleen suggests that Fe released from the reticuloendothelial system is not blocked. The high LIBC as well as the rapid plasma clearance of an injected dose of ^{59}Fe is evidence that the Fe transport system is fully functional. The high level of ^{59}Fe which reappears in the red blood cells within 2 days of an i.v. dose of ^{59}Fe demonstrates the hemoglobin producing capacity of the erythropoietic system. A likely lesion thus appears to be an impairment in the absorption of Fe from the gastrointestinal tract. The level of Fe in a small section of the duodenum was markedly reduced in the presence of the high level of dietary copper. This may be an indication of reduced amounts of Fe being transported across the intestinal wall.

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Brief Communication: Ascorbic Acid Blocking of Aminopyrine Nitrosation in NZO/Bl Mice [1,2]

Melvin Greenblatt, M.D.,[3] *National Cancer Research Laboratories, University of Otago Medical School, Dunedin, New Zealand*

SUMMARY—NZO/Bl mice were given a single gavage of 17.3 μmoles 4-dimethylaminoantipyrine and 58 μmoles sodium nitrite ($NaNO_2$), a dose that produced acute centrilobular necrosis of the liver in 48 hours, due to dimethylnitrosamine formation. The effect of the addition of ascorbic acid in varying concentrations to the gavage mixture was studied. High concentrations of ascorbic acid (2 times the molar concentration of $NaNO_2$) prevented hepatic necrosis. When the concentration of ascorbic acid was reduced to equimolar with $NaNO_2$, the protective effect was incomplete.—J Natl Cancer Inst 50: 1055–1056, 1973.

THE ANALGESIC and antipyretic drug 4-dimethylaminoantipyrine (also called aminopyrine) (AP), a tertiary amine, when administered with sodium nitrite ($NaNO_2$) to rats causes acute centrilobular hepatic necrosis. This pathologic effect is caused by production of *N*-dimethylnitrosamine (DMN), a hepatotoxic and carcinogenic nitrosamine identified in vitro (*1*) in rat stomach contents after AP and nitrite gavage.

Mirvish et al. (*2*) reported that the nitrosation of several secondary and tertiary amines is blocked in vitro by the addition of ascorbic acid. They suggested that this effect may be useful in preventing the production of carcinogenic *N*-nitroso compounds when foods containing a high nitrite content are eaten with secondary or tertiary amines.

I have studied the effect in vivo of the addition of ascorbic acid to mixtures of AP and nitrite in mice, and have used hepatic necrosis as an index of DMN production. As little as 0.125 mg DMN/mouse (1.6 μmole/mouse) produced centrilobular necrosis when administered once in animals killed 48 hours after gavage.

MATERIALS AND METHODS

Male NZO/Bl mice (*3*), inbred for 95 generations, were 6–8 weeks old at the beginning of the experiment and weighed 20–30 g. In each 200 cubic-inch metal cage, 2–4 animals were housed and fed a standard animal ration and water ad libitum before and after treatment. Each experimental group had 4 mice. I obtained AP from Aldrich Chemical Co., Milwaukee, Wisconsin, and used it without further purification. $NaNO_2$ and ascorbic acid (analytical reagent grades) were obtained from BDH Chemicals Ltd., Poole, England. Stock solutions of $NaNO_2$ and AP were prepared and kept in the refrigerator. Ascorbic acid solution was prepared immediately before use.

The amount of $NaNO_2$ given each mouse was close to the maximum tolerated dose. The concentrations of the other reactants were fixed in ratios comparable to Mirvish's in vitro study.

Treatment consisted of intragastric intubation of the solutions indicated in table 1. Treatment was given once, in the morning. All treatment solutions were kept on ice before gavage. Mice were killed with coal gas 48 hours later, and the livers were examined grossly and microscopically by frozen section and conventional histologic techniques.

DISCUSSION AND CONCLUSIONS

These data are consistent with the in vitro observations of Mirvish et al. and show that, in this mouse stomach model, liver necrosis, caused by production of DMN, is produced when AP and nitrite are administered together. High concentrations (twice the molar concentration of $NaNO_2$) of ascorbic acid block the nitrosative dealkylation of

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TABLE 1.—*Experimental design and results of treatment**

Group	$NaNO_2$	AP	Ascorbic acid	DMN	Hepatic necrosis†
1	58	—	—	—	0
2	—	17.3	—	—	0
3	—	—	114	—	0
4	58	17.3	—	—	+++
5	58	17.3	114	—	0
6	58	17.3	57	—	+
7	—	—	114	1.6	+
8	—	—	—	1.6	+

*All values are in μmoles of compound given by gavage. Total volume was 0.4 ml/mouse; each group had 4 mice.

†Measured semiquantitatively 48 hours after gastric intubation. +: Individual cells necrotic about central vein; ++: all cells around central vein necrotic and one-third of lobule involved; +++: pronounced centrilobular necrosis involving more than one-third of lobule.

AP and prevent the production of DMN, at least in sufficient quantities to produce hepatic necrosis. Because the sensitivity dose of this morphologic method is approximately 5 mg DMN/kg mouse, <1.6 μmoles DMN was produced when a mixture of 17.3 μmoles AP and 58 μmoles $NaNO_2$ were administered with 114 μmoles ascorbic acid. Therefore, $<1.6/17.3$ (i.e., 9.2%) AP was converted to DMN under these conditions.

However, when the concentration of ascorbic acid was decreased so that it was equimolar with $NaNO_2$, the protective effect of the ascorbic acid was incomplete. Ascorbic acid given with DMN did not potentiate or antagonize the hepatotoxic effect. Although we confirm Mirvish's data, in practical terms, the high amount of ascorbic acid required to prevent nitrosation of AP would limit the usefulness of this technique in diminishing the potential hazards associated with concurrent administration of $NaNO_2$ and nitrosatable compounds (*4–6*). The biochemical effect of the addition in vivo of reducing agents such as ascorbic acid to mixtures containing DMN deserves further study.

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Optimum Vitamin C Intake as a Factor in the Preservation of Disc Integrity*

Preliminary Report

JAMES GREENWOOD, JR., M.D., F.A.C.S.
Clinical Professor of Neurosurgery, Baylor University College of Medicine

ALBERT EINSTEIN, probably the greatest genius of all time, repeatedly mentioned in his writings that the accumulation of knowledge only further revealed the hopelessness of knowing or understanding more than a small bit about the universe. The same thing is true of medicine. With our increasing knowledge the understanding of the intricacies of the chemistry of the body becomes more hopeless. This does not mean that present knowledge should not be used to its greatest extent, but it does indicate that one should not be forced to wait for complete scientific knowledge before using methods of treatment which are not harmful and which show hope of success. For example, consider the hidden work on Prontosil® which lay in the literature for many years, was finally reviewed, and eventually led to the development of antibiotic and chemotherapeutic methods. In 1935 I was greatly shocked in doing some studies on hypoglycemia to find that the action of insulin was little understood. The prevention of smallpox by Jenner's method of vaccination, though incompletely understood, was nevertheless a boon to mankind. This report on vitamin C therapy is a preliminary one and perhaps premature, but considerable evidence has been accumulated in 6 years to indicate that worthwhile progress has been made in the prevention of disc disruption and postoperative disc recurrences.

This study really began in 1957 when I started to take increasingly larger doses of vitamin C because of a bad back with severe lumbosacral pain which had gradually become more disabling over a period of 10 years. Golf games were missed because of back pain and rigidity, and quite a few evenings and nights were spent on a heating pad. To hit a bag of practice golf balls (40 or 50) would cause disability for several days. The relationship of vitamin C to connective tissue and ground substance of the body, including bones, cartilage, and teeth, was well documented, and I started with 100 mg. of ascorbic acid 3 times daily. This greatly improved the condition, and in about 4 months I was quite comfortable and could take extra exercise with little difficulty. I soon forgot the vitamin C and within an additional 3 months was again in difficulty. Resumption of the vitamin C produced a similar improvement, and it was at this time that I disclosed my experience to Dr. Paul Harrington, one of my close friends and a widely known orthopedic surgeon, who informed me that he had been giving large doses of vitamin C in his "back" cases for 2 or 3 years and was taking it himself with similar results.[1] He suggested that I increase the dose to 250 mg. 4 times daily. At present we recommend 500 mg. a day in 2 doses with an increase to 1,000 mg. daily if there is any discomfort or if heavy exercise, such as hunting, sail-boating, etc., is anticipated. Evidence from most patients indicates that muscular soreness experienced with exercise has been greatly reduced by the large doses of vitamin C and becomes obvious again when the vitamin is forgotten.

The administration of vitamins to some extent has become a cult, and this aspect should be examined in the case of the administration of large doses of vitamin C; however, there is no indication in the literature that any harm can come from larger doses, and the material is relatively cheap and may be purchased by the patient without a prescription, not only from drug stores but also from food markets.

Vitamin C Characteristics

Vitamin C or L-ascorbic acid is a water-soluble vitamin which is not stored in the body

* Presented at a special Scientific Symposium, March 13, 1964, which was held in honor of Dr. James W. Watts' Sixtieth Birthday at the George Washington University Hospital, Washington, D.C.

and is synthesized by most animals. Only in the guinea pig and the primates do there occur evidences of C deficiency, exhibiting its greatest manifestation in scurvy. Excess vitamin is excreted in the urine, feces, and perspiration, and a recent paper indicates that it is excreted to some extent in expired air. Minimal requirements have variously been set at 30 to 70 mg. a day and deficiency manifests itself only after a period of time, since the strong tissues which have been developed under adequate vitamin C intake do not immediately break down when the vitamin is withdrawn. Vitamin C is important in the maintenance of proper texture of teeth, bone, cartilage, blood vessels, and connective tissues throughout the body. Ingelmark[2] states, "Articular cartilage initially reacts to functional strain by comparative rapid cell proliferation in enlargement of the chondrocyte. This phase is followed by production of intercellular substance (collagen and vitamin C) on a scale so large that the number of chondrocytes per unit volume decreases and becomes lower than it was originally. This suggests that the amount of regenerated intercellular substance is dependent upon the quantity of intercellular substance available. Unless the latter is adequate, intercellular substance can never be produced in amounts large enough to cause appreciable hypertrophy."

In infection vitamin C is depleted, migrating to the white blood cells and adrenal glands. It is generally assumed that vitamin C affects various enzymes through reactions mediated by its oxidation-reduction potential.

The question of optimal levels of vitamin C is controversial. Animals who synthesize vitamin C maintain levels of saturation. The effects of exercise, injury, fatigue, exhaustion, and infection are all known but have never been successfully evaluated. Although much is known of the chemistry of vitamin C and its interrelationship to adrenal cortical activity, the basic knowledge of this chemistry still remains a mystery, and additional experiments must be done, particularly with respect to infection, work output, fatigue, exhaustion, and stress.

All of the available literature of the past 20 years has been reviewed, and this beginning of the story of vitamin C is perhaps the most dramatic of all the vitamins in many ways and continues to be one of the most mysterious. The relationship of vitamin C to the mucopolysaccharides in the formation of connective tissue, healing, the integrity of all connective-tissue structures, including bones, cartilages, teeth, and particularly the relationship to the adrenal gland, which itself contains 1 per cent of the total vitamin C in the body in spite of its small size, offers a very fertile field for additional work. Most investigators seem to progress to a point and then seem to founder. This is no reason not to try on an empirical basis the effects of this apparently harmless vitamin in areas where it might be effective.

Clinical Procedure

During the past 5 years gradually increasing numbers of patients with early disc lesions, with either simple lumbosacral strain or definite sciatic nerve involvement, were placed on vitamin C, 750 to 1,000 mg. daily.

Patients with severe disc involvement were given at least a short trial of vitamin C therapy in spite of the obvious need for surgery. A small percentage of these recovered without surgery. Some recurred later.

All patients with chronic disease, particularly those with generalized disc involvement, were placed on vitamin C.

All postoperative patients were placed on vitamin C and told to take 500 mg. daily indefinitely, increasing the dose to 750 or 1,000 mg. when there was any discomfort.

Results in Over 500 Cases

It can be stated with reasonable assurance that a significant percentage of patients with early disc lesions were able to avoid surgery by the use of large doses of vitamin C. Many of these patients after a few months or years stopped their vitamin C and symptoms recurred. When they were placed back on the vitamin the symptoms disappeared. Some, of course, eventually came to surgery.

The number of reoperations on the same patient for recurrence at the same level or a new disc lesion at a different level has been greatly reduced. Also, to some extent the standard statement that "1 out of 20 patients who has a ruptured disc operated upon will return within 5 years for operation of the same disc or for a second disc at a different level" has been altered. We are reviewing our disc cases for the past 5 years for evidence of recurrence and are comparing them with previous years. This would include something over 300 patients operated on during this period and a greater number of unoperated ones who we feel have been helped by

this routine. Initial surveys indicate a significant reduction in reoperations as well as a more satisfactory postoperative course.

Interrelationship Between Adrenal Function and Vitamin C

1. Normal adrenal and normal C—normal healing.
2. Normal adrenal and reduced C—poor healing.
3. Normal adrenal and scorbutic—no healing.
4. Cortisone excess and normal C—no healing.
5. Cortisone excess and scorbutic—healing imperfect.
6. Adrenalectomy and scorbutic—no healing.
7. Adrenalectomy and normal C—healing imperfect.
8. Cortisone and excessive C equals?—no studies.

One common denominator in this interplay between these 2 functions seems to be that adequate vitamin C plus normal or suppressed adrenal activity results in normal connective tissue and healing reactions, and that vitamin C deficiency plus an excess of adrenal activity or cortisone excess results in normal connective tissue reaction. Vitamin C is detrimental only when excessive cortisone is administered. The interrelationship is not clear.

Discussion

This is a paper based on empirical application of a considerable amount of knowledge gained by many workers on vitamin C through the years and its effect on connective tissue, ground substance, bone, teeth, and cartilage. Although the available information is great, it represents less knowledge than is needed to thoroughly understand the effect on cartilage degeneration and regeneration in our disc cases. I have previously stated that this is a metabolic disease in which trauma plays only a minor part except in rare instances. While I must apologize for the fact that this cannot be a scientific paper, the results, I believe, when completely tabulated will show that significant benefit is obtained by the treatment of disc conditions by vitamin C therapy.

Vitamin C therapy is at least a factor in the disc problem, and its availability in adequate amounts seems necessary to disc metabolism and maintenance as it is for all tissue ground-substance. The optimum level is higher than previous estimates and much higher than ascorbic levels. The optimum level is indicated by many studies to vary considerably with stress, muscular activity, infection, or injury.

It is thought that the postoperative course in most cases has been improved, recurrences reduced, and surgery occasionally avoided, and that greater confidence in the operation may be expected as a result of vitamin C administration.

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Clinical manifestations of ascorbic acid deficiency in man[1, 2]

Robert E. Hodges,[3] M.D., James Hood,[4] M.D., John E. Canham,[5] M.D., Howerde E. Sauberlich,[6] Ph.D., and Eugene M. Baker,[7] Ph.D.

The metabolism of ascorbic acid has been studied in normal men receiving adequate intakes of this vitamin by Baker et al. (1, 2). Prior to 1966, no study utilizing isotopic techniques had been performed in experimental human scurvy to define the metabolism of this vitamin in the face of severe dietary deficiency. We recently designed and completed two separate studies of experimentally induced scurvy in man. The results of the first have been published (3–5) and the observations made during the second study constitute the substance of this report.

Earlier studies of experimental scurvy by Pijoan and Lozner (6) suggested that after initial saturation with vitamin C, the body stores were sufficient to protect against scurvy for a period of deprivation lasting as long as 5 to 6 months. The British Medical Research Council (7) reviewed previous experimental studies and concluded that healthy men have stores of ascorbic acid sufficient to enable them to exist on deficient diets for periods ranging from 160 to 200 days without developing overt scurvy. In our two studies during which the subjects developed scurvy, it was apparent that the onset of this disorder began in less than 90 days, even though the participants were initially fed a diet containing 77.5 mg vitamin C. During the second study, we compared the body pool size with the blood and plasma levels of ascorbic acid and with the onset of clinical scurvy.

The British Medical Research Council demonstrated that a daily dose of 10 mg L-ascorbic acid was sufficient to prevent scurvy for as long as 424 days (7) and that the same dose was enough to cure scurvy once it had appeared. In a study performed by the Royal Canadian Air Force, Johnstone et al. (8) did not mention the development of clinical signs of scurvy in a group of 33 recruits who received a daily intake of 7.9 mg ascorbic acid for a period of 6 to 8 months. We previously observed (3, 4) that an intake of 6.5 mg ascorbic acid daily was sufficient to cause slow but steady disappearance of scurvy in a single subject. This low intake of ascorbic acid (except during periods of emotional stress) was enough to cause a gradual repletion in the body pool of ascorbic acid in one man.

The major objectives of this second study of experimental scurvy in man were to obtain additional information regarding *1*) the size of the body pool of ascorbic acid, *2*) the rate of catabolism of this vitamin, *3*) the correlation between body pool size and blood plasma levels, and *4*) the relationship of all three to clinical signs and symptoms of scurvy. In addition, by giving both tritium-labeled ascorbic acid and ^{14}C-labeled ascorbic acid, we were able to study the metabolism of this vitamin more precisely. Finally, we wished to observe the rate of repletion of body pool size and the cure of scurvy in men receiving a greater range of daily doses of this vitamin.

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[1] From the Department of Internal Medicine, University Hospitals, University of Iowa, Iowa City, Iowa 52240, and the U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colorado 80240.

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[3] Professor, Department of Internal Medicine, University of Iowa. [4] Formerly, Department of Internal Medicine, University of Iowa. Current address: 1328 2nd Ave., S. E., Cedar Rapids, Iowa 52403. [5] Commanding Officer and Director, U. S. Army Medical Research and Nutrition Laboratory. [6] Chief, Chemistry Division, U. S. Army Medical Research and Nutritional Laboratory. [7] Assistant Chief, Chemistry Division, U. S. Army Medical Research and Nutrition Laboratory.

TABLE 1
List of subjects, period of depletion and level of intake on repletion

Volunteer	Age	Height, cm	Initial weight, kg	Period of complete deprivation of ascorbic acid, days	Daily intake of ascorbic acid from diet and supplements	
					Day	*mg*
H	26	184.2	84.1	91	1 to 13	77.5
					14 to 104	0
					105 to 110	4
					111 to 227	6.5
					228 to 258	600
P	33	175.3	87.3	91	1 to 13	77.5
					14 to 104	0
					105 to 110	4
					111 to 227	6.5
					228 to 258	600
S	37	182.9	82.7	97	1 to 13	77.5
					14 to 97	0
					98 to 110	2.5
					111 to 227	66.5
					228 to 258	600
R	52	167.6	65.2	97	1 to 13	77.5
					14 to 110	0
					111 to 227	66.5
					228 to 258	350
Mc	34	170.2	68.9	84	1 to 13	77.5
					14 to 97	0
					98 to 110	128
					111 to 227	130.5
					228 to 258	350

Methods

Volunteers from the Iowa State Penitentiary at Fort Madison, Iowa, were recruited and their informed consent was obtained. They then were housed on the Metabolic Ward of University Hospitals in Iowa City, Iowa, for the duration of the experiment. This second study began with six volunteers, but one subject requested to withdraw from the experiment prior to the development of clinical scurvy. The remaining five men were deprived of ascorbic acid for periods ranging from 84 to 97 days following an initial control period (*days 1* to *13*) during which they were fed a solid diet of soy protein products containing 2.5 mg ascorbic acid daily and supplemented by an additional 75 mg ascorbic acid. On *day 14* their diet was changed to a liquid formula containing essentially no ascorbic acid. This formula, based on vitamin-free casein, has been previously described by Hodges et al. (3).[8] Because of the unpalatability of this formula, the men took it thrice daily via polyethylene gastric tube (3). Fifteen percent of their calories were supplied from protein, 40% from fat, and 45% from carbohydrates. Absence of L-ascorbic acid and L-dehydroascorbic acid from the diet was confirmed by chemical analysis (4). Mineral supplements and the amounts of essential vitamins recommended by the National Research Council (9) (with the exception of ascorbic acid) were given daily. The mineral supplement was inadvertently omitted from the diets during the first 34 days of the depletion period (*days 14* to *47*).

Isotopic labeling of the body pools with L-ascorbic-1-^{14}C acid was accomplished in all men 1 week before commencing depletion. Two men (*S* and *P*) received, in addition, tritium-labeled L-ascorbic acid as described elsewhere (10).

After the period of complete deprivation of ascorbic acid, and at a time when the men had obvious evidence of clinical scurvy, we began to replete them with differing intakes of vitamin C. A solid diet based on soy protein foods and containing 2.5 mg L-ascorbic acid was fed from *day 98* to *day 227* to *subject S* and from *day 111* to *227* to all the other men. The percent of calories derived from protein, fats, and carbohydrates remained the same in the soy protein diet as in the liquid formula diet. In the final phase of the study, beginning on *day 228* and lasting until *day 258*, the men were fed a regular diet providing approximately 100 mg ascorbic acid plus a supplement of either 250 or 500 mg daily and an ad libitum intake of calories. The duration of deprivation and the total daily intakes of ascorbic acid from the diet and from the oral supplements are summarized in Table 1.

[8] The formula contained 30 mg ferrous fumarate, not 30 g as shown in Table 1 of reference 3.

TABLE 2
Signs and symptoms of scurvy in the five subjects

H	Hyperkeratosis Congested follicles Petechiae Ecchymoses Subconjunctival hemorrhage Joint effusions Dyspnea on exertion Edema Femoral neuropathy
Mc	Hyperkeratosis Congested follicles Petechiae Ecchymoses Swollen gums Arthralgia
P	Hyperkeratosis Congested follicles Coiled hairs Petechiae Bleeding swollen gums Joint effusions
R	Hyperkeratosis Congested follicles Coiled hairs Ecchymoses Joint effusions Dyspnea on exertion
S	Hyperkeratosis Redness of gum margins Petechiae

Temperature, pulse, and respiratory rates were recorded four times daily and blood pressure twice daily. The men were examined each day by an internist and at intervals by an ophthalmologist (11). Chest X-rays and electrocardiograms were taken at intervals. Twenty-four hour collections of urine and feces were made daily for determinations of urinary and fecal nitrogen and for radiometric assay (10). Samples of expired air were collected for radiometric assay (10) subsequent to administration of the isotopically labeled L-ascorbic acid. Weekly hematologic studies included hemoglobin, white and red cell counts, hematocrit, and erythrocyte sedimentation rate.

Aliquots of the diet were assayed weekly for ascorbic acid content (4). Plasma and whole blood ascorbic acid levels were determined weekly (4). The daily rate of excretion of the labeled ascorbic acid or metabolites in the urine was measured (4) and serum vitamin A and carotene levels were determined weekly (12). Additional measurements included total serum proteins and serum protein electrophoresis, cholesterol, creatinine, erythrocyte hemolysis and tryptophan load tests (13). Insulin tolerance tests (0.1 unit/kg body wt subcutaneously) and adrenalin tolerance tests (0.0025 mg/kg body wt subcutaneously) were performed at monthly intervals. During these tests the concentration of glucose was measured in the blood at 0, 30, 60, 90, 120, 180, and 240 minutes.

The men were exposed in a cold climate-control room (50 F, 45% humidity) over a 4-hr period each day for 1 to 4 days beginning on *day 112*. Psychological tests were administered and measurements of psychomotor performance were made at intervals (14). Vascular reactivity was studied during deficiency and again after repletion (15). The immunologic response to injections of typhoid antigens was assessed during deficiency and after repletion; agglutination titers were determined by the method of Widal (16).

Results (clinical)

Despite a somewhat shorter period of deprivation in the second scurvy study, (84 to 97 days) than in the first (99 days), the subjects in the second study developed a more severe degree of scurvy. Joint effusions, petechial hemorrhages, small ecchymotic lesions, a large subconjunctival hemorrhage and edema and dyspnea were observed in addition to the hyperkeratosis, perifollicular congestion, gum changes, and occasional petechial hemorrhages observed in the first study. The signs and symptoms of scurvy noted in each subject are listed in Table 2.

The increased severity of scurvy that occurred in the second study can be accounted for by individual differences in the rate of depletion of the body pool(s) of ascorbic acid (Table 3).

In the first study, the body pool(s) of ascorbic acid were labeled isotopically by oral administration of L-ascorbic-1-^{14}C acid

TABLE 3
Rate of catabolism of ascorbic acid

	Subject	Percent available body pool of ascorbic acid catabolized/day
Second study	*H*	4.1
	P	3.5
	Mc	3.2
	R	2.8
	S	2.6
First (4) study	*N*	2.7
	K	2.5
	S	2.4
	L	2.2

on the 23rd day of depletion (4). In the second study, isotopic labeling was accomplished 7 days prior to the beginning of the depletion period and again on the 14th, 15th, and 16th days of deprivation (10).

The most rapid rate of depletion of ascorbic acid, 4.1% of the available body pool/day, occurred in *subject H* who also developed the most severe degree of clinical scurvy. Initially the pool size of each man approximated 1,500 mg.

The first sign of scurvy to appear in both studies was petechial hemorrhage. These petechiae occurred in four of the five men in the second study and appeared as early as the 29th day of depletion. They first appeared when the plasma ascorbic acid ranged from 0.13 to 0.24 mg/100 ml and the pool size from 96 to 490 mg. Spontaneous ecchymoses occurred in four of the men, but they were always small in size and appeared only on the legs.

Coiled hairs were observed in two of the men and first appeared on the 42nd and 74th days, respectively. Gum changes appeared only in the four men who had their own teeth; there were none in the one subject who was edentulous. As in the first study, these changes were most marked in those men who initially had gingivitis. The subject who developed the most severe scurvy initially had healthy gums and he showed only minor gum changes at the peak of deficiency. The first definite abnormalities of the gums appeared between the 43rd and 84th days of depletion and progressed after the plasma ascorbic acid levels fell below 0.16 mg/100 ml and the pool size below 360 mg.

Hyperkeratosis, which was present in all five subjects, was marked in three of them and slight in two. This first appeared or became more apparent between the 45th and 100th days. Follicular congestion also developed in all five of the men between the 49th and 90th days.

Although none of the subjects in the first scurvy study developed arthralgia, this was a complaint in four of the five men who participated in the second scurvy study. The onset of joint pains began between the 67th and 96th days. Joint effusions appeared between the 68th and 103rd days by which time the pool size was below 110 mg and the plasma ascorbic acid level was less than 0.16 mg/100 ml. Joint pain occurred in the knees of three of the five men, in the ankles of two, and in the elbows, wrists, and shoulders of one man each. Effusions in both knees occurred in three men, one of whom also had swelling of both of his ankles.

The onset of signs and symptoms, the levels of plasma and whole blood ascorbic acid, and the size of the body pool(s) of this vitamin are summarized in Table 4. It is apparent that arthralgia and joint effusions were relatively late manifestations of clinical scurvy and that the onset of joint involve-

TABLE 4
Onset of signs of scurvy

Signs of scurvy	Day of study	Day of depletion	Plasma ascorbic acid, mg/100 ml	Whole blood ascorbic acid, mg/100 ml	Pool size, mg
Petechiae 4/5	42 to 79	29 to 66	0.13 to 0.24	0.32 to 0.40	96 to 490
Ecchymoses 4/5	49 to 116	36 to 103	0.06 to 0.30	0.27 to 0.73	19 to 438
Coiled hairs 2/5	55 to 87	42 to 74	0.14 to 0.17	0.33 to 0.50	94 to 166
Gum changes 4/5	56 to 97	43 to 84	0.09 to 0.16	0.30 to 0.50	63 to 360
Hyperkeratosis 5/5	58 to 113	45 to 100	0.00 to 0.16	0.29 to 0.47	64 to 342
Congested follicles 5/5	62 to 103	49 to 90	0.00 to 0.16	0.20 to 0.40	32 to 324
Sicca syndrome 5/5	71 to 136	58 to 123	0.06 to 0.18	0.30 to 0.42	24 to 138
Dyspnea 2/5	76 to 103	63 to 90	0.16 to 0.16	0.34 to 0.37	34 to 78
Arthralgia 4/5	80 to 109	67 to 96	0.04 to 0.16	0.30 to 0.50	45 to 217
Joint effusions 3/5	81 to 116	68 to 103	0.07 to 0.16	0.20 to 0.34	39 to 110
Neuropathy 1/5	84	71	0.15	0.37	80
Marked edema 1/5	114	101	0.15	0.47	67

Numbers after signs of scurvy refer to the number of men out of the total (5) in whom a particular symptom appeared.

ment was always preceded by other signs of scurvy.

Edema was noted in three of the men, but in two of them there was only minimal pretibial pitting on pressure or some slight puffiness of the feet. Beginning on the 88th day of deprivation there was a rapid increase in weight followed by swelling of the legs in the third man (*H*), who had the most severe degree of scurvy. Repletion with 4 mg ascorbic acid day was commenced 4 days later on *day 105* (see Table 5). Oliguria followed and his urinary output dropped to 340 ml/day on *day 109*. His intake of ascorbic acid was then increased to 6.5 mg/day on *day 111* and after a brief initial increase in edema, he had a profound diuresis with complete disappearance of edema by *day 133*.

An unusual complication of scurvy that occurred in one subject (*H*) was bilateral femoral neuropathy (17). The onset of this neuropathy occurred early in the course of scurvy on the 71st day of deprivation. At this time his plasma ascorbic acid was 0.15 mg/100 ml and his body pool size was only 80 mg. Except for two perifollicular hemorrhages on one ankle between the 67th and the 73rd days, he had no other evidence of scurvy until the 89th day of depletion when effusions appeared in both knees. His neuropathy was attributed to hemorrhage into the sheaths of both femoral nerves. Recovery was complete 4 months after the beginning of repletion with ascorbic acid.

Another interesting clinical observation made during the second scurvy study was the development of Sjögren's (sicca) syndrome (11), a condition usually associated with one of the diffuse connective tissue disorders. All five men in the second study developed one or more of the component features of this syndrome and two men (*H* and *P*) developed the complete syndrome. *Subject H* had xerostomia and keratoconjunctivitis sicca and *subject P* had enlargement of his submandibular salivary glands in addition to keratoconjunctivitis sicca. Dryness of the eyes was present in a third subject (*S*) and another

TABLE 5
Water retention in *subject H*

Day of study	Day of deprivation	Daily intake ascorbic acid, mg	Urine output, ml	Weight, kg	Ascorbic acid		
					Pool size, mg	Plasma, mg/100 ml	Whole blood, mg/100 ml
0				84.1			
101	88		720	84.7	37		
102	89	0	760	85.8	34		
103	90		710	86.5	32		
104	91		760	86.8	30		
105			895	86.9	33	0.14	0.2
106			560	86.0	37		
107			500	86.5	42		
108		4	490	85.8	46		
109			340	86.1	50		
110			410	86.6	54		
111			600	86.0	57		
112			790	86.9	60	0.15	0.47
113			990	87.8	64		
114			1,200	88.8	67		
115		6.5	2,200	88.8	70		
116			1,910	87.8	73		
117			2,900	86.7	76		
118			3,180	84.9	79		
119			2,260	82.9	83	0.10	0.27
120			2,160	81.9	86		
121			2,270	81.0	89		
133				77.2	128	0.18	0.30

man (*Mc*) had transient parotid gland enlargement. Other features of the sicca syndrome included dental caries, recurrent loss of new dental fillings and tenderness of the buccal mucosa. Cutaneous changes included pruritus, scaling ichthyotic or eczematous appearance, and pigmentation of the ichthyotic areas. The men also noted excessive loss of hair.

Hematologic studies showed a slight degree of anemia as manifested by a decline of hemoglobin and hematocrit values. In *subject H*, this decline was greater when he had water retention and returned to normal after diuresis.

Urinary excretion of ascorbic acid declined rapidly during depletion and was essentially zero after 1 month. Average values for plasma ascorbate fell rapidly during the first month of depletion. Subsequently the majority of the values obtained remained below 0.2 mg/100 ml until repletion was begun. Results for *subjects R* and *S* who were repleted with 66.5 mg of ascorbic acid daily are shown in Fig. 1. In Fig. 2, one can see that the rate of depletion of ascorbic acid in *subjects P* and *H* was similar to that in Fig. 1. However, the plasma levels for *subject H* did not remain below 0.2 mg/100 ml until after the second month of depletion. During repletion on a level of 6.5 mg ascorbic acid/day, the plasma levels of *subjects P* and *H* generally remained low.

Serum cholesterol values initially averaged 280 mg/100 ml on blood drawn at the prison. During the control period the average serum cholesterol value fell to about 200 mg/100 ml and continued to fall during the depletion period to an average level of about 180 mg/100 ml. Beginning with the repletion period, the cholesterol value began to rise before there was any other change in diet and continued to rise until the end of the study when it reached 300 mg/100 ml. Of course, during the final 30 days of the study the men ate foods that would be expected to increase serum cholesterol values (Fig. 3).

The men all had been previously immunized with typhoid antigen during military service. They were given "booster injec-

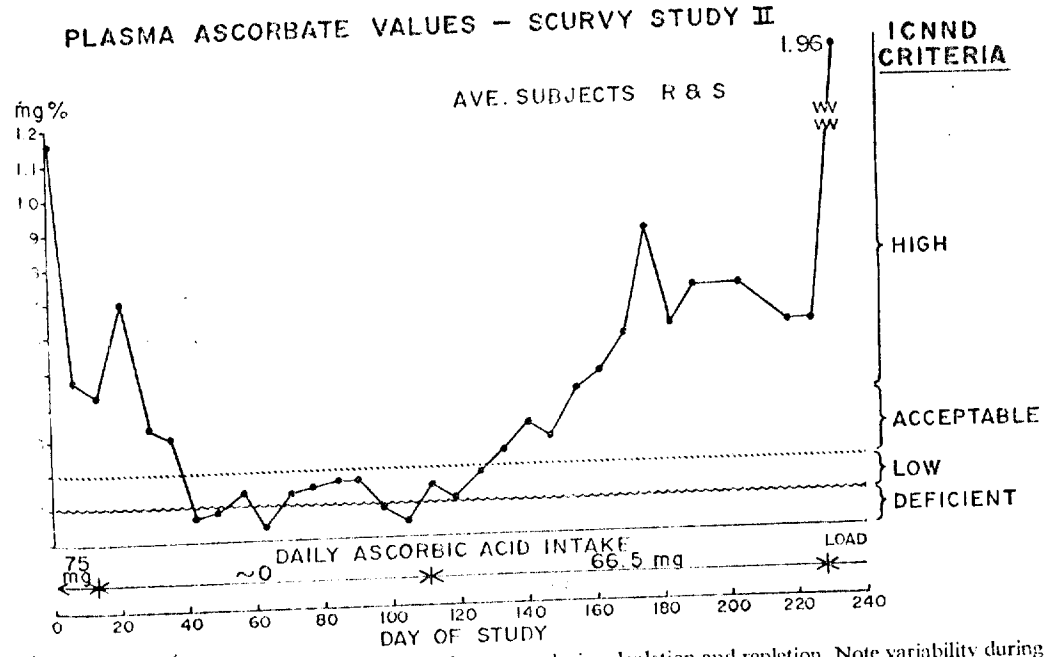

FIG. 1. Average plasma ascorbate values of two men during depletion and repletion. Note variability during period of depletion. Repletion with 66.5 mg daily resulted in a rapid rise in plasma levels. Note also that during deficiency many of the plasma values fell within the low range. The ICNND criteria (24) are based upon serum ascorbate values. In our laboratories parallel studies indicated no significant difference between serum and plasma ascorbate values under the conditions of the study.

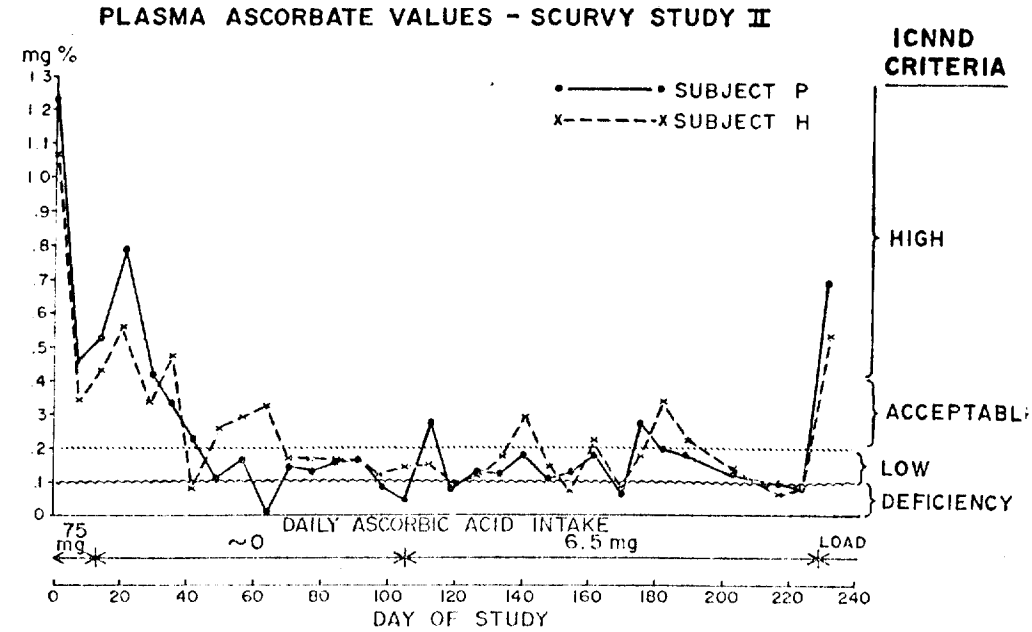

FIG. 2. Average plasma ascorbate values of two men repleted with only 6.5 mg ascorbic acid daily. Repletion with this amount had a negligible effect on plasma levels. Note the frequency with which plasma values fell above the deficiency range.

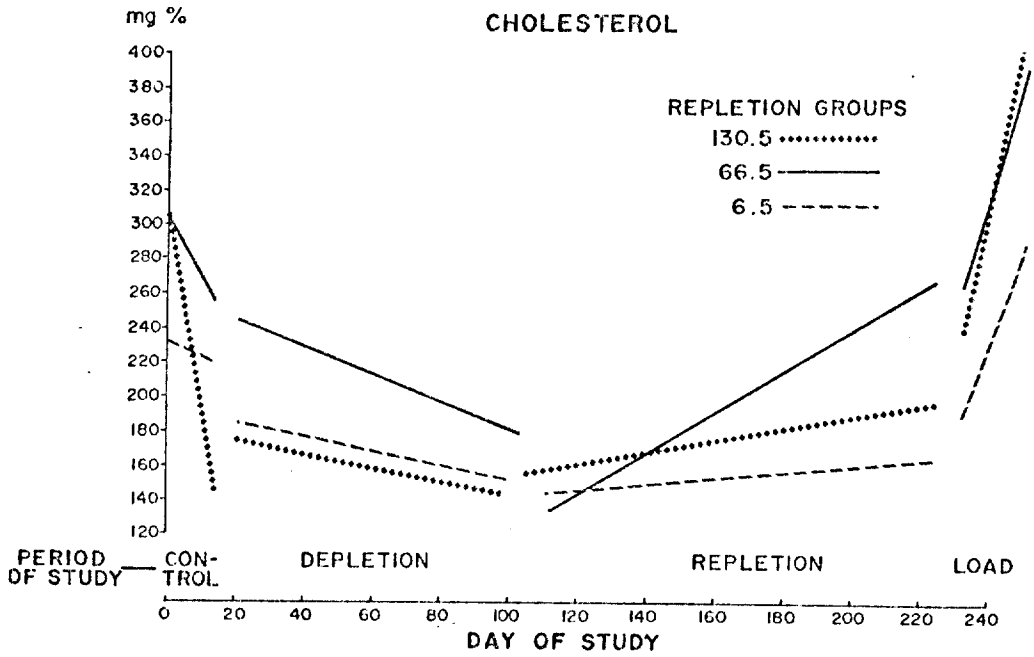

FIG. 3. The regression curves of the cholesterol values obtained during the control, depletion, repletion, and load phases of the study are depicted. The coefficient of correlation for the four respective experimental phases for groups receiving 6.5, 66.5 and 130.5 mg ascorbic acid/day were as follows: 6.5 mg—0.276, 0.642, 0.307, 0.768; 66.5 mg—0.363, 0.813, 0.732, 0.883; and 130.5 mg—0.954, 0.535, 0.468, 0.905.

tions," 0.5 ml of antigen (typhoid vaccine, USP, Eli Lilly and Company, Indianapolis, Indiana) subcutaneously, at the time of maximal depletion and again during the period when saturation doses of vitamin C were given. Their response to immunization during clinical scurvy was normal with a quadruple rise in antibody titer (Fig. 4).

Insulin tolerance curves during scurvy showed a significant decrease in blood glucose values at 2, 3, and 4 hr. These changes were reversed by repletion with ascorbic acid (Fig. 5).

Adrenalin tolerance tests also showed a significant decrease in blood glucose values throughout a period of 240 min during which the men were scorbutic, yet the glycogenolytic response remained intact. Repletion with ascorbic acid resulted in a rise in glucose values throughout the test period (Fig. 6).

Discussion

Comparison between the two Iowa City studies and the British Medical Research Council study gives evidence that very small amounts of ascorbic acid may protect against the onset of scurvy for rather long periods

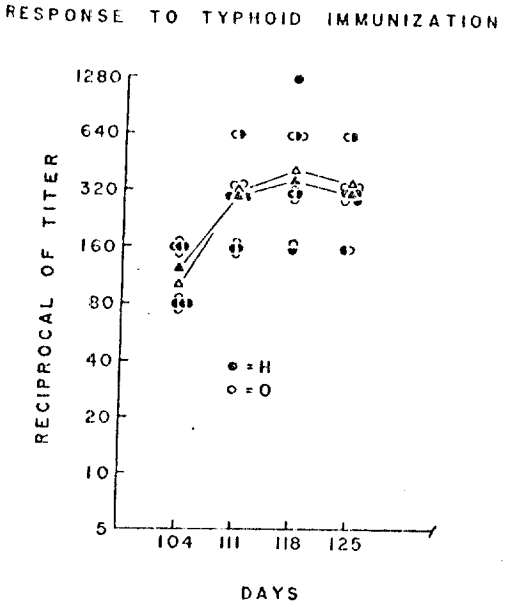

FIG. 4. Antibody response to typhoid antigen during end of depletion and the early phase of repletion was normal.

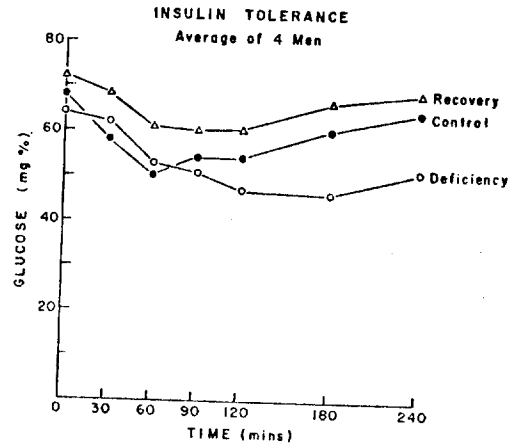

FIG. 5. Insulin tolerance curves during scurvy showed a statistically significant decrease in blood glucose at 2, 3, and 4 hr. *Subject R* became clinically hypoglycemic. These changes were reversed by repletion with ascorbic acid.

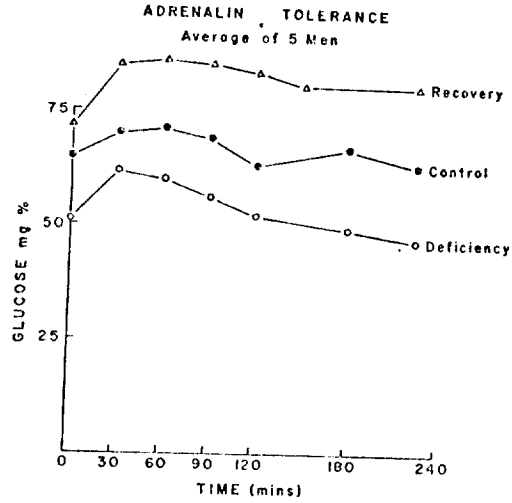

FIG. 6. Blood glucose values declined significantly during scurvy and rose again after administration of ascorbic acid, yet the glycogenolytic effect of adrenalin persisted.

of time. The British had estimated that their diets contained approximately 1 mg ascorbic acid in a day's ration, yet it required a much longer period of time for their subjects to develop the same signs and symptoms than those that appeared in our subjects (Table 6). These observations suggest the possibility that the British diet might have contained substantially more than the estimated 1 mg

TABLE 6
Comparison between Sheffield and Iowa City studies

	Sheffield study	Iowa City studies I and II
No change	Up to 119 days	Up to 29 days
Petechial hemorrhages (not perifollicular)		26 to 66 days
Follicular hyperkeratosis	82 to 149 days	45 to 100 days
Aching of limbs	149 to 168 days	64 to 96 days
Swollen or bleeding gums	163 to 254 days	38 to 105 days
Perifollicular hemorrhage	182 to 238 days	49 to 90 days
Acne	114 to 210 days	No change
Joint effusions	210 to 216 days	68 to 103 days
Dyspnea	210 to 228 days	63 to 90 days
Edema, slight to marked		33 to 101 days
Neuropathy		71 days
Sicca syndrome		58 to 123 days
Ocular hemorrhages		84 to 95 days

ascorbic acid in a day's ration. We know that a dose of 6.5 mg daily was barely enough to ameliorate scurvy in three subjects. We also observed that the catabolism of the vitamin was more rapid at the beginning of the period of deprivation when the subjects were deprived of the mineral supplement than it was later in the experiment when the subjects received an adequate mineral intake (10).

The Iowa City studies confirm the previous reports that the principal signs and symptoms of scurvy consist of follicular hyperkeratosis, hemorrhagic manifestations, fatigue, muscular aches and pains, swollen joints, swollen bleeding gums, and peripheral edema. Other little-recognized syndromes made their appearance and are reported elsewhere. In the first scurvy study (5), one of us (JH) noted the appearance of minute hemorrhages and small aneurysms in the bulbar conjunctivae. Ocular lesions are reported uncommonly in scurvy, perhaps as a result of the rather slow loss of ascorbic acid from the eye. Another little-known feature of scurvy was observed in the subjects participating in Scurvy II. This has been referred to variously as the sicca syndrome or Sjögren's syndrome (11). The occurrence of peripheral neuropathy in one man was an unexpected finding (17). His recovery was gradual and complete after restoration of ascorbic acid.

In this second study of scurvy in man, we have repeated the experiment conducted in Scurvy I (3), but extended it and achieved a more severe state of clinical scurvy, as evidenced by *subject H* who became almost unable to walk as a result of the rapid onset of arthropathy superimposed on bilateral femoral neuropathy. The onset of scurvy signaled a period of potentially rapid deterioration. The relatively short time that elapsed between the onset of illness in one subject (*H*) and the development of serious evidences of scurvy warns against procrastination in treatment of this condition.

Historical accounts of scurvy have repeatedly made mention of mental depression and other emotional changes occurring in this disorder (18). Psychological studies conducted by experimental psychologists from the U. S. Army Medical Research and Nutrition Laboratory in Denver did indeed confirm abnormalities in emotional responses. These results will be reported separately (14).

Administration of radioactively tagged L-ascorbic-1-^{14}C acid permitted us once again to observe the rate of destruction of this vitamin and to estimate its pool size in previously healthy men. Comparison of the pool size and the blood levels of ascorbic acid with the development of signs and symptoms was thus made possible. Repletion was accomplished at several dose levels ranging from 6.5 to 130.5 mg daily and once again demonstrated that a certain pool size must be attained before urinary excretion of the free reduced L-ascorbic acid occurs (10). Furthermore, the rate of recovery from signs and symptoms of scurvy seemingly was proportional to the repletion dose.

Although other investigators have reported abnormal vascular reactivity and vasomotor and structural abnormalities of autonomic ganglion cells in scorbutic animals, no previous studies of vascular reactivity have been reported in scorbutic man. In Scurvy II the appearance of cyanosis and edema of the lower extremities, incident to exposure in a cold room, triggered investigations that demonstrated abnormal vascular reactivity without evidence of significant depletion of endogenous norepinephrine or defective synthesis of norepinephrine (15). It was obvious that forearm vascular responsiveness to intraarterial norepinephrine and tyramine and to lower body negative pressure became abnormal. The mechanisms, however, are not yet clear. These abnormalities conceivably might help to explain the sudden death so often reported in historical accounts of scurvy.

Another interesting point relates to the differences between experimental and spontaneous scurvy. Scurvy, as it occurred historically and as it still appears in scattered areas throughout the world, generally is more severe and involves more systems than experimental scurvy. One might speculate that this results from multiple deficiencies or from climatic extremes plus a high level of physical activity or from associated infectious diseases. Nonetheless, the similarities between experimental and spontaneous scurvy are remarkably close.

In Scurvy I, we reported the characteristics of follicular hyperkeratosis, perifollicular erythema, petechial hemorrhages, and swollen bleeding gums. In Scurvy II, the same signs and symptoms were again present, but were more marked than in the first study. Joint swelling and pain made themselves evident in Scurvy II, but had not been observed in the subjects participating in Scurvy I.

The observations made in *subject H* demonstrate another curious function of ascorbic acid. During the time when he was developing dependent edema of his lower extremities, he had accompanying oliguria and a progressive rise in body weight. Subsequent to administration of enough ascorbic acid to reverse some of his scorbutic symptoms, he had prompt diuresis accompanied by weight loss and subsidence of edema. No doubt this phenomenon had been observed by others and presumably may have led to the oft-quoted statement that ascorbic acid has a diuretic effect (19). In this instance, it definitely did.

Nutritionists have endeavored to set arbitrary levels which would enable differentiation between well-nourished, adequately nourished, poorly nourished, and deficient individuals. In the case of ascorbic acid, great reliance has been placed on the Interdepartmental Committee on Nutrition for National Defense (24) Standards for Nutrition Surveys which are; high = > 0.4, acceptable = 0.2 to 0.4, low from 0.1 to 0.2, and deficient < 0.1 mg/100 ml. It is important to note in Figs. 1 and 2 that the subjects in Scurvy II had plasma values well above the deficiency level throughout the time they had frank scurvy. Furthermore, a comparison between plasma levels of ascorbate and pool sizes showed a very poor correlation. Whole blood values were somewhat more dependable in providing an estimate of pool size (10). In general, it is fair to say that scurvy appeared when the body pool size fell below 300 mg (10) and the whole blood level below 0.3 mg/100 ml.

The mild anemia may have resulted from the volume of blood drawn for laboratory purposes. There was little evidence of correlation between the severity of scurvy and the degree of anemia.

The changes in cholesterol are the opposite of those that one might expect from reports by Russian investigators of a reduction in serum cholesterol concentrations as a result of therapy with ascorbic acid (20). The men in the present study had a fall in serum cholesterol values probably as a result of the formula diet that contained no cholesterol and large amounts of polyunsaturated fats. At a time when they were being repleted, while still on a cholesterol-free diet, they had a significant rise in cholesterol levels. This rise became more marked at the close of the study when they were fed a diet of choice, high in cholesterol and saturated fats. Bronte-Stewart (21) also noted low serum cholesterol levels in scorbutic Bantus, and found that cholesterol

levels rose with the administration of ascorbic acid alone.

Changes in serum proteins have been reported in scorbutic animals to show a decrease in albumin and a rise in alpha-1 globulin (22). The similar fall in albumin in our subjects was accompanied by a rise in alpha-2 and gamma globulins (10). The observation in Scurvy I that nitrogen losses in the urine were greater than normal suggests that impaired protein metabolism is a feature of scurvy. The immune responses of scorbutic animals have been reported to be normal (23). This observation was confirmed in the present human study.

Our observations in a total of nine subjects with experimentally induced scurvy suggest that rather small doses of ascorbic acid can cure scurvy, given sufficient time. Certainly the administration of modest doses (66.5 mg) resulted in rapid disappearance of symptoms and signs of scurvy and in prompt repletion of the body pool. Since this vitamin is readily absorbed from the gastrointestinal tract there seems little reason to treat scurvy with parenteral ascorbic acid or with massive oral doses.

Summary

Six healthy volunteers from the Iowa State Penitentiary at Fort Madison, Iowa, participated in studies of human scurvy. They were hospitalized on the Metabolic Ward of University Hospitals in Iowa City, Iowa, and fed a diet totally devoid of vitamin C.

One of the men withdrew from the study because of personal reasons. The remaining five subjects developed clinical scurvy in 84 to 97 days, manifested by signs and symptoms of fatigue, hemorrhagic phenomena, swollen joints, swollen bleeding gums, follicular hyperkeratosis, muscular aches and pains, and emotional changes.

Urinary ascorbic acid rapidly declined to undetectable levels early in the course of depletion and blood levels progressively became too low to measure accurately. Serum protein abnormalities appeared that consisted primarily of a decrease in albumin and an increase in alpha-2 and gamma globulins. Other changes occurred in serum lipids.

Radioisotopic studies indicated progressive depletion of the body pools during the depletion phase of the study and repletion in proportion to the amount of ascorbic acid administered daily. This study confirms and extends the observations made in our earlier study that the full clinical syndrome does not appear until the normal body pool has been depleted to less than 300 mg.

The minimal amount of ascorbic acid necessary to prevent or cure scurvy appears to be slightly less than 10 mg daily. Once again our observations are in accord with those of the British Medical Research Council. Estimates of the optimal intake of ascorbic acid must be made on the basis of these data plus a knowledge of the biologic and physiological variables of mankind.

The authors wish to express their gratitude for the strong support provided by all members of the Nursing Service, the Laboratory Service, and Dietetic Service of the Metabolic Unit, Department of Medicine, University of Iowa Medical School, and by members of the Chemistry Division, U. S. Army Medical Research and Nutrition Laboratory. The continuing encouragement and support provided by various members of the U. S. Army Medical Research and Development Command, particularly that of Colonel I. C. Plough, were greatly appreciated.

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Ascorbic acid concentrations in leukocytes and selected organs of guinea pigs in response to increasing ascorbic acid intake[1]

M. O. Keith, M.Sc., and O. Pelletier, Ph.D.

ABSTRACT The concentration of ascorbic acid in leukocytes, adrenals, brain, spleen, and heart of growing male guinea pigs was investigated after the animals had received daily oral injections of L-ascorbic acid ranging from 0.2 to 10.0 mg/100 g body weight for 42 days while being maintained on an ascorbic acid-free semipurified diet. The concentration of ascorbic acid in leukocytes and organs increased most rapidly with increasing oral intake of the vitamin until the animals' requirement was reached. The dose-response curves were similar in shape for ascorbic acid in leukocytes and the organs, a similarity which was confirmed by high correlation coefficients. The results indicated that leukocytes reflect tissue concentration and hence vitamin C status in growing guinea pigs. *Am. J. Clin. Nutr.* 27: 368–372, 1974.

Scientific investigations have indicated that blood leukocyte concentration of ascorbic acid reflects the vitamin C status of humans more accurately than serum because of the less rapid drop in leukocyte ascorbic acid concentration with advancing deficiency (1, 2). Other early reports (3, 4) indicated that leukocyte ascorbic acid concentration could be used to show vitamin C status of humans.

Among numerous studies that have used vitamin C concentration in leukocytes as a measure of vitamin C status, two have shown low correlations between ascorbic acid intake and leukocyte ascorbic acid level in elderly patients (5, 6). This poor correlation casts doubt on the ability of leukocytes to reflect ascorbic acid status.

Chevillard and Hamon (7) showed that leukocyte ascorbic acid concentration corresponded to the concentration in the adrenals, bone marrow, and spinal cord of guinea pigs.

Although the ascorbic acid concentration has been shown to increase in the liver, spleen, kidney, brain, and heart with increasing intake of the vitamin by guinea pigs (8), no similarly controlled experiments have shown how leukocyte ascorbic acid concentration responds to increases in intake. This was the purpose of the present work.

Materials and methods

Male albino guinea pigs (supplied by High Oak Ranch, Goodwood, Ontario) were fed a natural pelleted diet (Guinea Pig Chow, Ralston Purina of Canada, Ltd., Woodstock, Ontario) and lettuce for 1 week followed by 1 week on a semipurified diet (Table 1) containing 1 g of L-ascorbic acid/kg.

Forty animals averaging 360 g body weight were then randomly allotted in groups of four to stock cages and fed the semipurified diet containing no ascorbic acid for 42 days. During this time, L-ascorbic acid was given orally at 0.2, 0.5, 1.0, 1.6, or 10.0 mg/100 g body weight daily. Each dosage level was given to eight animals. The ascorbic acid was dissolved in a solution containing 2 mg saccharin/ml to enhance the flavor of the vitamin solution and thus facilitate oral dosing.

The animal room was maintained at 22 C and 55% relative humidity with 12 hr of light (from 7 AM) and 12 hr of darkness (from 7 PM).

Feed and water were provided ad libitum; body weight was recorded weekly.

Each animal was orally dosed with 3 μCi ascorbic acid-1-^{14}C (New England Nuclear Canada, Dorval, Quebec) in 0.4 mg ascorbic acid (specific activity 1.667×10^4 disintegrations/min per μg ascorbic acid) 72 hr before being killed. The radioactive dose was given just before the regular ascorbic acid dose which was reduced by 0.4 mg for that day. No ascorbic acid was given on the day the animals were killed. The animals were anesthetized with ether.

Blood was collected from the abdominal aorta in a disposable plastic syringe and transferred into tubes containing 1 mg disodium-EDTA/ml blood. The blood was allowed to stand at room temperature for 10 to 20 min. Leukocytes and platelets were then separated from the whole blood by a method that employs a solution of bovine fibrinogen (9).

Approximately 5 ml blood was added to duplicate

[1]From the Food Research Laboratories, Health Protection Branch, National Health and Welfare, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2.

TABLE 1
Guinea pig diet

Component	g/kg diet
Vitamin-free casein	300
Sucrose	200
Corn starch	196
Alphacel	150
Corn oil	74
Salt mix (USP XIV)	40
Potassium acetate	25
Magnesium oxide	5
Vitamin mix[a]	10

[a] Provided in grams/kilogram diet: meso-inositol 2.000; niacin 0.200; para-aminobenzoic acid 0.100; calcium pantothenate 0.080; riboflavin 0.030; thiamin·HCl 0.020; pyridoxine·HCl 0.020; folic acid 0.010; biotin 0.010; vitamin B_{12} (3,000 μg/g) 0.040; vitamin D_2 (500,000 USP units/g) 0.003; vitamin A (20,000 USP units/g) 0.873; menadione 0.010; vitamin E (250 IU/g as DL-α-tocopherol acetate) 0.264; choline bitartrate 6.340.

20- by 150-mm test tubes each containing 5 ml of a solution of 6% bovine fibrinogen (Bovine Fibrinogen-Fraction 1, Nutritional Biochemicals Corp., Cleveland, Ohio) in 0.9% sodium chloride. The tubes were mixed by inversion 10 times and placed in a vertical position for 8 min. The top layers containing leukocytes and platelets were then removed by aspiration, pooled in a 15-ml conical, graduated centrifuge tube with stopper and thoroughly mixed. A 0.5-ml aliquot was diluted in 1 ml 0.9% sodium chloride and shaken momentarily; the leukocytes in this solution were counted (Coulter Counter, Model F_N; Coulter Electronics, Hialeah, Florida) immediately because they showed a slight tendency to clump with platelets if allowed to stand. The volume of the remaining leukocyte solution was recorded after centrifuging at 1,200 × g for 15 min in a clinical centrifuge; the clear supernatant was discarded. Approximately 1 ml of 2% metaphosphoric acid was added to the precipitate which was transferred to the vessel of a tissue grinder (Borosilicate glass vessel, 4 ml, size AA, with Teflon pestle; A. H. Thomas Co., Philadelphia, Pa.), using a stainless steel spatula and a Vortex (Vortex Jr. Mixer, Scientific Industries Inc., Queen's Village, New York) mixer. The total volume in the vessel was approximately 4 ml after rinsing the centrifuge tube with three portions of almost 1 ml 2% metaphosphoric acid. The homogenized solution was then returned to the centrifuge tube. The total volume approximated 8 ml after rinsing the vessel with 2% metaphosphoric acid. The exact volume was recorded after the sample was centrifuged at 1,200 × g for 15 min. The clear supernatant was frozen in polyethylene vials until assayed for TAA.

The adrenals, brain, heart, and spleen were excised, weighed, wrapped in parafilm, and immediately frozen on Dry Ice. They were later thawed and homogenized the same day in 4% metaphosphoric acid, which was diluted to 2% in the final assay solution. The homogenates were filtered; the brain tissue required centrifugation at 1,100 × g before filtration. Homogenates of the organs and leukocytes were frozen until assayed for total ascorbic plus dehydroascorbic acid (TAA) by the method of Pelletier (10).

Radioactivity of the extracts was measured on a Packard Tri-Carb Liquid Scintillation Spectrometer Model 3320. PPO (2,5-diphenyloxazole) and POPOP (1,4-*bis*[2-(5-phenyloxazolyl)]-benzene) were dissolved in toulene and mixed with Triton X-100 as described by Patterson and Green (11). Efficiency of counting was determined in each extract using toluene-^{14}C as the internal standard.

An analysis of variance and Duncan's Multiple Range Test were performed on the data. Correlation analysis of the mean TAA concentration, disintegrations per minute (dpm), and specific activity in leukocytes with the mean TAA concentration, DPM, and specific activity in each organ was carried out.

Results

The average body weight gain per day ± the standard deviation for all animals was 5.78 ± 1.37 g and was not affected ($P > 0.05$) by ascorbic acid intake.

The concentration of ascorbic acid in leukocytes, adrenals, spleen, brain, and heart increased with an increased dose of ascorbic acid (Fig. 1). Increases in TAA concentration with an increased dose were often significant ($P < 0.05$) (Table 2).

The decrease in specific activity with increasing ascorbic acid dose was similar in leukocytes and organs (Fig. 2). Brain tissue showed lower activity than the other organs.

Correlation coefficients for TAA, carbon-14 activity, and specific activity in leukocytes with TAA, carbon-14 activity, and specific activity in the organs were high (Table 3).

Discussion

The increasing TAA concentration in adrenals, spleen, brain, and heart with increasing ascorbic acid intake (Table 2) has been previously demonstrated by Kuether et al. (8). The corresponding increase in leukocyte TAA concentration has not been demonstrated previously (Table 2, Fig. 1). The high correlation coefficients of leukocyte TAA, dpm, and specific activity with TAA, dpm, and specific activity in each organ (Table 3) emphasize the similarity in response to increasing ascorbic acid dose by leukocytes and the organs studied. Chevillard and Hamon (7) demonstrated a tendency for leukocyte ascorbic acid level to show a similar response to ascorbic acid dose to that shown by adrenals, bone marrow, spinal cord, and visceral ganglia in guinea pigs.

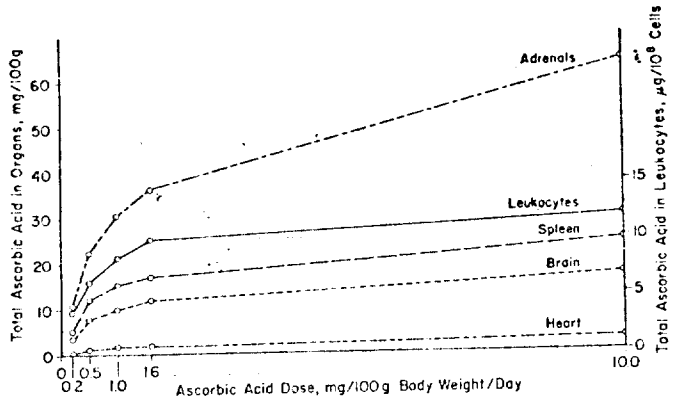

FIG. 1. Total ascorbic acid in leukocytes and organs of guinea pigs receiving oral doses of ascorbic acid. Refer to Table 2 for significant differences between dose levels.

TABLE 2
Total ascorbic acid concentration in leukocytes and organs of guinea pigs*

	Ascorbic acid dose, mg/100 g body weight				
	0.2	0.5	1.0	1.6	10.0
Leukocytes, µg/10⁸ cells	3.83 ± 1.38^{a}	6.40 ± 1.55^{ab}	8.48 ± 2.86^{bc}	10.10 ± 4.92^{cd}	12.04 ± 3.24^{d}
Adrenals, mg/100 g	10.65 ± 1.67^{a}	22.42 ± 6.22^{b}	30.65 ± 8.70^{bc}	36.60 ± 8.73^{c}	64.11 ± 16.95^{d}
Spleen, mg/100 g	5.30 ± 1.58^{a}	12.00 ± 2.47^{b}	15.15 ± 4.56^{bc}	16.90 ± 3.29^{c}	24.52 ± 5.38^{d}
Brain, mg/100 g	3.49 ± 0.61^{a}	7.76 ± 1.74^{b}	9.80 ± 1.66^{c}	11.92 ± 1.19^{d}	17.06 ± 1.52^{e}
Heart, mg/100 g	0.48 ± 0.19^{a}	1.26 ± 0.21^{b}	1.56 ± 0.57^{bc}	1.71 ± 0.46^{c}	2.90 ± 0.48^{d}

* Each mean ± SD is based on eight observations except for leukocyte TAA concentration at a dose of 0.2 mg/100 g body weight, n = 7; and at 10.0 mg/100 g body weight, n = 6. Means with the same superscript are not significantly different ($P > 0.05$) at each site of measurement.

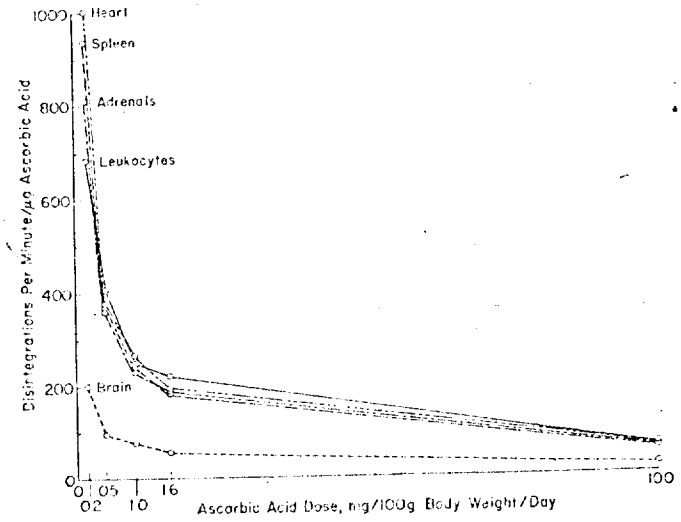

FIG. 2. Specific activity of ascorbic acid in leukocytes and organs of guinea pigs 3 days after receiving ascorbic acid-1-^{14}C.

TABLE 3
Correlations of TAA, disintegrations per minute (dpm), and specific activity in leukocytes with TAA, DPM and specific activity in organs

Leukocytes correlated with:	TAA	DPM	Specific activity
Adrenals	0.96	0.98	0.99
Spleen	1.00	0.96	0.98
Brain	0.98	0.96	0.99
Heart	0.97	0.98	0.98

The concentration of TAA in leukocytes increased gradually until the intake of the vitamin approximated the animals' daily requirement of 1.6 mg/100 g body weight (12). At 10 mg/100 g body weight, ascorbic acid continued to accumulate but to a lesser extent. At daily intakes less than 1.6 mg/100 g body weight, the slope of the line of leukocyte ascorbic acid concentration versus intake is greater and therefore the most sensitive.

In an adjunct to the main experiment, the mean leukocyte ascorbic acid concentration in seven animals showing scorbutic signs of varying degrees of severity was 0.9 $\mu g/10^8$ leukocytes (unpublished observations). This indicates that the leukocyte concentration will reflect nutritional status of the vitamin when the daily intake is less than 0.2 mg/100 g body weight in guinea pigs (Fig. 1).

The dose-response curves showing TAA concentration in leukocytes, adrenals, spleen, brain, and heart (Fig. 1) and specific activity in the same tissues (Fig. 2) indicate that leukocyte ascorbic acid concentration reflects not only the ascorbic acid stored in the organs, but also organ activity in utilizing recently ingested ascorbic acid represented by ascorbic acid-1-^{14}C. The guinea pigs utilized little of the ascorbic acid above the daily requirement of 1.6 mg/100 g body weight.

Several investigators have successfully used leukocyte ascorbic acid concentration to show differences in nutritional status of vitamin C, but it is recognized that the technique of measuring ascorbic acid concentration in leukocytes is complicated and may give variable results (13).

Loh and Wilson (14) and Loh (15) suggested that leukocyte ascorbic acid concentration alone does not reliably reflect tissue status and that both plasma and leukocyte ascorbic acid concentrations should be used to do this.

Various methods have been used to isolate leukocytes from whole blood. The method of Denson and Bowers (16) using dextran has been most widely used. The fibrinogen method requires less time than the dextran method to separate leukocytes from whole blood and the resulting leukocyte solution is less contaminated with erythrocytes. Leukocytes can take up ascorbic acid from the surrounding medium (17). Erythrocytes can contain significant amounts of ascorbic acid (1, 18). Erythrocyte contamination and the time required for separation of leukocytes from the blood are variables that could influence the leukocyte ascorbic acid concentration.

Present results indicate that leukocyte ascorbic acid concentration can be used to indicate nutritional status of the vitamin. However, comparisons of data between laboratories will continue to have limited significance until an efficient standard procedure for leukocyte isolation and measurement is used.

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Source: Int. J. Vitamin Nutr. Res. 42: 528-544, 1972
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Tolerance of High Ascorbic Acid Doses

W. F. Körner and F. Weber (Division of Vitamin and Nutrition Research, F. Hoffmann-LaRoche & Co. A. G., 4000 Basle, Switzerland)

Summary

"A review is given of the toxicity and tolerance of ascorbic acid based on data from the literature and results from recent investigations. Critical evaluation of the numerous clinical and experimental findings gave no indication of any effect prejudicial to health caused by vitamin C, even if administered in high doses. Occasionally, slight gastro-intestinal disturbances due to a laxative effect of the ascorbic acid, and in exceptional cases, allergic symptoms were observed. Nevertheless, the frequency of these phenomena is not greater than that of the signs of intolerance caused by other essential nutrients."

Introduction

For almost 40 years, vitamin C has been used in medicine as a remedy (drug), after Reichstein *et al*. (122) and Hirst, Haworth *et al*. (12, 65, 67) had carried out almost at the same time (in 1933) the synthetic preparation of ascorbic acid. The administered doses of vitamin C range from 50 mg to 5 g, and in special cases even amount to 10-20 g and more per day, since ascorbic acid is generally considered as a non-toxic substance (2, 45, 81, 84, 117). As a result of the steadily increasing "health consciousness" of large segments of the population during the past 10 years, harmless natural products, which are used as drugs in the same way as vitamin C, have assumed increasing importance outside the boundaries of medical practice.

As long as the intake of vitamin C takes place within the range of the recommended daily dosage (39, 44, 43), no problems naturally arise in regard to the tolerance of ascorbic acid. At the same time, the daily vitamin C intake with food can vary within wide limits, i.e. in the the range of several hundred milligrams. This intake can even occasionally exceed the magnitude of 1 g ascorbic acid per day, when one takes into

account that, for example, 100 g of black currant berries contain up to 400 mg (according to their origin), the same amount of red pepper contains up to 235 mg, and 100 g of tomatoes contain up to 100 mg vitamin C.

2. Body Pool and Requirement

The body pool of an adult was determined at 20-50 mg/kg BW (body weight), the daily catabolism under non-stress conditions at about 3% of the amount of vitamin C present in the body (11, 14-16, 36, 66, 157, 168). Accordingly, the daily requirement necessary to maintain tissue saturation is equal to over 1 mg/kg BW, and was determined for an adult at 1-1.7 mg/kg BW (19, 156). For children and adolescents, the corresponding vitamin C requirements amount to 6.5-7 mg/kg BW (126, 143), and are definitely higher, apparently on the basis of a stronger catabolism due to a higher metabolism. These figures do not include (take into account) losses, such as arise, for example, under stress situations. If one considers the above upper limit of the human body pool (50 mg/kg BW) for ascorbic acid, then we find that it is almost identical with that of the guinea pig (54 mg/kg BW) (49), which, in case of a free selection of food, however, takes up (consumes) a daily amount of about 50 mg vitamin C (corresponding to ab. 17 mg/kg BW) (48). The gorilla, which like primates and the guinea pig is not endowed with an endogenous vitamin C synthesis, also consumes under the same conditions with its daily food larger amounts of ascorbic acid; Bourne (22) calculated for the gorilla a daily intake of 4.5 g, corresponding to ab. 36 mg/kg BW for an animal weighing 125 kg. Other members of the animal kingdom, which are endowed with their own synthesis of vitamin C and thus are able to satisfy their requirements independently of the intake with food, exhibit higher tissue concentrations of ascorbic acid. Thus, the body pool of the rat, with a daily synthesis of 26 mg/kg BW, is twice as high as that of the guinea pig and man, and is namely equal to 107 mg/kg BW (25, 49).

The reason why man, apes and guinea pigs are dependent upon an external (exogenous) supply of vitamin C is due to the hereditary lack of at least one enzyme system (L-gulono-γ-lactone oxidase). For this reason, Stone (148-150) is of the opinion that the lack of an endogenous vitamin C synthesis should be considered as an "inborn error of metabolism". This specific genetic defect, which, in case of average vitamin C supply (as compared to species with their own vitamin C synthesis), is expressed in the form of low ascorbic acid concentrations in the blood, could thus be

designated as a so-called "hypoascorbinemia", which under extreme conditions results in scurvy. According to Stone (148), an exogenous supply of ascorbic acid in the same amount as the one endogenously synthesized would represent a complete correction of this genetically induced disease. According to this concept, the optimal ascorbic acid requirement of man does not differ greatly from that of other mammals which are capable of satisfying their requirement by means of their own synthesis, and this even under stress situations (3, 85, 89, 99), where the requirement is considerably higher. For example, the rat produces under stress conditions (probably by enzyme induction) up to 215 mg/kg BW/day (30).

3. Toxicology

In view of the increasing tendency, manifested in recent years, to take vitamin C in gram doses over a longer period of time, the question constantly raised about the possible toxicity and tolerance of this natural product is understandable. The following findings are available in this respect.

3.1. Acute Toxicity (LD_{50}/24 hours)

Animal species	Mode of administration				Literature reference
	Oral	Subcutaneous	Intravenous	intraperitoneal	
Mouse	8021	5000	1058	2000	Bachtold, H.P., Demole, V., 1934, Gaudiano, et al.
Rat	>5000	5000	1000		Demole, V., 1934
Guinea pig	>5000	>1000	500	2000	Demole, V., 1934
Rabbit	>2000	>1000	>1000	>1000	Demole, V., 1934
Cat	>1000	> 500	> 500		Demole, V., 1934
Dog	>5000	> 200	> 200		Demole, V., 1934

Demole (33) found that the guinea pig can tolerate a daily dose, without any side effects, equal to 500-1000 times the daily requirement (4-5 mg/kg BW).

3.2. Subchronic Toxicity (LD_{50})

Animal species	Mode of administration (mg/kg/day)				Duration (days)	Literature reference
	Oral	Subcutaneous	Intravenous	intraperitoneal		
Mouse	8021				10	Bachtold, H.P.
Mouse			1058		10	Bachtold, H.P.
Rat	> 6500				6	Lang, K.
Rat		>>600			28	Gineste, P.J.
Guinea pig	> 8900				6	Lang, K.
Guinea pig		500			7	Demole, V. 1935
Guinea pig				100	16	Villard, P. et al.
Rabbit			500		7	Demole, V. 1935
Rabbit				100	16	Villard, P. et al.
Cat			> 500		9	Edwards, W.C.
Dog	100				7	Demole, V. 1935
?			> 2000		3	Leveque, J.I.

Burlina and Lippi (23) studied the effect of a daily subcutaneous administration of 100 mg/kg BW ascorbic acid, given for 6 days, on the liver function of the guinea pig. As compared to controls, they found, along with a moderate decrease of the alkaline phosphatase and lipase activity, an increase in the amount of ribo-and desoxyribonucleic acids. The liver parenchyma exhibited slight regenerative changes. The distribution and concentration of fats, lipids and neutral polysaccharides were unchanged. A reduced glycogen content was found in the liver lobes. Vozar (164) was able to confirm the absence of changes in the concentration of fats and neutral fats in the liver after a daily administration, for 20 days, of up to 15 mg/kg BW vitamin C in the guinea pig, whereas a definite increase in the fat content was observed in the rat.

Chronic Toxicity

Diacono (35) carried out tests, over a period of 4 months, on young rabbits and guinea pigs with parenteral daily doses of 100 mg ascorbic acid per animal (corresponding to 200 and 500 mg/kg BW). In the rabbit, only transient subconjunctival skin hemorrhages were found, without any kind of other symptoms, and particularly no disturbances in the different organ systems. Growth development and weight gain were normal. The guinea pig only showed a slight olive-brown coloration of the sclera, a disruption (disturbance) of hair growth, diarrhea, and a relative drop of the blood calcium content. No disturbances in the skeletal system were found during X-ray examination.

During the past 10 years, the question of the chronic tolerance of high vitamin C doses became important, and appropriate animal tests were carried out by different groups of workers. Kieckebush et al. (81) and Lang (88) performed 6-week long toxicity tests on rats. Daily oral administration of 6.5 g/kg BW ascorbic acid (calculated on the basis of the initial weight of the animals) did not disclose any injurious effects whatsoever. The weight gain, mortality, protein efficiency, food intake and histopathological findings did not differ from the figures found on controls. On the other hand, daily doses greater than 25 g/kg BW were found to be definitely toxic. From these results, the above authors draw the conclusion that the not yet toxic limit dose for the rat is equal to 10 g/kg BW. On the basis of this figure, a safety range (margin) of 1:700 is obtained for man at a daily dose of 1 g ascorbic acid (corresponding to 14-15 mg/kg BW). Daily doses of 8.9 g/kg BW, given during 14 weeks, were also found to be nontoxic for the guinea pig (80, 88).

A further indication for the good tolerance of ascorbic acid is provided by new data obtained in a study of chronic toxicity (151). Three groups of 26 rats of both sex each received, as compared to an adequate control group, a daily oral dose of 1, 1.5 and 2 g/kg BW ascorbic acid added to a standard diet for 2 years. All dosages were tolerated without any disturbances of the general condition and growth development. No differences were noted in the mortality rates, and no deviations from the untreated controls were observed during the course of hematological, blood chemical and histological examinations. The amount tolerated without any symptoms of 2 g/kg/day, noted in the highest dosage group, would thus correspond in a 70-kg man to

the daily intake of 140 g ascorbic acid.

4. General Tolerance in Man

Numerous studies have been published during the course of many years on the systemic use of high vitamin C doses in children, adults and old people (17, 27, 37, 56-58, 68, 70, 82, 86, 93, 107, 121, 140, 144, 146, 161, 163). Among others, daily doses of 3-6 g were given orally for 15 months, up to 35 g was injected intravenously during 1.5 months, and up to 80 g was given orally for 6-10 days without observing any side effects or any hypersensitivity reactions. In one case, a patient suffering from Kahler's disease (multiple myeloma) received, along with other therapeutic treatment, a daily dose of 1 g ascorbic acid up to a total dose of 2.37 kg. No side effects were observed, which could have been attributed to the vitamin C medication (142).

However, side effects of minor importance have been described by other authors; such cases always involve isolated observations and not controlled studies, so that proof of a causal relationship with ascorbic acid administration is usually lacking.

4.1. Gastric and Intestinal Disorders

As a result of a certain laxative effect of high ascorbic acid doses, diarrhea sometimes occurs, which can be accompanied by increased intestinal peristalsis and gastralgia (pain in the stomach) (63, 112, 124, 137, 160, 165, 166). These symptoms appear to be more strongly expressed during oral intake of free ascorbic acid than after intake of sodium ascorbate, which explains why these symptoms arise only after a considerably greater dosage when vitamin C medication is given in the form of effervescent tablets (containing $NaHCo_3$). However, Greer (57), who treated 5 poliomyelitis patients with daily oral doses of 50-80 g vitamin C for 6-10 days, observed, at this excessively high dosage, only a light gastroenteritis and anal irritation. Widenbauer (165), in connection with his observations on nursing infants, points out that this side effect of ascorbic acid can be used advantageously in constipation by stimulation of peristalsis.

4.2. Vagotonic Reactions

In a series of patients with special vegetative characteristics (traits), isolated vagotonic reactions have been noted after ascorbic acid administration, which were manifested in the form of hyperexcitability, restlessness,

insomnia, heat sensation, flushes (congestion), headache, slowing of the pulse, dermographia and edema formation (4, 46, 55, 59, 73, 94, 101, 124, 137, 139, 165, 166). These symptoms were mainly observed in nursing infants and growing children, and also occasionally in adults. The gastric sensations observed sometimes by Hasselbach (63) during treatment of tuberculosis patients, following intravenous injection of sodium ascorbate, can probably be classified in this group.

These vagotonic reactions apparently take place independently of the dose level, and appear to be a little more marked in children than in adults. The almost always described reduction of the pulse rate averaged 10 beats/minute and was sometimes accompanied by a rise in blood pressure (4). On the contrary, Hochwald (69) noted a hypotensive effect in man in the course of the administration of "high" doses of ascorbic acid. The lowering of the systolic blood pressure observed by him amounted, in normotensive persons, to about 20 mm Hg after intravenous injection of 500 mg sodium ascorbate. Contrary to the frequently described stimulating (exciting) effect (insomnia) is the report of Maurer et al. (95), who successfully administered over a longer period of time a daily dose of 1-3 g vitamin C to patients suffering from insomnia.

4.3. Questionable Side Effects

Other side effects, which were rarely observed during the course of vitamin C therapy, are abnormalities in menstruation, in the sense of alterations in the time and amount of menses (63, 64, 158) and urine retention (60). Papayanopulos and Schroeder (116) found, in a series of patients with various diseases accompanied by a thrombocytopenia, a significant increase in the thrombocyte count after parenteral daily administration of 500 mg vitamin C for 1-7 days, and considered this thrombocytosis as the expression of a stimulation of the blood-forming bone marrow resulting from the pharmacological effect of ascorbic acid. An increase in thrombocytes was also found by Rietschel and Mensching (124, 125) in a series of healthy infants and young children after 9 to 20 days during daily supply of 100-200 mg vitamin C, and also in adults which had received 500 mg vitamin C daily for about one week. After the vitamin C medication was discontinued, the increase in thrombocytes subsided (regressed) again. The above authors consider this effect as the result of a vitamin C overdosage. These findings are contrasted with the observations made by Gertler et al. (46): 16 patients with lupus vulgaris (tuberculosis of the

skin), aged from 15 to 65 years, received a daily intravenous injection of 1 g sodium ascorbate for 1-6 months. After an initial slight rise of the blood pressure by 30-50 mm Hg, an increase of the blood sedimentation rate (BSR) by 50-20 mm, an increase in the number of granulocytes, eosinophils and thrombocytes (up to 400,000 per microliter) during the first and second week, a gradual drop of the blood pressure back to normal values, a return of the BSR to its initial value, and a reduction of the leucocyte and thrombocyte count back to normal or below normal limits was noted during the third week.

5. Allergies

As is known for numerous drugs and natural products, vitamin C can also induce allergic reactions in rare cases, which are manifested mainly as rubeola (rubella)-like, morbilliform and scarlatiniform erythemas, urticaria and occasionally edemas (61, 114, 165-167). Among the 9 cases reported by Widenbauer (165), including 5 adults and 4 infants, there were 2 cases, in which an idiosyncrasy towards quinine was diagnosed from the case history, and in another an idiosyncrasy towards atropine, strawberries and crustaceans. A further patient, who showed an allergic reaction to both citrus fruit and ascorbic acid, is mentioned by Campbell (26). Rust (129) was able to identify in 7 cases positive skin reactions to ascorbic acid with the scarification test, and Panzani (115) was able to identify such reactions in one case.

In a 40-year old female patient with thalassemia, in whom every common illness induced a hemolytic crisis in recent years, a case history inquiry disclosed that this patient had taken, prior to every such crisis, "high" doses of vitamin C for the prevention of colds. After she stopped taking ascorbic acid, no such crises occurred (50). On the other hand, Choremis and Gregoriu (29) found in children with thalassemia a relative deficiency of vitamin C. Loading tests with 50 mg/kg BW ascorbic acid showed that these patients exhibited a considerably greater retention of the administered vitamin C than did control subjects.

6. Catabolism of Ascorbic Acid

The principal metabolic products (metabolites) of ascorbic acid which are excreted, along with the unaltered vitamin, are dehydroascorbic acid, 2,3-diketogulonic acid and oxalic acid. The percentage fractions of these

metabolites vary from one species to another and also depending on the amount of ascorbic acid supplied. The guinea pig excretes up to ab. 40% of an administered vitamin C dose as CO_2 within 24 hours; a further 7% is eliminated as oxalate, and ab. 3% as ascorbic acid, dehydroascorbic acid and 2,3-diketogulonic acid in the urine. Excretion in the feces amounts to barely 1%. Only after administration of a very high vitamin C dose (150 mg/kg) does the excretion of ascorbic acid and its metabolites in urine increase up to 40% of the amount administered, whereby the oxalate fraction remains unchanged (constant) at ab. 7% of the administered dose (1, 24). Rats excrete about 40% of the endogenously synthesized ascorbic acid as such or as its metabolites with the urine (132). In contrast to the guinea pig, ascorbic acid is not broken down to CO_2 in the human organism, and it is excreted mainly in the urine. This total excretion amounts to about 3% of the body pool and is divided into ascorbic and dehydroascorbic acid (ab. 25%), 2,3-diketogulonic acid (ab. 20%) and oxalate (ab. 55%) (66). About half of the average daily excretion of 35 mg oxalate probably originates (is supplied) from the ascorbic acid catabolism (78). The amount of oxalate formed from ascorbic acid is, however, limited to a certain extent, since practically no increase in oxalate excretion was noted after daily administration of 4 g ascorbic acid. Only after a daily administration of 9 g acid did the oxalate excretion increase on the average to 95 mg/24 hrs (86, 152). In this connection, long-term tests were carried out by Takiguchi et al. (153, 154) on 30 healthy test subjects, which received a daily oral dose of 1-2 g ascor ascorbic acid during 90 to 180 days. The oxalate excretion, which prior to the test period averaged 29.3-29.8 mg/24 hrs, was not increased by the chronic vitamin C administration. In the urine, ascorbic acid was found as the main fraction, along with smaller amounts of dehydroascorbic acid and 2,3-diketogulonic acid. Earlier tests with daily doses of 250 and 500 mg, and also 2, 4, 8 and 9 g, during one week showed that only at a daily dose of 8 g ascorbic acid the oxalate excretion increased two-fold, and at 9 g/day it increased to 3 times the normal value (86). These findings agree with those reported by Ryoichi (130), El-Dakhakhny and El-Sayed (40), and McLaurin and Beisel (98). These data thus indicate that the intake of large doses of ascorbic acid does not result in a pathological hyperoxaluria,

which is characterized by oxalate excretion values of 300-400 mg/24 hrs (138).

Various other studies on patients with primary hyperoxaluria have shown that ascorbic acid medication does not even lead to an increase of an oxalosis which is already present. Thus Atkins et al. (10) administered labeled ascorbic acid to 2 patients and determined during 100 days the oxalate excretion in 24-hour urine. The oxalate fraction originating from vitamin C catabolism amounted to only 10% of the total oxalate excretion. It was concluded from this fact that externally supplied ascorbic acid does not cause an increase in a hyperoxaluria already present. Similar are the observations made by Munzingor et al. (105), who did not note any changes whatsoever in the oxalate excretion of a 4.5-year old child with nephrolithic (nephrolithiasis) oxalosis receiving a daily dose of 1 g ascorbic acid for 5 days. Only Hosli et al. (71) found in a 9-year old girl with an oxalosis detected by biopsy, after administration of 2 g ascorbic acid for 5 days, a definite increase of the previously already pathologically higher oxalate excretion in urine, whereas in 2 healthy children and one girl with urolithiasis resulting from an idiopathic hypercalcinuria the excretion values of oxalic acid remained unchanged after 3 g vitamin C.

7. Acid-Base Balance (Equilibrium)

The question arises as to whether a high dosage intake of vitamin C can cause a disruption of the acid-base balance in the sense of a hyperacidity (overacidification). Normally, there is an equilibrium in arterial blood between base and acid equivalents, which results in a pH of 7.38-7.42 with a total base and/or acid content of 151 mval/l serum. Of this amount, organic acids make up only 6 mval, i.e. barely 4%; the alkali reserve (standard bicarbonate) amounts, however, to 22-28 mval HCO_3^-/l.

The strength of an acid is characterized by its dissociation, i.e. the stronger the acid, the smaller are its dissociation constants (pk). The values for both constants for ascorbic acid are equal to 4.25 (9,18) and 11.79 (20, 21, 108, 109). For comparison purposes, the tribasic citric acid, with pk values of 3.0, 4.29 and 5.49, and lactic acid with a pk value of 3.90 exhibit a considerably higher degree of acidity. The same also applies to β-hydroxybutyric acid and acetoacetic acid, which cause a keto-

acidosis in diabetic coma.

Studies available sofar indicate that the intake of vitamin C contributes only to a insignificant extent to an acid loading of the organism; this fact becomes particularly clear when we keep in mind that considerable amounts of other carboxylic acids (malic and citric acids) are often absorbed with the food. In a healthy person, after loading with 3-6 g ascorbic acid, the total acid excretion in the urine, and therefore also the excretion of NH_4^+, exhibits only a moderate increase; the titratable acidity and the pH value remain unchanged (62, 106, 107, 159). The alkaline pH of 24-hour urine, observed under pathological conditions (e.g. in chronic urinary tract infections), can be shifted to the acid side already by a slight acid loading of the urine such as can be achieved by ascorbic acid excretion (97, 145). On the other hand, the pH of blood remains unchanged after a single dose of 3-6 g vitamin C (106, 107) or a daily administration of 1 g sodium ascorbate for 14 days (83). No changes were also noted in the CO_2 partial pressure and the standard bicarbonate (alkali reserve) of blood. Thus, the assumption expressed by Perrault et al. (118) that a prolonged vitamin C intake could cause acidosis is also not supported by the facts.

8. Electrolyte Metabolism

Ferrini et al. (42) found in rats which received daily for 10 days 10 mg (corresponding to ab. 60 mg/kg BW) of ascorbic acid a definite increase of the intracellular calcium concentration, whereas sodium showed a slight decrease. In plasma, on the other hand, the concentrations of both electrolytes remained unchanged. Similar findings are reported by Pokras'on and Sokolova (119), according to which daily intravenous injections of 35 mg/kg BW ascorbic acid during 7 days in vitamin C-deficient guinea pigs increased the K^+ concentration in the blood, brain and myocardium (heart muscle), while the Na^+ and Cl^- concentrations in these tissues remained unchanged. The observations of Murphy and Zelman (106), according to which daily doses of 3-6 g vitamin C in man should reduce the Na^+ excretion in urine by about 50% which would correspond to a noteworthy retention of Na^+, could not be confirmed during reexamination (62).

Whereas ascorbic acid, therefore, does not affect the electrolyte balance in a negative sense, certain disturbances can possibly be caused

by auxiliary products. Thus, a certain amount of sodium bicarbonate is required to achieve the effervescent effect of effervescent vitamin C tablets. In general, an effervescent tablet contains ab. 1 g $NaHCO_3$ per gm of vitamin C (100), i.e. 1 millimole (mMole) of $NaHCO_3$ corresponds to 0.93 mMole acid. The amount of Na^+ (11.9 mval) added to 1 g vitamin C in an effervescent tablet is equivalent to the Na^+ amount present in ab. 0.7 g common salt (sodium chloride). This amount of common salt equivalents can be disregarded even when 4 effervescent tablets of 1 g vitamin C are taken daily by a healthy person, since in the average diet the daily intake of Na^+ varies between 8 and 12 g sodium chloride equivalents. In the case of patients on a diet low in common salt, on the other hand, it would be significant to take the above fact into account when using high-dosage vitamin C medication in the form of effervescent tablets, in order not to exceed the daily amount of 3 g common salt (equal to 51 mval Na^+). Along with 1 g vitamin C effervescent tablets, tablets are also available commercially which contain about 1/4 of the vitamin C dose and the same sodium bicarbonate content of 1 g. If one wants to supply a larger amount (gram doses) of vitamin C to the organism with these relatively low-dosage administration forms, the uptake of common salt equivalents will consequently be increased several times, and this could lead to detrimental results in patients on a low sodium chloride diet. In principle, this problem is encountered in all effervescent powders used for the preparation of sparkling lemonades, the main component of which is sodium bicarbonate.

9. Blood Coagulation

In scurvy, blood coagulation is disrupted as a result of the lack of available coagulation factors and particularly in view of the lowered thrombocyte count (5-7, 31, 72). According to Sahud and Aggeler (131), there is direct relation between the extent of thrombocyte aggregation and the vitamin C content of the platelets. Therefore, ascorbic acid is an essential factor for a normal blood coagulation. Aptekar and Lorie (8) observed, after high dosages of vitamin C, in man and rabbits a shortening of the coagulation time. On the other hand, this finding could not be confirmed in other studies (75, 96). McCraw and Desmarais (96) found in the guinea pig, as a vitamin C effect, only a shortening of the bleeding time. According to results of animal tests (141) and accidental observations

on man (127, 155), vitamin C is believed to interfere with anticoagulants of the dicumarol type and to abolish in part the inhibition of coagulations. However, Hume et al. (74) found in 5 patients, who had been treated for a long time with warfarin, that a daily dose of 1 g vitamin C given for 14 days had no effect on the coagulation status. The opposite result was obtained in tests on guinea pigs (32), namely that, in case of vitamin C deficiency, the coagulation time after warfarin application, is greatly increased (prolonged) and the mortality rate of the animals is significantly higher. The pattern and rate of excretion of warfarin metabolites, however, remained unaffected both by a lack of vitamin C and administration of the vitamin. In the adrenal gland of the rat, the concentration of ascorbic acid remained unchanged after administration of warfarin (28).

10. Does Prolonged Intake of High Vitamin C Doses Result in a Higher Requirement?

It is known from numerous studies that products supplied to the body can effect the induction of microsomal enzymes, which in turn (on their part) promote the catabolism and elimination of these products. In the case of ascorbic acid, this question arose in connection with the observations of Jakowlew (76) during the course of the siege of Leningrad during World War II. The impression was gained at that time that those members of the population who were better fed suffered more frequently from scurvy, during the hunger period, than those who were poorly fed, although no exact data are available on the level of food intake and vitamin C supply in these two groups of the population. In a subsequent experimental study, Jakowlew (76, 77) attempted to find a scientific basis for his previous observation. He gave guinea pigs a daily dose of 5 mg ascorbic acid (corresponding to ab. 15-20 mg/kg BW) for 6 months; a second group of animals received a dose of vitamin C twice as high (10 mg). The animals were then placed on a scorbutigenic diet (causing scurvy). In the low-dosage group, animals died on the average after 33 days, while in the group receiving 10 mg/animal, the animals died after 30 days. This difference is not significant; in addition, there is lacking a true control group kept on a standard diet. On the basis of the same hypothesis, Gordonoff (51-54) carried out similar tests. Guinea pigs on a standard diet received in addition 500 mg ascorbic acid per day as sodium ascorbate (corresponding to ab. 1.5/kg BW) subcutaneously for 4 weeks. The controls (also on a standard diet) were untreated. At the

end of the treatment period, all animals were placed on a scorbutigenic diet, and the time until the appearance of scurvy symptoms was determined. The results, however, cannot be considered as conclusive, since the vitamin C group was exposed to a sort of prolonged (long-term) stress as a result of the daily subcutaneous injections, as opposed to the controls. In addition, the amount of vitamin C administered lies in the vicinity of the toxicity limit for the selected mode of application. On the other hand, the requirements of a controlled test were fulfilled by Kieckebusch (80) and Lang (88) in reexamination (checking over) of the results obtained by Gordonoff. In this test, the guinea pigs received, together with a standard diet, oral doses of vitamin C of 1.7-9.5 g/kg BW in the form of sodium ascorbate for 14 weeks, followed by administration of a scorbutigenic diet. All other conditions were the same for animals of the test and control groups. The high vitamin C doses added to the diet did not exhibit any kind of negative effects with respect to growth, food intake, protein efficiency, scurvy and survival rates.

The observations recently reported by Rhead and Schrauzer (123) on the occurrence of scurvy in 2 persons after previous long-term intake of vitamin C in the form of orange juice are just as poorly substantiated as those made by Jakowlew (76), since no information is given on the intake and composition of food following the previous vitamin C period of one to several years duration. It is evident that an organism very well saturated with vitamin C, in case of insufficient vitamin C supply, also becomes depleted after a certain time, if one takes into account that the daily rate of decomposition (breakdown) of vitamin C is equal to about 3% of the body pool.

11. Reproduction

In the 1940's, Kappelli (79) and Neuweiler (110), and later Fuhrmann (44) reported that vitamin C, when administered ante partum, promotes labor. Stojanov (147) observed, however, an opposite effect, and this effect also could not be confirmed in animal tests (128). According to Samborskaya and Ferdman (136), high vitamin C doses given to pregnant women in the early stage are supposed to cause a premature termination of pregnancy. Lecoq *et al.* (90, 91, 118) see the cause of this fact in an increased acidosis effected by ascorbic acid administration; but this is not the case, on the basis of recent findings (compare Section 7). Subsequently, experimental studies were carried out by different research groups to

clarify the question as to the extent to which high doses of vitamin C affect fertility and pregnancy (87, 102-104, 111, 133-136). In the various studies, different high ascorbic acid doses of 75 to ab. 750 mg vitamin C/kg/day were administered partly in the food, partly by the parenteral route for different periods of time. Control groups were not used, the fertility of the animals was not known and the beginning of pregnancy (by control of the vaginal plug) was not determined. The results were therefore widely divergent. These contradictory findings can be explained in part at least by the fact that about 25% of the female guinea pigs did not become pregnant at all (120). Nevertheless, in all tests no general toxic symptoms of the high-dosage vitamin C intake could be noted in the animals.

More differentiated were the tests carried out by Samborskaya and Ferdman (136) on already pregnant rats with subcutaneous daily doses of 150 mg sodium ascorbate (corresponding to ab. 800 mg/kg BW), in comparison to an isotonic NaCl solution. Out of 14 animals, only in 3 cases was the pregnancy prematurely terminated on the 13th to 15th day of pregnancy. Post-mortem examination of the uterus did not disclose either dead fetuses or signs of abortion. In a further experimental study (113), the absence of any negative effects of high ascorbic acid doses on the fertility of female guinea pigs could also be confirmed. Two groups of animals were kept in pairs on a standard diet. In the test group, 0.5% ascorbic acid was administered by addition to the diet. The average daily amount of vitamin C ingested was equal, on the basis of food amount measurements, to about 163 mg/animal (corresponding to ab. 500 mg/kg BW); the total amount, during the course of the 10-month observation period, was 48-50 g animal. During the entire test period, the number and size of the litters was recorded for each animal. As compared to the controls, no statistically divergent results or noticeable differences in the sense of a reduced fertility could be noted.

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Ascorbinsäure
-Bedarf
-Body-pool
-Toxizität
-Toleranz
-Katabolismus
-Säure-Basen-Haushalt
-Elektrolyt-Stoffwechsel
-Blutgerinnung
-Fertilität
-Hohe Dosen

Zur Toleranz hoher Ascorbinsäuredosen

W. F. Körner und F. Weber

Abteilung für Vitamin- und Ernährungsforschung
F. Hoffmann-La Roche & Co. AG, 4000 Basel

Zusammenfassung: Bei kritischer Wertung der recht zahlreich vorliegenden klinischen und experimentellen Befunde ergeben sich keine Anhaltspunkte dafür, dass Vitamin C, auch bei Zufuhr hoher Dosen, gesundheitsschädigend wirkt. Hin und wieder werden nach Verabreichung sehr grosser Mengen leichte Magen-Darm-Störungen, die im wesentlichen auf einem laxativen Effekt der Ascorbinsäure beruhen, sowie in Ausnahmefällen Allergien beobachtet. Diese Erscheinungen liegen zahlenmässig im Rahmen der Intoleranzerscheinungen, wie man sie von anderen Nährstoffen des täglichen Bedarfs kennt.

1. Einleitung

Seit fast vier Dezennien wird Vitamin C in der Medizin als Heilmittel angewandt, nachdem Reichstein *et al.* [122] sowie Hirst, Haworth *et al.* [12, 65, 67] nahezu gleichzeitig im Jahre 1933 die synthetische Darstellung der Ascorbinsäure gelungen war. Die verabreichten Dosen schwanken zwischen 50 mg und 5 g und betragen in besonders gelagerten Fällen sogar 10–20 g und mehr pro Tag, da die Ascorbinsäure im allgemeinen als eine atoxische Substanz betrachtet wird [2, 45, 81, 84, 117]. Infolge des seit dem letzten Dezennium laufend zunehmenden «Gesundheitsbewusstseins» breiter Bevölkerungskreise erlangten harmlose Naturstoffe, die wie das Vitamin C auch als Heilmittel verwendet werden, ausserhalb der ärztlichen Praxis vermehrt an Bedeutung.

Solange die Vitamin-C-Zufuhr im Rahmen der empfohlenen Tagesmengen [39, 41, 43] erfolgt, ergeben sich hinsichtlich der Verträglichkeit der Ascorbinsäure verständlicherweise keine Probleme. Dabei kann die tägliche Vitamin-C-Aufnahme mit der Nahrung in weiten Grenzen, d. h. im Bereich einiger hundert Milligramme, schwanken. Sie kann sogar die Grössenordnung von 1 g Ascorbinsäure pro Tag hin und wieder überschreiten, wenn man berücksichtigt, dass z. B. 100 g schwarze Johannisbeeren, je nach Provenienz, bis zu 400 mg, die gleiche Menge roten Paprikas bis zu 235 mg und 100 g Tomaten bis zu 100 mg Vitamin C enthalten.

2. Body-pool und Bedarf

Der body-pool des erwachsenen Menschen wurde mit 20–50 mg/kg Körpergewicht (KG), der tägliche Katabolismus unter Nicht-Stressbedingungen mit ca. 3% der im Körper vorhandenen Vitamin-C-Menge ermittelt [11, 14–16, 36, 66, 157, 168]. Dementsprechend ergibt sich für die Aufrechterhaltung der Gewebssättigung ein Tagesbedarf, der über 1 mg/kg KG liegt und mit 1–1,7 mg/kg KG für den Erwachsenen ermittelt wurde [19, 156]. Für Kinder und Jugendliche liegen die entsprechenden Bedarfsziffern mit 6,5–7 mg/kg KG [126, 143] – offenbar auf Grund eines stoffwechselmässig bedingten stärkeren Katabolismus – deutlich höher. In diesen Zahlen sind Verluste, wie sie z. B. unter Stress-Situationen entstehen, nicht berücksichtigt. Betrachtet man die obere genannte Grenze des menschlichen body-pools (50 mg/kg KG) für Ascorbinsäure, so stellt man fest, dass er mit demjenigen des Meerschweinchens von 54 mg/kg KG nahezu identisch ist [49], welches bei freier Nahrungswahl jedoch eine Tagesmenge von ca. 50 mg Vitamin C (entsprechend ca. 17 mg/kg KG) aufnimmt [48]. Auch der Gorilla, der wie alle Primaten und das Meerschweinchen über keine endogene Vitamin-C-Synthese verfügt, nimmt unter gleichen Bedingungen mit der täglichen Nahrung grössere Mengen Ascorbinsäure auf. Bourne [22] errechnete eine Tageszufuhr von 4,5 g, entsprechend ca. 36 mg/kg KG für ein 125 kg schweres Tier. Andere Vertreter des Tierreichs, die über eine Eigensynthese für Vitamin C verfügen und somit ihren Bedarf unabhängig von der Aufnahme mit der Nahrung decken können, weisen höhere Gewebskonzentrationen an Ascorbinsäure auf. So beträgt der body-pool der Ratte bei einer Tagessyntheseleistung von 26 mg/kg KG das Doppelte desjenigen des Meerschweinchens und des Menschen, nämlich 107 mg/kg KG [25, 49].

Die Abhängigkeit des Menschen, Affen und Meerschweinchens von exogenem Vitamin C ist ursächlich durch einen hereditären Mangel von mindestens einem

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Enzymsystem (L-Gulono-γ-lacton-oxidase) bedingt. Stone [148–150] vertritt daher die Auffassung, dass man den Ausfall der endogenen Vitamin-C-Synthese als «inborn error of metabolism» betrachten sollte. Dieser spezifisch-genetische Defekt, der sich bei durchschnittlicher Vitamin-C-Zufuhr – im Vergleich zu Spezies mit eigener Vitamin-C-Synthese – in niedrigen Ascorbinsäurekonzentrationen im Blut äussert, wäre somit als sog. «Hypoascorbinämie» zu bezeichnen, die unter extremen Bedingungen zum Skorbut führt. Nach Stone [148] würde volle Korrektur dieser genetisch entstandenen Erkrankung bedeuten, dass Ascorbinsäure in dem Umfang exogen zugeführt wird, wie sie endogen synthetisiert würde. Nach dieser Auffassung unterscheidet sich der optimale Ascorbinsäurebedarf des Menschen nicht wesentlich von demjenigen anderer Mammalia, die in der Lage sind, ihren Bedarf durch Eigensynthese zu decken, dies selbst in Stress-Situationen [3, 85, 89, 99], wo der Bedarf wesentlich höher ist. Die Ratte z. B. produziert unter Stressbedingungen – wahrscheinlich durch Enzyminduktion – bis zu 215 mg/kg KG und Tag [30].

3. *Toxikologie*

Im Hinblick auf die in den letzten Jahren stärker zu Tage tretende Tendenz, Vitamin C in Gramm-Dosen über längere Zeit einzunehmen, ist die immer wieder aufgeworfene Frage nach der möglichen Toxizität und Toleranz dieses Naturstoffes verständlich. Hierzu liegen die folgenden Befunde vor:

3.1. *Akute Toxizität* (LD_{50}/24 h)

Tierspezies	Applikationsart (mg/kg)				Lit.-Zitat
	oral	subcutan	intravenös	intra-peritoneal	
Maus	8021	5000	1058	2000	[13, 33, 45]
Ratte	> 5000	5000	1000		[33]
Meerschweinchen	> 5000	> 1000	500	2000	[33]
Kaninchen	> 2000	> 1000	> 1000	> 1000	[33]
Katze	> 1000	> 500	> 500		[33]
Hund	> 5000	> 200	> 200		[33]

Demole [33] kam zu dem Ergebnis, dass das Meerschweinchen das 500–1000-fache der Tagesbedarfsmenge (4–5 mg/kg KG) täglich ohne Nebenwirkung verträgt.

3.2 *Subchronische Toxizität* (LD_{50})

Tierspezies	Applikationsart (mg/kg/Tag)				Dauer (Tage)	Lit.-Zitat
	oral	subcutan	intravenös	intra-peritoneal		
Maus	8021				10	[13]
Maus			1058		10	[13]
Ratte	> 6500				6	[88]
Ratte		> 600			28	[47]
Meerschweinchen	> 8000				6	[88]
Meerschweinchen		500			7	[34]
Meerschweinchen				100	16	[162]
Kaninchen			500		7	[34]
Kaninchen				100	16	[162]
Katze			> 500		9	[38]
Hund	100				7	[34]
Hund			> 2000		3	[92]

Burlina und Lippi [23] untersuchten den Einfluss einer 6tägigen täglichen s. c. Verabreichung von 100 mg Ascorbinsäure pro kg KG auf die Leberfunktion des Meerschweinchens. Im Vergleich zu Kontrollen fanden sie bei einem mässigen Abfall der alkalischen Phosphatase- und Lipaseaktivität eine Zunahme der Ribo- und Desoxyribonukleinsäuren. Das Leberparenchym wies leichte regenerative Veränderungen auf. Verteilung und Konzentration der Fette, Lipide und neutralen Polysaccharide waren unverändert. In den Leberlappen fand sich eine Abnahme des Glykogengehalts. Vozár [164] konnte das Ausbleiben von Veränderungen der Konzentration der Fette und Neutralfette in der Leber nach 20tägiger Zufuhr von täglich bis zu 15 mg Vitamin C pro kg KG beim Meerschweinchen bestätigen, während er bei der Ratte unter gleichen Versuchsbedingungen eine deutliche Zunahme konstatierte.

Chronische Toxizität

Diacono [35] führte an jungen Kaninchen und Meerschweinchen Versuche mit parenteralen Tagesgaben von 100 mg Ascorbinsäure pro Tier (entsprechend 200 bzw. 500 mg/kg KG) während 4 Monaten durch. Beim Kaninchen fanden sich nach dieser Zeit nur flüchtige subkonjunktivale Hautblutungen ohne jegliche andere Manifestationen, insbesondere keine Störungen der verschiedenen Organsysteme. Grössenwachstum und Gewichtszunahmen waren normal. Das

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Meerschweinchen zeigte nur leichte oliv-braune Färbung der Skleren, Störung des Haarwachstums, Diarrhöe und relative Abnahme des Blutkalziumgehalts. Röntgenologisch waren keine Störungen im Skelettsystem festzustellen.

Während der letzten zehn Jahre gewann die Frage der chronischen Verträglichkeit hoher Vitamin-C-Dosen an Aktualität, und von verschiedenen Arbeitsgruppen wurden entsprechende Tierversuche durchgeführt. Kieckebusch *et al.* [81] und Lang [88] führten 6-Wochen-Toxizitätsversuche an Ratten durch. Die tägliche orale Zufuhr von 6,5 g Ascorbinsäure pro kg KG (berechnet auf das Ausgangskörpergewicht der Tiere) liess keinerlei schädliche Wirkungen erkennen. Gewichtszunahme, Mortalität, Protein-Efficiency, Nahrungsaufnahme und histopathologischer Befund unterschieden sich nicht von den Daten der Kontrollen. Hingegen erwiesen sich Tagesdosen von > 25 g/kg KG als deutlich toxisch. Die Autoren ziehen aus ihren Befunden den Schluss, dass die noch nicht toxische Grenzdosis für die Ratte bei 10 g/kg KG liegt. Daraus resultiert bei einer Tagesdosis von 1 g Ascorbinsäure beim Menschen (entsprechend 14–15 mg/kg KG) eine Sicherheitsspanne von 1 : 700. Auch für das Meerschweinchen erwiesen sich Tagesgaben von 8,9 g/kg KG während 14 Wochen als atoxisch [80, 88].

Einen weiteren Hinweis für die gute Verträglichkeit der Ascorbinsäure liefern neue Daten aus einer Studie über chronische Toxizität [151]. Drei Gruppen zu je 26 Ratten beiderlei Geschlechts erhielten im Vergleich zu einer adäquaten Kontrollgruppe während zwei Jahren täglich 1, 1,5 und 2 g Ascorbinsäure pro kg KG per os zusätzlich zur Standarddiät. Alle Dosierungen wurden ohne Störungen des Allgemeinzustandes und der Gewichtsentwicklung vertragen. In den Absterberaten zeigten sich keine Unterschiede. Auch bei den hämatologischen, blutchemischen und histologischen Untersuchungen fanden sich keine Abweichungen gegenüber den unbehandelten Kontrollen. Die in der höchsten Dosisgruppe symptomlos tolerierte Menge von 2 g/kg/Tag entspräche damit beim Menschen (70 kg) einer täglichen Aufnahme von 140 g Ascorbinsäure.

4. *Allgemeine Verträglichkeit beim Menschen*

Über die systemische Anwendung hoher Vitamin-C-Dosen bei Kindern, Erwachsenen und alten Leuten erschienen im Laufe der Jahre zahlreiche Arbeiten [17, 27, 37, 56–58, 68, 70, 82, 86, 93, 107, 121, 140, 144, 146, 161, 168]. Unter anderem wurden Tagesdosen von 3–6 g während 15 Monaten oral, bis zu 35 g i. v. während 1½ Monaten und bis zu 80 g oral während 6 bis 10 Tagen verabreicht, ohne dass Nebenwirkungen oder Überempfindlichkeitsreaktionen beobachtet wurden. In einem Fall erhielt ein an Morbus Kahler erkrankter Patient, neben

anderen therapeutischen Massnahmen, täglich 1 g Ascorbinsäure bis zu einer Gesamtdosis von 2,37 kg. Nebenwirkungen, welche auf die Vitamin-C-Medikation hätten zurückgeführt werden können, wurden nicht beobachtet [142].

Es sind jedoch von anderen Autoren Nebenwirkungen von untergeordneter Bedeutung beschrieben worden. Dabei handelt es sich immer um Einzelbeobachtungen und nicht um kontrollierte Studien, so dass der Beweis für einen ursächlichen Zusammenhang mit der Ascorbinsäureverabreichung meistens fehlt.

4.1. *Magen-Darm-Störungen*

Als Folge einer gewissen laxativen Wirkung höherer Ascorbinsäuredosen treten gelegentlich Diarrhöen auf, die von gesteigerter Darmperistaltik und Gastralgien begleitet sein können [63, 112, 124, 137, 160, 165, 166]. Diese Symptome scheinen bei oraler Zufuhr der freien Ascorbinsäure stärker ausgeprägt zu sein als nach Einnahme von Natriumascorbat, woraus sich erklärt, dass sie bei Medikation von Vitamin C in Form von Brausetabletten (Gehalt an $NaHCO_3$) erst nach wesentlich höherer Dosierung auftreten. Greer [57] jedoch, der 5 Poliomyelitis-Patienten mit täglich 50–80 g Vitamin C oral während 6 bis 10 Tagen behandelte, beobachtete unter dieser exzessiv hohen Gabe nur leichte Gastroenteritiden und Analirritationen. Widenbauer [165] weist in Zusammenhang mit seinen Beobachtungen an Säuglingen darauf hin, dass dieser Nebeneffekt der Ascorbinsäure bei Darmträgheit durch die Anregung der Peristaltik vorteilhaft ausgenutzt werden kann.

4.2. *Vagotone Reaktionen*

Bei einer Reihe vegetativ stigmatisierter Patienten sah man vereinzelt nach Ascorbinsäurezufuhr vagotone Reaktionen, die sich in Übererregbarkeit, Unruhe, Schlaflosigkeit, Hitzegefühl, Wallungen, Kopfschmerzen, Pulsverlangsamung, Dermographismus und Ödembildung äusserten [4, 46, 55, 59, 73, 94, 101, 124, 137, 139, 165, 166]. Diese Erscheinungen wurden vorwiegend bei Säuglingen und Kindern im Wachstumsalter, jedoch nur gelegentlich bei Erwachsenen beobachtet. Auch die von Hasselbach [63] bei der Behandlung von Tuberkulosekranken gelegentlich beobachteten Magensensationen nach i. v. Gaben von Natriumascorbat dürften sich in diese Gruppe einordnen lassen.

Diese vagotonen Reaktionen erfolgen offenbar unabhängig von der Dosishöhe, scheinen aber bei Kindern ein wenig ausgeprägter zu sein als bei Erwachsenen. Die fast immer beschriebene Verringerung der Pulsfrequenz betrug im Durchschnitt 10 str/min und war gelegentlich von einer Blutdrucksteigerung begleitet [4]. Demgegenüber sah Hochwald [69] im Verlauf der Zufuhr «hoher» Ascor-

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binsäuredosen am Menschen einen blutdrucksenkenden Effekt. Die von ihm beobachteten Senkungen des systolischen Blutdrucks betrugen bei Normotonikern ca. 20 mm Hg nach i. v. Gabe von 500 mg Natriumascorbat. Im Gegensatz zu dem mehrfach beschriebenen erregenden Effekt (Schlaflosigkeit) steht die Mitteilung von Maurer *et al.* [95], die täglich 1–3 g Vitamin C über längere Zeit bei Patienten, die an Schlaflosigkeit litten, mit Erfolg verabreichten.

4.3. *Fragliche Nebenwirkungen*

Andere Nebeneffekte, die im Verlauf der Vitamin-C-Therapie selten zur Beobachtung gelangten, sind Menstruationsanomalien im Sinne von zeitlich und mengenmässig veränderten Regelblutungen [63, 64, 158] und Urinverhalten [60]. Papayanopulos und Schroeder [116] stellten bei einer Reihe von Patienten mit diversen Erkrankungen, die mit einer Thrombozytopenie einhergingen, nach 1 bis 7tägiger parenteraler Verabreichung von täglich 500 mg Vitamin C eine signifikante Zunahme der Thrombozytenzahlen fest und betrachteten diese Thrombozytose als Ausdruck einer Reizung des blutbildenden Knochenmarks infolge eines pharmakologischen Effekts der Ascorbinsäure. Zunahme der Thrombozyten fanden Rietschel und Menching [124, 125] auch bei einer Reihe gesunder Säuglinge und Kleinkinder nach 9 bis 20 Tagen währender täglicher Vitamin-C-Zufuhr von 100–200 mg sowie bei Erwachsenen, die 500 mg Vitamin C pro Tag während ca. einer Woche erhalten hatten. Nach Absetzen der Vitamin-C-Medikation ging der Thrombozytenanstieg wieder zurück. Die Autoren betrachten diesen Effekt als Folge einer Vitamin-C-Überdosierung. Diesen Befunden stehen die Beobachtungen von Gertler *et al.* [46] gegenüber. Sechzehn Lupus-vulgaris-Patienten (Alter: 15 bis 65 Jahre) erhielten täglich während 1 bis 6 Monaten 1 g Natriumascorbat i. v. Nach initialem leichtem Blutdruckanstieg um 30–50 mm Hg, Erhöhung der BSR um 5–20 mm, Zunahme der Granulozyten, Eosinophilen und Thrombozyten (bis 400 000/µl) während der ersten bis zweiten Woche setzte in der dritten Woche allmählicher Blutdruckabfall zur Norm, Rückkehr der BSR in die Ausgangslage und Abnahme der Leukozyten- und Thrombozytenzahl zur bzw. unter die Normgrenze ein.

5. *Allergien*

Wie von zahlreichen Arzneimitteln und Naturstoffen bekannt, so vermag auch Vitamin C in seltenen Fällen allergische Reaktionen auszulösen, die sich hauptsächlich in rubeoli-, morbilli- und scarlatiformen Erythemen, Urticaria und gelegentlich Ödemen äussern [61, 114, 165–167]. Unter den von Widenbauer [165]

mitgeteilten neun Fällen (5 Erwachsene, 4 Säuglinge) befanden sich zwei, in denen in einem Fall anamnestisch eine Idiosynkrasie gegen Chinin, im anderen eine solche gegen Atropin, Erdbeeren und Crustaceen vorhanden war. Ein weiterer Patient, der sowohl auf Zitrusfrüchte als auch Ascorbinsäure allergisch reagierte, wird von Campbell [26] erwähnt. Rust [129] konnte in sieben, Panzani [115] in einem Fall im Scarifikationstest mit Ascorbinsäure positive Hautreaktionen nachweisen.

Bei einer 40jährigen Patientin mit Thalassämie, bei welcher in den letzten Jahren jede banale Erkrankung eine hämolytische Krise auslöste, ergab die anamnestische Erhebung, dass diese Patientin vor jeder solchen Krise «hohe» Dosen Vitamin C zur Erkältungsprophylaxe eingenommen hatte. Nachdem sie keine Ascorbinsäure mehr einnahm, traten keine Krisen mehr auf [50]. Demgegenüber fanden Choremis und Gregoriu [29] bei Kindern mit Thalassämie ein relatives Vitamin-C-Defizit. Belastungsversuche mit 50 mg Ascorbinsäure pro kg KG i. m. ergaben bei diesen Patienten eine beträchtliche grössere Retention des verabreichten Vitamin C als bei den Kontrollen.

6. *Katabolismus der Ascorbinsäure*

Als hauptsächlichste Stoffwechselprodukte der Ascorbinsäure werden neben dem unveränderten Vitamin Dehydroascorbinsäure, 2,3-Diketogulonsäure und Oxalsäure ausgeschieden. Die prozentualen Anteile variieren von Spezies zu Spezies sowie in Abhängigkeit von der Menge der zugeführten Ascorbinsäure. Das Meerschweinchen scheidet bis zu ca. 40% einer verabreichten Vitamin-C-Dosis im Verlauf von 24 Stunden als CO_2 aus; weitere 7% werden in Form von Oxalat und ca. 3% als Ascorbinsäure, Dehydroascorbinsäure und 2,3-Diketogulonsäure mit dem Harn eliminiert. Die Ausscheidung mit den Faeces beträgt knapp 1%. Nur nach Verabreichung einer sehr hohen Vitamin-C-Dosis (150 mg/kg) steigt die Ausscheidung der Ascorbinsäure und ihrer Metaboliten im Urin bis auf 40% der verabreichten Menge an, wobei der Oxalatanteil mit ca. 7% der verabreichten Dosis unverändert bleibt [1, 24]. Ratten scheiden etwa 40% der endogen synthetisierten Ascorbinsäure als solche oder als Metaboliten mit dem Harn aus [132]. Im Gegensatz zum Meerschweinchen wird vom menschlichen Organismus die Ascorbinsäure nicht bis zum CO_2 abgebaut. Die Ausscheidung erfolgt im wesentlichen mit dem Harn. Sie beträgt total ca. 3% des bodypools und verteilt sich auf Ascorbinsäure + Dehydroascorbinsäure (ca. 25%), 2,3-Diketogulonsäure (ca. 20%) und Oxalat (ca. 55%) [66]. Wahrscheinlich stammt etwa die Hälfte der durchschnittlichen täglichen Ausscheidung von

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35 mg Oxalat aus dem Ascorbinsäurekatabolismus [78]. Die aus Ascorbinsäure entstehende Menge Oxalat ist jedoch in gewissem Umfang limitiert, da nach Gabe von täglich 4 g Ascorbinsäure praktisch keine Zunahme der Oxalatausscheidung zu verzeichnen war. Erst nach Gabe von 9 g/Tag stieg die Oxalatexkretion auf durchschnittlich 95 mg/24 h an [86, 152]. Längerfristige Versuche in diesem Zusammenhang führten Takiguchi *et al.* [153, 154] an 30 gesunden Versuchspersonen durch, die während 90 bis 180 Tagen täglich 1–2 g Ascorbinsäure per os erhielten. Die Ausscheidung von Oxalat, die vor der Versuchsperiode im Mittel 29,3–29,8 mg/24 h betrug, wurde durch die chronische Vitamin-C-Verabreichung nicht gesteigert. Im Harn wurde als Hauptfraktion Ascorbinsäure, daneben in geringeren Quantitäten Dehydroascorbinsäure und 2,3-Diketogulonsäure gefunden. Frühere Versuche mit Tagesgaben von 250 und 500 mg sowie 2, 4, 8 und 9 g während einer Woche ergaben, dass erst unter 8 g Ascorbinsäure pro Tag die Oxalatausscheidung auf das Doppelte, unter 9 g/Tag auf knapp das Dreifache des Normwertes ansteigt [86]. Diese Befunde decken sich mit denjenigen von Ryoichi [130], El-Dakhakhny und El-Sayed [40] sowie McLaurin und Beisel [98]. Die Daten ergeben somit, dass Ascorbinsäurezufuhr in hohen Dosen nicht zu einer pathologischen Hyperoxalurie führt, die durch Ausscheidungswerte von 300–400 mg/24 h charakterisiert ist [138].

Aus verschiedenen anderen Studien an Patienten mit primärer Hyperoxalurie geht hervor, dass Ascorbinsäuremedikation selbst bestehende Oxalose nicht verstärkt. So verabreichten Atkins *et al.* [10] an zwei Patienten markierte Ascorbinsäure und bestimmten während 100 Tagen die Oxalatausscheidung im 24-Stunden-Harn. Der aus dem Vitamin-C-Katabolismus herrührende Oxalatanteil betrug nur 10 % der Gesamtoxalatausscheidung. Daraus wurde geschlossen, dass extern zugeführte Ascorbinsäure eine bestehende Hyperoxalurie nicht verstärkt. Ähnlich sind auch die Beobachtungen von Munzinger *et al.* [105], die bei einem 4½ Jahre alten Kind mit nephrolithiatischer Oxalose unter Belastung mit täglich 1 g Ascorbinsäure während 5 Tagen keinerlei Veränderung der Oxalatausscheidung konstatieren konnten. Nur Hösli *et al.* [71] fanden bei einem 9 Jahre alten Mädchen mit bioptisch nachgewiesener Oxalose nach Belastung mit 2 g Ascorbinsäure während 5 Tagen eine deutliche Zunahme der zuvor schon pathologisch erhöhten Oxalatausscheidung im Harn, während bei zwei gesunden Kindern und einem Mädchen mit Urolithiasis infolge idiopathischer Hyperkalzurie nach 3 g Vitamin C die Ausscheidungswerte für Oxalsäure unbeeinflusst blieben.

7. *Säure-Basen-Haushalt*

Es stellt sich die Frage, ob die hochdosierte Vitamin-C-Zufuhr zu einer Störung des Säure-Basen-Haushalts im Sinne einer Übersäuerung führt. Normalerweise besteht im arteriellen Blut ein Gleichgewicht zwischen Basen- und Säureäquivalenten, woraus ein pH von 7,38–7,42 mit einem Totalbasen- bzw. Säurengehalt von 151 mval/l Serum resultiert. Auf die organischen Säuren entfallen dabei nur 6 mval, d. h. knapp 4 %; die Alkalireserve (Standardbicarbonat) beträgt jedoch 22–28 mval HCO_3^-/l.

Die Stärke einer Säure ist durch ihre Dissoziation charakterisiert, d. h. eine Säure ist um so stärker, je kleiner ihre Dissoziationskonstanten (pK) sind. Die Werte für die beiden Konstanten betragen für Ascorbinsäure 4,25 [9, 18] und 11,79 [20, 21, 108, 109]. Vergleichsweise weisen die dreibasische Zitronensäure mit pK-Werten von 3,0, 4,29 und 5,49 sowie die Milchsäure mit einem pK-Wert von 3,90 einen wesentlich höheren Säuregrad auf. Das gleiche gilt auch für die β-Hydroxybuttersäure und die Acetessigsäure, welche die Ketoazidose beim diabetischen Coma bewirken.

Bisher vorliegende Untersuchungen lassen erkennen, dass Vitamin-C-Zufuhr nur unwesentlich zu einer Säurebelastung des Organismus beiträgt; dies wird besonders deutlich, wenn man bedenkt, dass mit der Nahrung oft erhebliche Mengen anderer Carbonsäuren (Äpfelsäure, Zitronensäure) aufgenommen werden. Im Harn nimmt nach Belastung mit 3–6 g Ascorbinsäure beim Gesunden die Gesamtsäure- und demzufolge die NH_4^+-Ausscheidung nur mässig zu; die titrierbare Azidität und der pH-Wert bleiben unbeeinflusst [62, 106, 107, 159]. Das unter pathologischen Bedingungen (z. B. chronischen Harnwegsinfektionen) vorkommende alkalische pH des 24-Stunden-Harns kann bereits durch eine geringe Säurebelastung des Urins, wie sie durch Ascorbinsäureausscheidung ermöglicht wird, in den sauren Bereich verschoben werden [97, 145]. Demgegenüber bleibt das pH des Blutes nach einmaliger Gabe von 3–6 g Vitamin C [106, 107] oder 14tägiger Verabreichung von täglich 1 g Natriumascorbat [83] unbeeinflusst. Auch für den CO_2-Partialdruck und das Standardbicarbonat (Alkalireserve) des Blutes fanden sich keine Veränderungen. Damit findet auch die von Perrault *et al.* [118] geäusserte Vermutung, dass längerdauernde Vitamin-C-Zufuhr eine Azidose bewirken könne, keine Stütze.

8. *Elektrolytstoffwechsel*

Ferrini *et al.* [42] fanden bei Ratten, die 10 Tage lang täglich 10 mg (entsprechend ca. 60 mg/kg KG) Ascorbinsäure erhalten hatten, eine deutliche Zunahme

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der intrazellulären Kaliumkonzentration, während Natrium eine leichte Abnahme zeigte. Im Plasma hingegen blieben die Konzentrationen der beiden Elektrolyte unverändert. Ähnliche Befunde liegen von Pokras'on und Sokolova [119] vor, wonach i. v. Gaben von 35 mg Ascorbinsäure pro kg KG täglich während 7 Tagen bei Vitamin-C-Mangel-Meerschweinchen die K^+-Konzentration im Blut, Gehirn und Myokard erhöhten, die Na^+- und Cl^--Konzentrationen in diesen Geweben aber unbeeinflusst liessen. Die Beobachtungen von Murphy und Zelman [106], denen zufolge Gaben von 3–6 g Vitamin C pro Tag beim Menschen die Na^+-Ausscheidung im Harn um etwa 50% reduzieren sollen, was einer beachtenswerten Na^+-Retention entspräche, konnten bei einer Nachprüfung nicht bestätigt werden [62].

Während Ascorbinsäure demnach den Elektrolythaushalt nicht im negativen Sinne beeinflusst, könnten allenfalls gewisse Störungen durch Hilfsstoffe hervorgerufen werden. So ist für die Erzielung des Brauseeffekts von Vitamin-C-Brausetabletten eine gewisse Menge Natriumbicarbonat erforderlich. Im allgemeinen enthält eine Brausetablette zu 1 g Vitamin C ca. 1 g $NaHCO_3$ [100], d. h. auf 1 mMol $NaHCO_3$ entfallen 0,93 mMol Säure. Die mit einer Brausetablette zu 1 g Vitamin C zugeführte Na^+-Menge (11,9 mval) ist derjenigen von ca. 0,7 g Kochsalz äquivalent. Diese Menge an Kochsalzäquivalenten fällt selbst bei Einnahme von vier Brausetabletten zu 1 g Vitamin C pro Tag bei Gesunden nicht ins Gewicht, da bei durchschnittlicher Ernährung die tägliche Na^+-Aufnahme zwischen 8 und 12 g Kochsalzäquivalenten schwankt. Bei Patienten mit kochsalzarmer Diät hingegen dürfte es sinnvoll sein, bei hochdosierter Vitamin-C-Medikation in Form von Brausetabletten der oben genannten Tatsache Rechnung zu tragen, um die Tagesmenge von 3 g Kochsalz (= 51 mval Na^+) nicht zu überschreiten. Neben Vitamin-C-Brausetabletten zu 1 g befinden sich aber auch solche mit ca. einem Viertel der Vitamin-C-Dosis im Handel bei gleichem Natriumbicarbonatgehalt von 1 g. Will man mit diesen relativ niedrig dosierten Darreichungsformen eine grössere Vitamin-C-Menge (Grammdosen) dem Organismus zuführen, wird folglich auch die Aufnahme von Kochsalzäquivalenten um ein Mehrfaches erhöht, was bei Patienten mit kochsalzarmer Diät nachteilige Folgen haben könnte. Dieses Problem stellt sich aber im Prinzip bei allen Brausepulvern zur Bereitung von Brauselimonaden, deren Hauptbestandteil das Natriumbicarbonat ist.

9. *Blutgerinnung*

Infolge mangelnder Bereitstellung von Gerinnungsfaktoren und insbesondere durch Absinken der Thrombozytenzahl ist im Skorbut die Blutgerinnung gestört

[5–7, 31, 72]. Nach Sahud und Aggeler [131] besteht zwischen dem Ausmass der Thrombozytenaggregation und dem Vitamin-C-Gehalt der Plättchen eine direkte Beziehung. Demnach ist die Ascorbinsäure ein essentieller Faktor für die normale Blutgerinnung. Aptekar und Lorie [8] beobachteten nach hochdosierten Vitamin-C-Gaben beim Menschen und Kaninchen eine Verkürzung der Gerinnungszeit. Demgegenüber konnte in anderen Untersuchungen dieser Befund nicht bestätigt werden [75, 93]. McCraw und Desmarais [96] fanden beim Meerschweinchen als Vitamin-C-Effekt nur eine Verkürzung der Blutungszeit. Nach tierexperimentellen Befunden [141] und Zufallsbeobachtungen am Menschen [127, 155] soll Vitamin C mit Antikoagulantien vom Dicumarol-Typus interferieren und die Gerinnungshemmung zum Teil aufheben. Hume *et al.* [74] fanden jedoch an 5 Patienten, die unter Langzeitbehandlung mit Warfarin standen, keinen Einfluss einer 11tägigen Gabe von täglich 1 g Vitamin C auf den Gerinnungsstatus. Umgekehrt geht aus den Versuchen an Meerschweinchen [32] hervor, dass unter Vitamin-C-Mangel die Gerinnungszeit nach Applikation von Warfarin stark verlängert und die Mortalitätsrate der Tiere signifikant erhöht sind. Ausscheidungsmuster und -rate der Warfarin-Metaboliten wurden durch Vitamin-C-Mangel jedoch ebensowenig beeinflusst wie durch Gaben von Vitamin C. In der Nebenniere der Ratte blieb die Ascorbinsäurekonzentration nach Verabreichung von Warfarin unverändert [28].

10. *Führt langdauernde Einnahme hoher Vitamin-C-Dosen zu einem höheren Bedarf?*

Aus zahlreichen Studien ist bekannt, dass dem Körper zugeführte Stoffe die Induktion mikrosomaler Enzyme bewirken können, die ihrerseits den Katabolismus und die Elimination dieser Stoffe fördern. Diese Frage ergab sich für die Ascorbinsäure im Anschluss an Beobachtungen von Jakowlew [76] im Verlauf der Belagerung Leningrads während des zweiten Weltkrieges. Es entstand damals der Eindruck, dass jene Bevölkerungskreise, die besser ernährt waren, im Verlauf der Hungerperiode häufiger an Skorbut erkrankten als die Bewohner mit schlechter Ernährung, wobei allerdings keinerlei exakte Daten über die Höhe der Nahrungszufuhr und Vitamin-C-Versorgung der beiden Gruppen vorliegen. In einer anschliessenden experimentellen Studie versuchte Jakowlew [76, 77] für seine Beobachtung eine wissenschaftliche Basis zu finden. Er verabreichte Meerschweinchen während 6 Monaten täglich 5 mg Ascorbinsäure (entsprechend ca. 15–20 mg/kg KG). Eine zweite Gruppe von Tieren erhielt die doppelte Vitamin-C-Dosis. Anschliessend wurden die Tiere auf skorbutogene Diät gesetzt.

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In der niedrig dosierten Gruppe verstarben die Tiere im Mittel nach 33 Tagen, in der Gruppe mit 10 mg/Tier nach 30 Tagen. Die Unterschiede sind nicht signifikant; darüber hinaus fehlt eine echte Kontrollgruppe auf Standarddiät. Basierend auf der gleichen Hypothese führte Gordonoff [51–54] ähnliche Versuche durch. Meerschweinchen auf Standarddiät erhielten zusätzlich pro Tag 500 mg Ascorbinsäure als Natriumascorbat (entsprechend ca. 1,5 g/kg KG) subcutan für die Dauer von 4 Wochen. Die Kontrollen (ebenfalls Standarddiät) blieben unbehandelt. Am Ende der Behandlungsperiode wurden alle Tiere auf skorbutogene Diät gesetzt und die Dauer bis zum Auftreten der Skorbutsymptome ermittelt. Die Resultate können jedoch nicht als beweiskräftig angesehen werden, da die Vitamin-C-Gruppe infolge der täglichen s. c. Injektion im Gegensatz zu den Kontrollen einer Art Dauerstress ausgesetzt waren. Abgesehen davon liegt die verabreichte Vitamin-C-Menge in der Nähe der Toxizitätsgrenze für die gewählte Applikationsart. Die Forderungen eines kontrollierten Versuchs wurden hingegen von Kieckebusch [80] und Lang [88] in einer Nachprüfung der Gordonoffschen Befunde erfüllt. In diesem Experiment erhielten Meerschweinchen zusammen mit einer Standarddiät orale Gaben von Vitamin C in Dosierungen von 1,7–9,5 g/kg KG in Form von Natriumascorbat für die Dauer von 14 Wochen, woran sich die Verabreichung einer skorbutogenen Diät anschloss. Alle sonstigen Bedingungen waren für Versuchs- und Kontrollgruppe gleich. Die hohen Vitamin-C-Zulagen zeigten keinerlei negative Auswirkungen in bezug auf Wachstum, Futteraufnahme, Protein-Efficiency, Skorbutrate und Überlebensrate.

Die neuerdings von Rhead und Schrauzer [123] mitgeteilten Beobachtungen über das Auftreten von Skorbut bei zwei Personen nach vorangegangener langdauender Vitamin-C-Aufnahme in Form von Orangensaft sind ebenso mangelhaft belegt wie diejenigen von Jakowlew [76], da jegliche Angaben über die Nahrungsaufnahme und -zusammensetzung im Anschluss an die vorangegangene ein- bis mehrjährige Vitamin-C-Periode fehlen. Es ist einleuchtend, dass auch ein sehr gut mit Vitamin C abgesättigter Organismus bei unzureichender Vitamin-C-Versorgung nach einer gewissen Zeit verarmt, wenn man berücksichtigt, dass die tägliche Abbaurate für Vitamin C ca. 3% des body-pools beträgt.

11. *Fortpflanzung*

In den 40er Jahren berichteten Käppeli [79] und Neuweiler [110], später Fuhrmann [44], dass Vitamin C, ante partum verabreicht, die Wehentätigkeit fördert. Stojanov [147] beobachtete jedoch einen gegenteiligen Effekt, und im

Tierversuch konnte diese Wirkung ebenfalls nicht bestätigt werden [128]. Nach Samborskaya und Ferdman [136] sollen hohe Vitamin-C-Dosen bei Graviden, in der Frühphase verabreicht, die Schwangerschaft vorzeitig beendigen können. Lecoq *et al.* [90, 91, 118] sehen die Ursache hierzu in einer durch die Ascorbinsäuregaben bewirkten verstärkten Azidose, was aber auf Grund neuerer Erkenntnisse nicht zutrifft (vgl. Abschnitt 7). In der Folge wurden von verschiedenen Forschergruppen experimentelle Untersuchungen an Meerschweinchen, Ratten und Mäusen zur Frage, inwieweit hohe Dosen Vitamin C sich auf die Fertilität und die Schwangerschaft auswirken, durchgeführt [87, 102–104, 111, 133–136]. In den diversen Studien wurden unterschiedlich hohe Ascorbinsäuredosen von 75 bis ca. 750 mg Vitamin C pro kg KG und Tag z. T. oral mit dem Futter, z. T. parenteral über verschieden lange Zeiträume verabreicht. Kontrollgruppen wurden nicht mitgeführt, die Fertilität der Tiere war nicht bekannt und der Beginn der Tragzeit (durch Kontrolle des Vaginalpfropfs) nicht determiniert. Die Resultate waren folglich divergierend. Die widersprechenden Befunde finden ihre Erklärung z. T. wenigstens darin, dass etwa 25% der weiblichen Meerschweinchen überhaupt nicht trächtig werden [120]. Immerhin konnten in allen Versuchen bei den Tieren allgemein toxische Erscheinungen der hochdosierten Vitamin-C-Zufuhr nicht festgestellt werden.

Differenzierter waren die Experimente von Samborskaya und Ferdman [136] an bereits trächtigen Ratten mit s. c. Tagesgaben von 150 mg Natriumascorbat (entsprechend ca. 800 mg/kg KG) im Vergleich zu isotonischer NaCl-Lösung. Von 14 Tieren war nur in 3 Fällen die Trächtigkeit vorzeitig am 13. bis 15. Trächtigkeitstag beendet. Die post mortem-Untersuchung der Uteri ergab weder abgestorbene Foeten noch Zeichen von Abort. Auch in einer weiteren experimentellen Studie [113] konnte das Fehlen negativer Auswirkungen hoher Ascorbinsäuredosen auf die Fertilität weiblicher Meerschweinchen bestätigt werden. Zwei Gruppen von Tieren wurden paarweise auf Standarddiät gehalten. Der Versuchsgruppe wurde zusätzlich 0,5% Ascorbinsäure mit dem Futter verabreicht. Die durchschnittliche täglich aufgenommene Vitamin-C-Menge betrug auf Grund der Futtermessungen ca. 163 mg/Tier (entsprechend ca. 500 mg/kg KG), im Verlauf der 10monatigen Beobachtungsperiode insgesamt 48–50 g/Tier. Während der gesamten Versuchszeit wurden Wurfzahl und Wurfgrösse für jedes einzelne Tier registriert. Gegenüber den Kontrollen ergaben sich weder statistisch abweichende Befunde noch angedeutete Unterschiede im Sinne einer Fertilitätsminderung.

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iew is given of the toxicity and tolerance of ascorbic acid n the literature and results from recent investigations. Critical numerous clinical and experimental findings gave no indica- prejudicial to health caused by vitamin C, even if administered in high doses. Occasionally, slight gastro-intestinal disturbances due to a laxative effect of the ascorbic acid, and in exceptional cases, allergic symptoms were observed. Nevertheless, the frequency of these phenomena is not greater than that of the signs of intolerance caused by other essential nutrients.

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Dr. W. F. Körner und Dr. F. Weber, Abteilung für Vitamin- und Ernährungsforschung
F. Hoffmann-La Roche & Co. AG, CH-4000 Basel

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Urinary Oxalate Excretion by Man Following Ascorbic Acid Ingestion.* (20827)

MERTON P. LAMDEN AND GLORIA A. CHRYSTOWSKI. (Introduced by H. B. Pierce.)

From the Department of Biochemistry, College of Medicine, University of Vermont, Burlington.

Oxalic acid has long been known to be a product of the *in vitro* oxidation of ascorbic acid(1-5). *In vivo*, Scheinkman(6) showed that ascorbic acid ingestion by guinea pigs resulted in increased urinary oxalate. Burns *et al.*(7) found that one of the end products of the metabolism of radioactive 1-C^{14}-L-ascorbic acid administered to guinea pigs was urinary oxalate. Since large doses of ascorbic acid are being prescribed by physicians(8,9), an investigation of the effect of ascorbic acid on the urinary oxalate excretion of man is warranted. This study is concerned with that investigation.

Procedure. In the first experiment, 11 healthy adult males on their regular diet collected 24 hour control urine samples. Each subject then ingested, in addition to his regular diet, 3 g of pure ascorbic acid at each mealtime (9 g/day) for 2 days, collected a 24 hour urine sample on the second day and another 24 hour control urine sample one week later. The results indicated that there was increased oxalate excretion with the daily ingestion of 9 g of extra-dietary ascorbic acid. A second experiment was conducted to determine at what minimum dosage of supplementary ingested ascorbic acid an increase in urinary oxalate occurred. Forty adult males were placed into groups ingesting different amounts of ascorbic acid ranging from 0.25 g to 8 g (divided into 3 doses as in the first experiment) daily for one week at the end of

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which each member collected a 24 hour urine sample. All subjects collected 24 hour control urine samples prior to receiving extra-dietary ascorbic acid and at the end of the first and second weeks following cessation of the ingestion of extra-dietary ascorbic acid. The groups taking 0.25, 0.50, and 2 g of ascorbic acid daily each consisted of 7 subjects. The groups ingesting 4 and 8 g of ascorbic acid each consisted of 6 subjects. The method for determining urinary oxalate was essentially that of Powers and Levatin(10). Care was taken to prevent the *in vitro* conversion of urinary ascorbic acid to oxalic acid. Urine was voided into a large bottle containing 225 ml of stabilizing solution (20% metaphosphoric acid and 3.3% thiourea). In order to test the procedure, 9 g of ascorbic acid were added to a composite urine sample containing the stabilizing solution. This preparation was then allowed to stand 24 hours at room temperature during which time the oxalate content increased only 1.2 mg (as oxalic acid) which amount was within the experimental error of the method. All urine samples were analyzed individually. The values for the oxalate content of the control urines for each subject were averaged. This average was subtracted from the value for the urinary oxalate excretion of the subject following ascorbic acid ingestion to determine the change, whether positive, negative, or zero.

Results. The mean oxalic acid excretion of 51 males without extra-dietary ascorbic acid was 38.3 ± 1.7 mg (standard error). This value was based on 142 determinations. The range of urinary oxalate output among the controls was 16 to 64 mg oxalic acid. Values reported in the literature for the normal 24 hour urinary oxalate excretion of man range from 14.0 to 56.0 mg with averages of 30 to 35 mg oxalic acid(2,11).

The remarkable constancy of the oxalic acid excretion for an individual on his regular diet without supplementary ascorbic acid is indicated by the average of the maximum variation among controls for each individual which was only 5.2 mg oxalic acid. The greatest variation among controls for an individual was 19 mg.

The effect of ingested ascorbic acid on urinary oxalate excretion is shown in Fig. 1

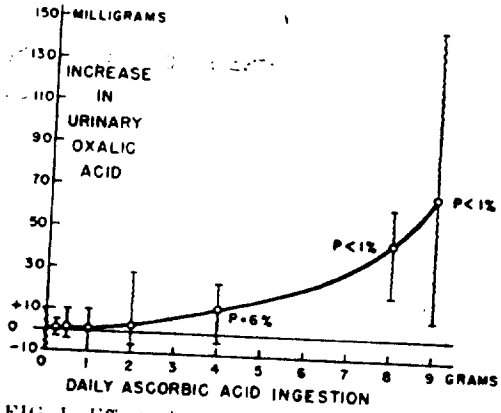

FIG. 1. Effect of amount of ascorbic acid ingested daily on increase in urinary oxalate (as oxalic acid). Circles on curve represent avg increase in excretion of urinary oxalic acid for each group. "Increase" in urinary oxalic acid is the difference between 24 hr urinary oxalic acid excretion of an individual following ingestion of ascorbic acid and avg of the values of his control urines collected when he received no extra-dietary ascorbic acid. The 0.25, 0.50, 1 and 2 g groups consisted of 7 subjects; 4 and 8 g groups, 6 subjects, and 9 g group, 11 subjects. Vertical wavy lines indicate maximum and minimum individual increases (sometimes negative) found within each group except in the case of controls (0 g extra-dietary ascorbic acid ingested) where they indicate the greatest variation between controls of a single individual in the whole study. P = probability level.

in which the increase in 24 hour urinary oxalic acid in mg is plotted against ascorbic acid ingested daily in grams. The ingestion of 4 g of ascorbic acid results in an average increase of 12 mg of excreted oxalic acid significant at the 6% level of probability; 8 g, an average increase of 45 mg of oxalic acid, and 9 g, an average increase of 68 mg of oxalic acid, these last two increases being statistically highly significant. The ingestion of less than 4 g of ascorbic acid daily resulted in a negligible increase in urinary oxalate.

Further investigation is being conducted on laboratory animals to determine whether urinary oxalate calculi and kidney changes may be a consequence of massive ascorbic acid ingestion over a long period of time.

Summary. An investigation was conducted to determine the urinary oxalate excretion of normal males on their regular diet, and to determine to what extent extra-dietary ascorbic acid would increase this excretion. The mean 24 hour urinary oxalic acid excretion of

51 healthy adult males on their regular diets was 38.3 ± 1.7 mg. The 24 hour excretion of any individual was remarkably constant over a three week period. The daily ingestion of 4 or more g of ascorbic acid increased urinary excretion of oxalic acid; whereas the daily ingestion of less than 4 g of ascorbic acid produced no significant increase in oxalate excretion.

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Ascorbic acid
Dietary intake
Leucocyte concentration
Haemoglobin level
Haemoglobin formation
Iron
Dynamic relationship
Different age groups
Intersex comparison

The Relationship between Leucocyte Ascorbic Acid and Haemoglobin Levels at Different Ages

H. S. Loh, M. B., D. C. H.
and
C. W. M. Wilson, M. D., B. Sc., Ph. D.

The Department of Pharmacology, University of Dublin, Ireland

Summary. Leucocyte ascorbic acid and haemoglobin values have been measured in healthy adolescent school children, adult factory employees and old people, and in geriatric patients in a hospital. The highest levels of leucocyte ascorbic acid were found in adolescence and the lowest in old age. The fall in level was greater with increasing age in females. There is evidence of a sex related difference in the utilisation of Vitamin C which appears at adolescence and diminishes in old age. The haemoglobin levels also showed a significant alteration with age. The levels were highest in adults significantly lower among school children and in old age in both sexes. Low haemoglobin values were associated with high ascorbic acid concentrations and vice versa in all the groups except the adult males and to a lesser extent in the school boys. In these two groups there was a positive correlation between the haemoglobin and leucocyte ascorbic acid values. The results suggets that there is a dynamic relationship between iron and ascorbic acid in haemoglobin formation, and that relative shortage of either will interfere with optimal haemoglobin formation. It is concluded that ascorbic acid in addition to iron plays a major role in haemoglobin formatin among large sections of the population.

Introduction

Ascorbic acid concentrations in the blood have been investigated in numerous studies. The most accurate estimate of the tissue status of the ascorbic acid is obtained by measuring its concentration in the buffy coat [3, 4, 2] which gives an

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indication of its level in the white blood cells under normal conditions [17]. Anaemia commonly is present in scurvy, and it is known that in the majority of cases ascorbic acid is an effective method of restoring the blood picture to normal [13]. The recommended dietary allowances of ascorbic acid to prevent scurvy in the United Kingdom is 30 mg daily for adolescent boys and girls over the age of 15, and for men and women [20]. It is generally assumed that this level of intake is attained in the population, and that there is no evidence indicating that higher levels could have a beneficial effect in normal individuals, except possibly in elderly people [7] and in females during ovulation and pregnancy [25].

In this investigation, leucocyte ascorbic acid concentrations and haemoglobin levels have been measured in boarding school children, adult employees in a factory, and in elderly subjects. The purpose of the investigation was to find out whether the ascorbic acid concentrations in the different groups could be related to their ages, and if there was any association between the ascorbic acid concentrations and the sexes in the groups. In addition, haemoglobin levels have been related to sexes and ages to compare the levels with those found in surveys carried out elsewhere. The relationship between ascorbic acid concentrations and haemoglobin levels has also been investigated.

Method

Leucocyte ascorbic acid concentrations and haemoglobin levels were measured in boarding school children, adult employees in a factory, and in elderly subjects in a geriatric institution attached to a hospital. All these were normal healthy subjects. A group of patients in a geriatric hospital was also investigated. Similar numbers of volunteers were recruited from each institution, and approximately equal numbers of males and females were included in the groups.

The children, who were resident in two boarding schools in Dublin (MB and MG), were volunteers with the consent of their school doctors and parents. They were aged fourteen years or over and the girls had attained their menarche. Ascorbic acid estimitions were carried out in mid January. The adult volunteers were from the staff of a pharmaceutical company in Dublin (B) and were examined in March. The geriatric subjects (Ml) were investigated during April. They cared for themselves, although all their meals were supplied from the hospital kitchen. They had no manifest signs or symptoms of disease apart from the degenerative changes normally found at this age. The geriatric patients in the K group were in the geriatric unit of another large hospital in Dublin. This group consisted of patients who were being treated for such conditions as hypertension, chronic respiratory disease, cerebrovascular disease, coronary insufficiency, and arthritis. The dietary Vitamin C intake was estimated in the adult employees and the geriatric subjects in the Ml group by the use of a questionary [10].

Leucocyte ascorbic acid concentrations were estimated by the method of Denson and Bowers [14] using a Coulter electronic counter for the leucocyte counts. The blood was removed from the antecubital vein of all subjects between 9.30 and 12 mid-day and placed in a saline/dextran/sequestrene diluent for leucocyte ascorbic acid estimations. The supernatant containing the leucocytes was removed from the tubes 45 minutes after the tubes had been placed on the bench when the red cells had settled. Leucocyte counts and ascorbic acid estimations were then carried

out in duplicate. Good correlation was obtained between the duplicates [26]. One ml of blood was placed in a sequestrene tube for haemoglobin estimations by the oxyhaemoglobin method using a Beckman spectrophotometer [32].

Results

The daily intake of Vitamin C by the adults and the geriatric M groups are shown in Table 1. The adult females had a significantly higher intake of Vitamin C than the males. There was no significant difference between the sexes of the Ml group in their intake of Vitamin C. The values for the haemoglobin levels and leucocyte ascorbic acid concentrations are also shown in Table 1 together with the ages and numbers of subjects in the different groups.

Tab. 1: Vitamin C dietary intake (mg/day), haemoglobin levels (g/100 ml), and leucocyte ascorbic acid concentrations (µg/10^8 cells) of the subjects in the different groups. Means, ranges and standard deviations are shown. The p values indicate the significance of the difference between the sexes.

	School Children		Adults		Geriatric Subjects			
	Boys MB	Girls MG	B Male	B Female	Ml Male	Ml Female	K Male	K Female
No. of Subjects	16	28	14	17	16	29	9	19
Ages Mean	14	15	39	34	71	74	80	75
Range	11–17	11–18	39–49	22–45	56–86	51–87	70–92	65–87
Dietary Intake Mean .	–	–	37.2	58.3	37.1	35.7	–	–
S. D.	–	–	19.1	33.8	16.9	17.3	–	–
Haemoglobin Mean ..	13.6	13.6	15.5	14.4	14.7	13.5	13.0	11.9
S. D.	0.8	1.3	1.1	1.3	1.4	1.1	2.2	1.7
Leuc. AA Mean	36.6	42.8	30.4	34.0	24.4	23.4	28.2	24.0
S. D.	9.1	12.6	8.7	10.9	13.6	11.3	15.3	8.5
Intersex Comparisons								
Dietary Intake p	–		< 0.002		> 0.05			
Haemoglobin p	> 0.05		< 0.05		< 0.01		> 0.05	
Leucocyte AA p	> 0.05 < 0.10		> 0.05		> 0.05		> 0.05	

In the school children there was no significant difference between the haemoglobin levels of boys and girls, but the difference between leucocyte ascorbic acid levels approached a significant level. In the adult employees, males had significantly higher haemoglobin levels than females, but though leucocyte ascorbic acid levels were higher in the females, this was not significant. In the ambulant geriatric group (Ml), males had significantly higher haemoglobin levels than females, but leucocyte ascorbic acid levels were not significantly different in the

two sexes. In the geriatric group (K), males had higher levels of both haemoglobin and leucocyte ascorbic acid than females, but neither reached significance.

The leucocyte ascorbic acid concentrations have been compared between the different groups in relation to their ages (Fig. 1). Males show an inverse correlation with age. School boys have significantly higher levels than adult factory workers who in turn have significantly higher levels than geriatric males in either group. The female subjects showed a similar but more pronounced trend, with significant differences between the groups. Linear regression lines have been calculated for the mean leucocyte ascorbic acid values against ages for each sex

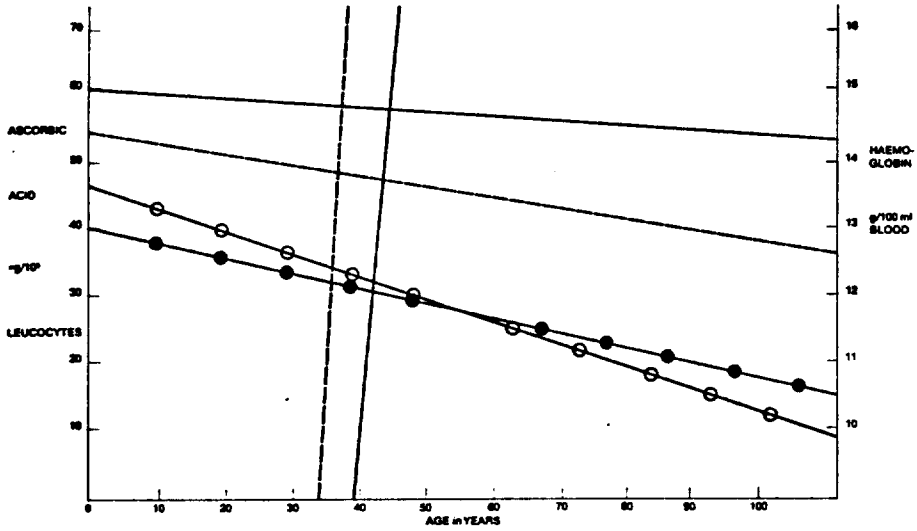

Fig. 1: Relationship between increasing age and leucocyte ascorbic acid concentrations and haemoglobin levels. Calculated regression lines are shown for the ascorbic acid concentrations in males (●—●) and females (○—○) against the ages of the different groups. Calculated lines relating haemoglobin levels with age above and below 34 years in females (— —) and 39 years in males (———)

in the different groups. The observed values correspond to the calculated regression lines for each sex ($p < 0.05$). The difference between the lines for males and females approached a significant value although it did not quite attain the 5% level. Below the age of 60 the females have higher ascorbic acid values than the males, but after this age the male concentrations exceed those in the females. The difference between the means of the school children and geriatric subjects was less in the males than in the females. The difference in the males was 63%

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of that in the females. These observations indicate that the change in leucocyte ascorbic acid concentrations between adolescence and old age was less in the males than in the females and that the higher value in the females disappears after the age of 60.

The haemoglobin levels also showed a significant alteration with age in both sexes (Fig. 1). The were at their highest in the adults. Among school children and in old age the levels were significantly lower in both sexes. A regression line fitted against the ages of the subjects and the haemoglobin levels departs significantly from the line obtained from the observed values. This indicates that in these sample populations the haemoglobin levels reach a peak in adult life and then begin to diminish as old age approaches. The heamoglobin levels of the adults and geriatric subjects decreased in parallel with the leucocyte ascorbic acid concentrations. The regression lines for both these variables against age, over the level of 35 years, are highly significant (Fig. 1).

There is evidence that ascorbic acid may play a role in haemoglobin formation [18]. If this is the case, a relationship may exist between haemoglobin levels and tissue ascorbic acid concentrations. The regression lines of the haemoglobin on the leucocyte ascorbic acid values for the eight different groups were therefore calculated. The correlation coefficient for the calculated lines approached significant levels for the adult males, and for the K group of male and female geriatric patients. The haemoglobin levels were at their maximum and minimum extremes in these groups. The regression coefficients, indicating the slopes of the lines, vary from a positive value for the adult males and school boys to a negative value for all the females, and the male geriatric groups (Table 2a). The significance of this progressive variation between haemoglobin and leucocyte ascorbic acid from a positive correlation at one extreme to a negative correlation at the other, has been examined by carrying out a regression analysis (Table 2b). The pooled regression was compared with the sum of the total regressions of the groups by an analysis of variance, and the F value was found to be 6.6839, which is highly significant. This indicates that there is a significant alteration in the regression coefficients between these two extremes over the range of regression variation through the groups.

Discussion

The groups were drawn from normal populations in Dublin except for the geriatric K group which consisted of elderly patients suffering from diagnosed diseases, in a geriatric hospital. The youngest group of boarding school boys and

Tab. 2: Comparison of Multiple Regression Lines and Analyses of Variance of Leucocyte Ascorbic Acid Concentrations and Haemoglobin Levels, Haemoglobin Levels being the Dependent Variable

Tab. 2 (a): Leucocyte ascorbic acid concentrations and haemoglobin levels arranged according to the diminishing values of the regression coefficients relating these variables in the different groups

	Leucocyte Ascorbic Acid	Haemoglobin	Regression Coefficient	Degrees of Freedom	Sum of Squares
Adult Males B	30.43 ± 8.65	15.5 ± 1.11	6.37	12	12.03688
School Boys MB	36.56 ± 9.10	13.6 ± 0.81	0.39	14	9.82143
Adult Females B	33.46 ± 10.57	14.4 ± 1.28	− 0.16	15	26.07419
Ger. Ml Females	23.42 ± 11.35	13.5 ± 1.07	− 0.35	27	32.22473
School Girls MG	42.80 ± 12.59	13.1 ± 1.30	− 0.97	26	45.25415
Ger. Ml Males	24.42 ± 13.59	14.7 ± 1.45	− 3.52	14	28.02133
Ger. K. Males	28.32 ± 15.26	13.0 ± 2.24	− 4.65	7	36.15086
Ger. K. Females	24.00 ± 8.47	11.9 ± 1.69	− 8.22	17	42.65904

Tab. 2 (b): Comparison of multiple regression lines, and analysis of variance of the regression coefficients. There is a significant shift in angles of regression as shown by the difference between the analysis of the sum of squares of the total groups and the pooled groups

	Leucocyte Ascorbic Acid	Haemoglobin	Regression Coefficient	Degrees of Freedom	Sum of Squares
Pooled groups	30.53 ± 12.97	13.6 ± 1.65	− 1.045	146	396.87964
	F value + 6.6839: $p < 0.001$				

girls had significantly higher leucocyte ascorbic acid levels than the older groups of either sex. A significant inverse relationship was demonstrated between the age and leucocyte ascorbic acid concentrations so that the lowest concentrations were associated with the oldest subjects. The regression lines relating these two variables in each sex were similar but there was a greater overall fall in ascorbic acid concentrations in the females with increasing age so that the males tended to develop higher values than the females after the age of 60. The adult females had a significantly higher intake of Vitamin C but this difference between the sexes also disappeared after the age of 60.

No difference was found between male and female ascorbic acid concentrations in old subjects contrary to the results reported from Farnborough [7] and London [1]. The observation that there was a difference which approached significance between adolescent boys and girls in their ascorbic acid levels sup-

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ports the suggestion that the sexes differ in their utilisation of Vitamin C at adolescence under normal dietary conditions [15]. At about 60 years a change appears in the metabolic pattern of the use of Vitamin C. A decline in blood ascorbic acid levels, and a tendency for the values of males and females to converge, occurs after this age [28]. Dietary differences may have a greater effect on ascorbic acid blood levels in old people than intersexual differences in the metabolism of ascorbic acid. The difference in leucocyte ascorbic acid concentrations between elderly men and women demontsrated under normal conditions is not maintained after supplementation with Vitamin C [19], although the difference is maintained during adolescence after daily supplementation with 200 mg Vitamin C for a three month period [30]. Brook and Grimshaw [8] attribute the abnormally low ascorbic acid values in their elderly subjects to a combination of inadequate dietary intake and metabolic effects arising from the ageing process. However the sex difference between leucocyte ascorbic acid values persisted into old age when the intake of Vitamin C was known to be low [1]. The intake of Vitamin C among old people at home in Dublin [31], as well as in institutions, is good. In consequence leucocyte ascorbic acid concentrations are relatively high. It is possible that the effect of an adequate dietary intake of Vitamin C among these old people in Dublin could outweigh the metabolically induced difference observed between the sexes in old age which has been reported elsewhere.

Numerous investigators [6, 9, 12, 21, 22] have shown that haemoglobin formation and oral administration of an appropriate dose of ascorbic acid are interdependent. These authors attributed the effect of the ascorbic acid to an increase in ferrous iron absorption from the gut in their anaemic patients. On the ohter hand, it has been shown that patients with idiopathic heamochromatosis and transfusional siderosis have low levels of white cell ascorbic acid [29]. This effect was attributed to irreversible oxidation of available ascorbic acid produced by the enlarged iron stores. When iron overload occurred, ascorbic acid levels diminished beacuse ascorbic acid catabolism took place. It has also been demonstrated that more than the usual amount of iron can be deposited in the liver and spleen in anaemia associated with ascorbic acid deficiency [23]. These observations together suggest that there may be a dynamic relationship between iron and ascorbic acid in haemoglobin formation. On the assumption that haemoglobin formation is dependent on an adequate supply of iron and ascorbic acid, it would be expected that a relative shortage of either would interfere with optimal haemoglobin formation. A similar limiting relationship occurs between Vitamin B_{12} and ascorbic acid in patients suffering from pernicious anaemia [12, 13]. Cox [11] suggests that

ascorbic acid therapy can correct erythropoiesis in this condition and produces an increased demand for pteroylglutamates, which, if it cannot be met from the tissues or diet, produces a folic acid deficiency.

The relationship between the leucocyte ascorbic acid concentrations and haemoglobin levels in the Irish samples can be interpreted if it is assumed that availability of iron and ascorbic acid are limiting factors in the production of haemoglobin. When both components are present in abundance, as in the adult male employees, haemoglobin formation is optimal. A significant positive relationship between haemoglobin formation and ascorbic acid concentration therefore existed. In the school boys the ascorbic acid concentration was adequate.

In all the other samples a negative relationship was found because either the iron or the ascorbic acid, were inadequate for optimal haemoglobin formation. In the adult females and the school girls the ascorbic acid concentrations were higher than in the adult males. It may therefore be concluded that iron absorption or utilisation was inadequate. This was more pronounced in the school girls because their haemoglobin levels were lower and their ascorbic acid levels were higher than in the adult females. It has been pointed out that menstruating girls have a lower haemoglobin level than non-menstruating girls [16] and the level remains low for two to three years in the menstruating girls [24]. The school girls in the Irish survey were found to have the high leucocyte ascorbic acid values which occur concomitantly with low adolescent haemoglobin values. This observation confirms that the inverse relationship, shown to exist between haemoglobin levels and ascorbic acid concentrations in haemochromatosis [29] also occurs under apparently normal conditions in the female adolescent menstruating population, and to a lesser degree in the adult female population. In consequence the school girls had a more negative regression coefficient than the adult females.

The geriatric subjects had negative regression coefficients on account of deficiency of ascorbic acid and of iron [5, 27] to varying degrees. The female geriatric patients in the K group had a relatively low haemoglobin level, which was associated with the lowest ascorbic acid concentrations of all the groups. In consequence, it can be concluded that ascorbic acid was one of the factors which, in association with inadequate iron, limited their attainment of an optimal haemoglobin level.

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Professor C. W. M. Wilson, Department of Pharmacology, University of Dublin, Trinity College, Dublin 2, Ireland

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THE LEUCOCYTE ASCORBIC ACID UPTAKE, DIRECT ANTIGENIC CHALLENGE TEST—A SENSITIVE IN-VITRO TEST FOR ATOPIC ALLERGY

H. S. Loh, K. Watters, C. W. M. Wilson, The Allergy and Immunology Research Clinic, Mercers Hospital, Dublin and Department of Pharmacology, University of Dublin, Trinity College, Dublin 2.

Immediate-type or atopic allergy is mediated by reaginic antibodies (IgE). Allergy of this type is usually detected *in vitro* by measurement of IgE directly, or indirectly by measurement of histamine release as in the test of the direct reaction to antigen in leucocytes (Assem and McAllen). Biological assay of histamine is difficult and so this useful *in vitro* test for immediate-type reactions can only be performed in specialist laboratories.

The leucocyte direct reaction to antigen test of Assem and McAllen has been modified by estimating the amount of ascorbic acid (AA) absorbed by leucocytes in the presence of antigen in 66 atopic subjects. The uptake of AA by leucocytes incubated *in vitro* in 3 mg% of AA buffered at pH7.4 for 2 hours was increased by 54 per cent from the resting blood level *in vivo*. In the presence of 1 per cent antigen solution (sensitivity to the antigen determined by skin tests), the absorption of AA by the leucocytes in the same solution of AA was only 15 per cent at 2 hours. The difference was highly significant ($P<0.001$).

There was no significant difference in AA uptake by the leucocytes when incubated in the presence of AA solution alone, or when incubated with non-sensitive antigen.

The uptake of AA by the leucocytes in 30 normal subjects was the same whether antigen was added to the incubating medium or not. This suggests that the leucocyte ascorbic acid uptake, direct antigen challenge test is a sensitive *in vitro* test for atopic allergy.

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Ascorbic Acid in Chronic Psychiatric Patients — A Controlled Trial

By G. MILNER

Anxiety and excitement have been shown by Maas (1961) to increase the rate of breakdown of ascorbic acid, and in schizophrenics this process may be exaggerated by an abnormality of adrenaline metabolism (Briggs, 1962). Schizophrenics receiving "adequate" dietary amounts of vitamin C, as judged by the requirements of the normal population, are commonly found to have low blood ascorbate levels. Evidence of low blood ascorbate levels in most psychiatric patients has been gathered by Horwitt (1942), Leitner and Church (1956) and others. It has been debated whether such laboratory findings are associated with a state of "subscurvy", where the individual has the complaints of excessive tiredness, depression, irritability and vague ill-health. Many authorities claim that there is no clinical evidence to justify the distinction of a state of "hypovitaminosis C". They claim that one either absorbs sufficient ascorbic acid to maintain health, or so little that the classical condition of scurvy supervenes.

The present trial was initiated in an attempt to clarify these conflicting views, and in order to satisfy the author's own feeling that subscurvy is both a clinical entity common in chronic psychiatric patients and one the psychological concomitants of which contribute to personality disturbance.

Design of Experiment

Forty chronic male patients agreed to take part in the investigation, although it had been decided that only 20 would have the active preparation. Their mean age was 52·6 years (range 29–69) and their mean length of hospitalization was 17·7 years (range 3–45). Four of the patients were suffering with manic-depressive psychosis, two with G.P.I., and 34 with schizophrenia. 16 of the patients (Group B) co-operated in the collection of 24-hour specimens of urine. The remainder of the patients constituted Group A.

The pharmacist prepared two solutions, identical as near as possible, except that one contained ascorbic acid and the other was inert. The solutions were put into medicine bottles with randomly given three-figure numbers, the pharmacist keeping a key to the active and inactive solutions. On the ward the bottles were allotted in random fashion to the patients, though it had been ensured that both groups would receive equal numbers of active and inert preparations. The patients received one dose of the preparation daily for three weeks, those in the active group having 1 G. of ascorbic acid per day.

Before and after the trial all the patients were examined for scorbutic stigmata, their Hb percentage checked, blood ascorbate levels and urinary excretion of ascorbic acid estimated. In order to assess any psychological changes, firstly, the depression scale of the M.M.P.I., which would be self-rating (depending on the patient's subjective interpretation of how he felt) was administered. Secondly, the Wittenborn Psychiatric Rating Scales were used as measures of overtly observable behaviour in the patient, as seen by both doctor and ward nurse. Both the M.M.P.I. and the W.P.R.S. were used before and after the trial.

With regard to the M.M.P.I., reliability coefficients of 0·80 (Holzberg and Alessi, 1949) have been obtained with psychiatric patients. Generally speaking, a high "Depression" score indicates poor morale of the emotional type, with a feeling of uselessness and an inability to

assume a normal optimism with regard to the future. McIntosh (1959) claims that mental depression is an early sign in scurvy and may be so marked as to present as a true depressive illness.

Six of the Wittenborn subscales were used—relating to manic state, depressed state, schizophrenic excitement, paranoid state, paranoid and hebephrenic schizophrenia. The reliability coefficients ("r") of these subscales are high and were based on different samples of 100 patients each (Wittenborn, 1951); they are shown in Table 2. These psychiatric rating scales are a procedure for recording the observed behaviour of mental patients and for describing them according to their current symptoms. The scales provide for the assignment of numerical values to indicate the presence and degree of pathological symptoms in a patient. In order that their repeated use may reveal changes in the patient, the scales are restricted to currently discernible behaviour. The statistic used for rating was a mean of the raw scores given by doctor and nurse for each patient.

Whilst taking the ascorbic acid or placebo, 24-hour specimens of urine were collected from the patients of Group B and their output of ascorbic acid measured.

Results

(1) *Physical Examination and Hb Percentage*

None of the patients were markedly anaemic and there were no significant changes in Hb percentage.

Twelve patients (eight in the active and four in the placebo group) had signs attributable to vitamin C deficiency before the trial. These signs (which included follicular hyperkeratosis, minute haemorrhages around hair follicles, haemorrhagic gingivitis and dyspepsia) cleared in five patients after saturation with ascorbic acid. The control group showed no change in this respect.

(2) *Psychological Testing*

The results with the patient's self-rating scale (M.M.P.I.) showed an improvement in the depression scale significant at the 1 per cent. level of probability (see Table I), in the active group.

Since the active and placebo groups, treated separately, were relatively small samples (N = 20), a technique of paired comparisons, whereby each patient is matched with himself, was used. A critical ratio was obtained by dividing the mean difference in scores by the standard error of the difference (S.E. Diff.).

TABLE I

M.M.P.I. Results. Depression Scale.

(These results are shown more graphically in Fig. 1.)

Group	Mean Standard Score		Mean Diff.	S.E. (m. diff.)		P
	Before	After				
Active ...	72·8	64·8	8·0	2·81	2·85	<0·01
Placebo ...	71·3	72·75	−1·45	2·5	0·58	Not Sig.

(This result was calculated after omitting the scores of one patient, who filled out the M.M.P.I. incorrectly.)

The Wittenborn results are shown in Table II and the changes in the rating scores are shown more graphically in Fig. 1. One of the most interesting features in this diagram is that the direction of change in the active group (shown by cross hatching in Fig. 1) is always towards improvement and always more than in the placebo group. There were three subscales, manic state, depressed and paranoid states,

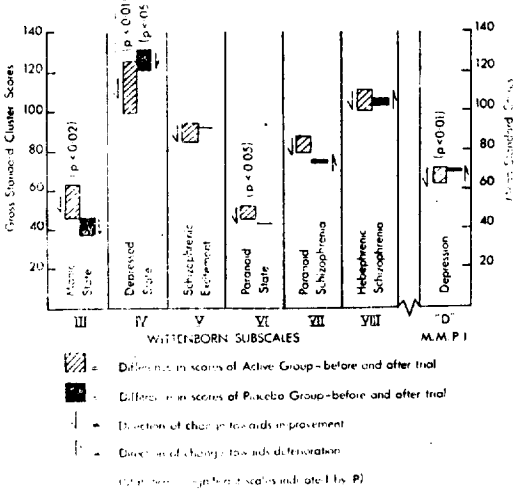


FIG. 1

TABLE II

Wittenborn Results

Symptom Complex	Group	Cluster Score Before	Cluster Score After	Mean Diff.	S.E. (m.d.)	t	P	Subscale "r"s
Manic state	Active	66	50	0·8	0·34	2·63	<0·02	0·92
	Placebo	49	42	0·35	0·22			
Depressed state	Active	117	89	1·4	0·302	4·64	<0·01	0·67
	Placebo	124	116	0·4	0·183	2·19	<0·05	
Schizophrenic excitement ..	Active	96	85	0·55	0·81			0·90
	Placebo	95	95	0	0·75			
MM								
Paranoid state	Active	53	46	0·45	0·211	2·13	<0·05	0·76
	Placebo	45	45	0	0·183			
Paranoid schizophrenia ..	Active	87	82	0·25	0·25			0·76
	Placebo	77	78	−0·05	0·235			
Hebephrenic schizophrenia..	Active	112	102	0·5	0·303			0·81
	Placebo	104	108	−0·2	0·213			

where this degree of improvement was statistically as well as clinically significant. This will be commented on in the discussion.

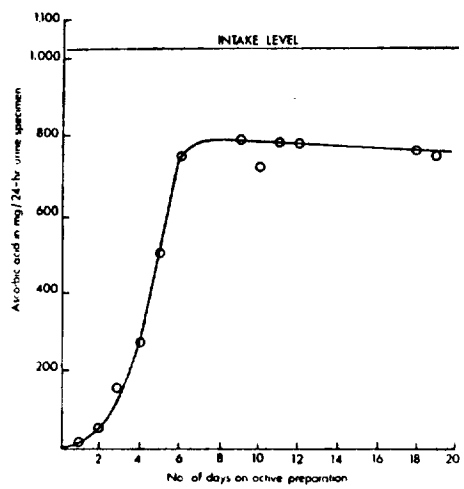
(3) *Subjective Results*

Subjective feelings of improvement in general well-being were reported by eight patients, five of these being in the active group. The nurse felt that five patients had obviously improved, while the ward doctor formed the impression, on general grounds, that four of those reported by the nurse had improved. All five patients proved to be in the active group. These subjective impressions were not submitted to statistical analysis.

(4) *Ascorbic Acid Excretion*

After the first 24-hour specimens of urine were collected, the patients were given the first dose of the test solution allotted to them.

The ascorbic acid excretion results are shown in Tables III and IV and Fig. 2. The mean urinary excretion of ascorbic acid by the patients of Group B in the placebo group was 15·2 mg. in 24 hours—at the very lowest limit of normal. The saturation curve for the active group patients is shown graphically in Fig. 2, where it can be seen that the mean time necessary for



(O REPRESENTS AVERAGE RESULT FOR 4 PATIENTS ON EACH DAY INDIVIDUAL EXCRETION RATES SHOWN IN TABLE 4)

FIG. 2

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TABLE III

24-hour Urinary Excretion of Ascorbic Acid in Members of Group B receiving Placebo Solution (Expressed as mg.)

Patient	Day of Collection												Mean
	1	2	3	4	5	6	9	10	11	12	18	19	
a	8·8		9·2		14·6		13·8		14·6		12·2		12·2
b	9·4		8·9		16·2		16·2		40·7		22·8		19·0
c	20·1		33·6		28·8		17·8		16·9		20·6		23·0
d	13·2		14·8		10·7		11·2		15·8		18·9		14·1
e		9·4		11·6		22·4		18·8		14·5		8·2	14·1
f		17·8		11·2		8·6		8·9		11·2		12·7	11·7
g		12·2		11·8		10·4		13·3		8·0		20·1	12·6
h		14·1		16·2		10·3		12·7		18		18·7	15·0
												Overall mean:	15·2

TABLE IV

24-hour Urinary Excretion of Ascorbic Acid in Members of Group B receiving Active Solution (Expressed as mg.)

Patient	Day of Collection											
	1	2	3	4	5	6	9	10	11	12	18	19
i	11·2		107		430		732		760		710	
j	17·8		197		517		800		764		803	
k	20·7		168		448		796		791		772	
l	30·2		152		602		830		824		786	
m		32·3		210		674		656		711		722
n		45·8		336		742		716		774		703
o		78·2		267		808		748		805		798
p		43·6		272		772		753		842		800
Mean =	19·9	49·8	156	271	496	739	789	719	785	783	763	753

saturation was 6 days. The consistently low readings on the 10th day were probably explained by some delay in the estimation of the urinary ascorbic acid concentration. The slight drop in excretion of the vitamin from the 8th to the 10th day of the trial was probably due to deterioration, under the influence of light, etc., of the vitamin C in the active preparation, though the bottles were protected as far as possible.

The mean urinary excretion of ascorbic acid by the patients in the placebo group was at the lowest limits of normal excretion rates. (Harris and Ray, 1935, found the minimum daily excretion in healthy subjects, having a little fruit each day, was 20 mg. The average delay before saturation with the vitamin was six days (see Fig. 2). Harris and Ray (1935, claimed that patients with scurvy or on the borderline of scurvy, required 7 to 10 days for saturation. Normal subjects can be saturated with the vitamin within 24–48 hours, by giving them 1 G. of the vitamin per day.

Discussion

The human organism depends on external sources for its supply of vitamin C, a daily intake of about 30–100 mg. being adequate for normal subjects (M.R.C. Report, 1948; McIntosh, 1959). Stress, anxiety and excitement accelerate the depletion of ascorbic acid, and the results of this trial indicate that these chronic psychiatric patients were suffering from subscurvy. Especially significant was the improvement in the depressed state of the patients. The depression scale on the M.M.P.I., seemed to reflect a general factor, as was described, and also the scales on the Wittenborn, where a significant improvement was found in the active group, were those where general personality functioning would be reflected, rather than the acuteness of any more specific psychiatric syndrome. Both active and placebo groups improved significantly with respect to their "depressed state", but the difference between the two groups was in itself statistically significant at the 1 per cent. level of probability.

The suggestion from the results is that saturation with vitamin C has brought about an improvement in overall personality functioning. Conversely, the psychological and physical improvement shown by these long-stay psychiatric patients after saturation with ascorbic acid indicates that their diet has been unsatisfactorily low in vitamin C content. This had led to a deficiency state—subscurvy—having quite definite psychiatric symptoms, the most important of which are marked depression and irritability.

It would seem likely that the now fairly general upgrading of hospital catering is not enough—psychiatric patients have greater demands for ascorbic acid than normal subjects, (Briggs, 1962). States of depression and anxiety associated with psychiatric disorders are probably accentuated by an inadequate intake of ascorbic acid. The administration of synthetic ascorbic acid to psychiatric patients would seem to be indicated.

Summary

(1) A controlled, blind trial of ascorbic acid saturation, involving 40 male, chronic psychiatric patients, is described.

(2) Standardized (objective and subjective) psychological techniques were used to assess changes dependent upon ascorbic acid intake.

(3) A clinical state of subscurvy was found in these patients.

(4) Psychiatric patients are shown to have an unusually high demand for ascorbic acid.

(5) Statistically significant improvement in the depressive, manic and paranoid symptom-complexes, together with an improvement in overall personality functioning, was obtained following saturation with ascorbic acid.

(6) It is suggested that chronic psychiatric patients would benefit from the administration of ascorbic acid.

Acknowledgments

Acknowledgments are made to Drs. H. B. Kidd and R. W. Lennon, Mr. D. F. Clark (Principal Clinical Psychologist) and Mr. T. Haines (Charge Nurse) for their most valuable assistance, as well as to Miss K. Smith and Mrs. J. Boldizsar, who did the secretarial work.

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6590

LARGE ASCORBIC ACID INTAKE

To the Editor: I wish to comment on the reference made regarding ascorbic acid as a causative factor of oxalic stones in the paper entitled, "Acquired Hyperoxaluria, Nephrolithiasis and Intestinal Disease" (N Engl J Med 286:1371, 1972).

I have used and prescribed large amounts of ascorbic acid for many years without experiencing the findings described. One case to be described is of particular interest. A chemist at the University of Chicago who was in his mid-thirties experienced diminishing visual acuity, which examination revealed to be due to lenticular opacities. Blood, urine and physical examinations were found to be normal. Since there is no specific therapy for this condition, large amounts of ascorbic acid were suggested. Following this suggestion, he took 4 g daily. Four months later, his visual acuity had increased from 20/40 to 20/20. Both lenses were found to be clear. He was advised to discontinue the ascorbic acid because either it or something else had restored his vision to normal. However, he was afraid to do so and kept on taking the same amount for 13 years, after which I lost contact with him. During all this time he had experienced no clinical symptoms of hyperoxaluria or nephrolithiasis. This is an exceptional case.

I have prescribed large amounts of ascorbic acid to many others over a period of years, without any clinical evidence of trouble. There must be other factors involved in the formation of oxalic acid.

Chicago, Ill. EDUARD POSER, M.D.

The above letter was referred to the authors of the article in question, one of whom offers the following reply:

To the Editor: L-ascorbic acid serves a precursor of urinary oxalate in man providing as much as 1/3 of the oxalate present in the urine.[1,2] This pathway apparently has a low capacity in that ingestion of large amounts of ascorbic acid causes only a modest increase in urinary oxalate (from 26 to 40 mg per day).[3] Since the solubility of calcium oxalate in urine is very limited, this modest increase in patients who had high normal or elevated levels of urinary oxalate (some "idiopathic stone-formers," acquired hyperoxaluria, or primary hyperoxaluria) could increase the tendency to formation of stones.

Rochester, Minn. LYNWOOD H. SMITH, M.D.
Mayo Clinic

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6938

Does Hypervitaminosis C Occur?
(Is there such a Thing as Hypervitaminosis C?)*

H. Rietschel
(From the Wurzburg University Children's Hospital)

In general, the existence of a hypervitaminosis C is rejected (refuted) by all authors who have expressed an opinion on this subject (Schroeder, Demole, Kahler and LaCroix, Willstaedt, among others). In his most recent book, "The Theory of Nutrition", Stepp does not even mention hypervitaminosis C. Only Widenbauer, in two articles published in this journal (Klin. Wochenschr. 1936, 815 and 1158) has pointed out that high, nonphysiological doses of vitamin C have given rise, in isolated cases, to "toxic side effects" in adults and infants. He observed the presence of transient erythemas and an increased intestinal peristalsis, and believes that these symptoms can be attributed to an increased tonus of the vagus. However, on the basis of these facts, he still does not want to admit the presence of a definite hypervitaminosis C, but believes rather that "individual people with special vegetative characteristics (traits) might perhaps possess a certain sensitivity to vitamin C". In principle, the following can be stated at first.

Every nutritive substance (and this includes, naturally, also vitamin C) has its own minimum, and the amount of the substance should not lie below this minimum for a long time, and further also an optimum, the range of which is different for every substance; finally, this optimum amount can be exceeded, and then we must actually expect that harmful effects can arise, provided that this nutrient substance is incorporated into the entire metabolism mechanism. Naturally, the organism is endowed with plentiful (numerous) regulations (or control systems) in order not to become immediately sick when the supply of every nutrient substance, and thus also of vitamin C, is too low or lacking, and also when the supply is too high. In the case of vitamin C, this self-aid of the organism can be seen in the fact that it burns up a large portion of the vitamin, and excess vitamin C is excreted as rapidly as possibly in the urine; however, it is quite probable, and

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*Report presented at the Southwest German Convention of Pediatricians in Heidelberg on 7 May, 1939.

actually necessary from a biological standpoint, that a continuous and increased supply of vitamin C, which greatly exceeds the optimum amount, cannot be indifferent for the organism. We could draw here a parallel with inorganic products, e.g. an increased supply of salt (NaCl) or potassium. In the case of vitamin D, it has been established with certainty that an excessive supply of even pure vitamin D can cause damage. I do not even have to mention hormones here, since their optimum range is in general very narrow (low), but the organism knows how to always stay in this narrow optimum range of hormones.

I have recently pointed out in an article published in this journal (1939, No. 5) that, after oral administration of high vitamin C doses (I want to thank Hoffmann-LaRoche and I. G. Farben industry for supplying the necessary amounts of ascorbic acid), an increase in the number of blood platelets (thrombocytes) was found in all infants which we have examined later. Papayanopulos and Schroeder have recently described the same fact in adults. Our studies on infants will be described here, which were carried out by Miss Leonhardt and Miss Imhof.

Together with Papayanopulos and Schroeder, we consider this increase in the thrombocyte count as a result of the high vitamin C administration. The Munich authors speak here about the "effect of a healing substance", but it would be more correct to speak about "a pharmacological effect". Whether this increase represents a favorable or unfavorable factor cannot be recognized at first. Papayanopulos and Schroeder have reported such findings on different human patients, and thus the concept that this increase in the thrombocyte count represents the "effect of a healing substance" is perhaps justified (or worth being advocated). However, we have observed this increase in the thrombocyte count in quite healthy infants, and here it must be really considered as something abnormal, namely pathological, since normally an infant does not have such a high thrombocyte count, and, in addition, a second factor was noted. Many infants exhibited, in the same way as Widenbauer has pointed out, a striking restlessness and insomnia, or also diarrheal stools. In the case of Ingrid F. (see table 1), the weight remained stationary after the daily administration of 200 mg, and the child lost its appetite and became so restless that we discontinued vitamin C after 3 days, and all these symptoms disappeared immediately. I must explicitly stress the fact that most of the infants did not exhibit any further pathological symptoms; however, along with restlessness, other

infants, e.g. Harald Schramm, also had crumbly stools with small pieces embedded in mucus. Widenbauer has also observed such symptoms, which actually indicate that vitamin C had an injurious effect here, and Baumann even reports that a particularly high content of vitamin C in human and cow's milk, when given over a longer period of time, gives rise more readily to dyspeptic stools (Z. Vit. 6, 23).

Table 1 -

No.	Patient	Daily vitamin C dose, mg	Thrombocytes/ml (counted according to Fonio)	Time of Determination
1	Otto V., 6 Mon.		124000	before the test
		100	118000	5-6 days
		200	194000	10-14 = for 5 days
		100	160000	15-17 = for 3 days
		200	202000	18-23 = for 6 days
			132000	after 3 days
2	Irmgard R., 3 1/2 mon.		120000	before the test
		100	127000	3-9 = for 7 days
		200	216000	10-14 = for 5 days
		100	160000 -	
			180000	15-17 = for 3 days
		200	224000	18-23 = for 6 days
			140000	after 3 days
3	Gerlinde D., 3 Mon.		265000	before the test
		100	220000 -	
			268000	8-15 = for 8 days
		200	328000 -	
			346000	16-18 = for 3 days
			196000	after 2 days
4	Ingrid F., 3 Mon.		185000	before the test
		100	199000	for 8 days
		200	472000	for 3 days
			191000	
5	Harald Schrb. 2 Mon.		114000	before the test
		100	124000	for 8 days
		200	280000	for 3 days
			112000	
6	Adelheid G., 6 Mon.		140000	before the test
		200	252000	after 12 days
7	Helga Sch., 4 Mon.		204000	before the test
		200	312000	after 12 days
8	Hans Kn., 4 Mon.		193000	before the test
		200	320000	after 12 days

Table 1 - cont.

No.	Patient	Daily vitamin C dose, mg	Thrombocytes/ml (counted according to Fonio)	Time of Determination
9	Peter Kn., 7 Mon.	200	122740 252000	before the test after 12 days
10	Klaus S., 3 1/2 Mon.	200	182000 289000	before the test after 12 days
11	Heinr. M., 15 Mon.	150	297000 478000	before the test after 3 days
12	Ferdinand L., 6 Mon.	250	244000 460000- 570000	before the test after 6 days
13	Georg R., 6 Mon.	250	241000 294000- 396000	before the test after 6 days

At this point it is perhaps proper to give a brief answer to the remarks made by Stepp and Schroeder in their article published in this journal (1939, 414). I want to note that I shall refrain in this connection from examining in detail all the charges made in their article, in order to avoid an unproductive controversy. Only one point shall be mentioned here.

Stepp and Schroeder see namely an argument for the high vitamin C requirement of man in the fact that mother's milk contains 4-6 mg % vitamin C, and the breast-fed infant, when drinking 1 liter of women's milk, thus takes up 40-60 mg vitamin C. These figures refer particularly to a study performed by Neubauer and to older data published by Harris, Bleyer et al. However, Baumann and Widenbauer have also determined the vitamin C content of women's milk and have obtained entirely different results. Baumann found in wet-nurses of the Basle Clinic values of 0.9-1-1.5-2.0 mg%, namely a considerably lower vitamin C content, and Widenbauer reports from Danzig that the vitamin C content of milk in all nursing women is equal to 0.5-2.2 mg%, and thus "is much lower than the normal figures given in the literature". This is not surprising, since the amount of vitamin C in milk is completely dependent upon the supply of vitamin C, as all authors unanimously point out and which is an obvious fact. Thus, we cannot speak about a normal content of vitamin

C in women's milk, since this content is a completely variable magnitude fully dependent upon the external supply of vitamin C. Baumann, who has checked the vitamin C content of the milk of nursing mothers in the city of Basle, found mostly low values in individual women during different months.

In the summer, various individual women showed fluctuations in the vitamin C content of 1.25-2.19, of 0.79-2.23, of 0.83-1.58, and of 0.42-0.91 (in December!). Some women showed in December and January (for the first time) amounts of 0.5, 0.6, 0.92, and 0.8 mg%. Only one value reached in summer 4.2 mg%, and only one, even in winter, reached 5.4 mg%. The latter woman was an Italian, who daily consumed rather large amounts of oranges. The wet nurses examined in our clinic also exhibited relatively low values. We found the following values in 5 wet nurses (examined in May-June on different days) with dichlorophenol indophenol (the lowest and highest values are always given): B.R. 1.76-2.15 mg%, I.R. 0.55-1.13 mg%, M.F. 1.83-2.39 mg%, G.N. 1.51-2.80 mg%, K.M. 1.22-3.27 mg%. We even believe that most nursing infants receive not more, but often less vitamin C from the mother's breast than when fed with cow's milk, especially in the winter and spring, since the vitamin C content of cow's milk is about 2 mg and is naturally much more constant. The statement that an infant drinks from the mother's breast milk with a vitamin C content of 4-6 mg% is not correct in this form and should disappear from the literature.

We have also carried out tests on adults and, in the same manner as the Munich authors but independently from them, have found an increase in the thrombocyte count, and in addition also general symptoms, which definitely indicate that high doses of vitamin C can exert a damaging effect. I personally, after taking 500 mg vitamin C daily for 5 days, have noticed, along with an increase in thrombocytes, such an extraordinary deterioration of sleep that I discontinued the test; the same fact was confirmed by other men and women in my clinic, who took a daily dose of 500 mg vitamin C in a self-test. I wish to report the following case history: Miss A (27 years old), healthy, thrombocyte count 110,000, which increased to 202,000 after 8 days of a daily administration of 500 mg vitamin C. In the last few days, had difficulty in falling asleep, along with a low depth of sleep, which gradually reached the state of insomnia. These complaints (troubles) vaninshed very soon after ascorbic acid was

discontinued.

Similar symptoms were noted in 5 other test subjects. Wordehoff has performed extensive experiments on high vitamin C intake at the Grafe clinic on adults and on himself, and has observed quite similar symptoms. Some people became restless and excited, had trouble falling asleep, their sleep was often interrupted, and in some persons diarrhea was noted, which immediately disappeared after the dosage of vitamin C was discontinued (Wordehoff will report these findings in a forthcoming article). There is no doubt, therefore, that a high (unphysiological) intake of vitamin C can cause disturbances (disorders) in the organism.

In this connection, the following question arises: are these disturbances the expression of a hypervitaminosis C? Widenbauer wants to carefully avoid this expression and speaks only about "toxic side effects in persons with special vegetative characteristics (traits)". We believe that we can use the term hypervitaminosis C to designate these symptoms. We are convinced that we could have induced these symptoms in a much larger number of people if we had administered even larger doses over a longer period of time. The fact that people have a different tolerance and that one person reacts with these symptoms earlier, and another person later, is not surprising and is a known fact for all products. However, since these symptoms always arise after large doses of vitamin C, they must be considered as the result (after-effect) of the high vitamin C doses, and we also consider the higher thrombocyte count as the result of a specific stimulus of vitamin C on the bone marrow, particularly on the genesis of thrombocytes. Thus, we cannot see anything desirable in this reaction in a healthy person. Since a healthy person (infant) normally has a smaller number of thrombocytes than after a high vitamin C dose administration, and since finally further certain pathological symptoms may appear (such as restlessness, insomnia, diarrhea, etc.), we must give an affirmative answer to the question of the existence of a hypervitaminosis C; however, the latter occurs only during prolonged use of unphysiologically high vitamin C doses, because man can protect himself quite well against it (by means of an increased combustion of ascorbic acid and excretion in the urine).

Numerous researchers always continuously report that higher doses of vitamin C cause an increase in performance (or an improvement of the person's physical health), that people feel more fresh, etc. We do not

want by any means to deny (or dispute) these observations, since every administration of vitamin C increases the catalytic processes (oxidative and reducing) in the organism. Strieck found in long-term cage tests on dogs that the total oxygen consumption is increased after intake of vitamin C. In healthy people, an effect of vitamin C on the basal metabolism could not always be achieved, although, according to Strieck, increases of up to 37% in the basal metabolism were noted in individual persons (Biochem. Zeitschr. 277, 279, 1935). Here, however, the condition (state) which we call hypervitaminosis C sets in,,although of course we cannot speak about an illness here. These states of an increase in the rate of catalytic processes caused by vitamin C are, under certain conditions, even useful and therapeutically desirable, although prolonged exposure to the effect of a high vitamin C dosage has, naturally, its own danger.

We agree, however, with all authors, that this danger of a hypervitaminosis C does not exist in the case of a usual (common) diet, even when this diet includes large amounts of vegetables and fruit, since such considerable amounts of vitamin C can hardly be supplied in the form of fruit and vegetables. The situation is different, however, when synthetic ascorbic acid is added daily to our diet in large amount. Even in this case, we do not consider that there is any great danger, since the requirement for vitamin C is higher during infectious diseases, in pregnancy and after muscular exertion, and nothing can be said against additional administration of vitamin C; however, a continuous daily administration of 200 to 300-500 mg over a long period of time cannot be considered as indifferent, and we would like to ask all critical physicians to pay attention to their patients in this respect. Nevertheless, we believe that a hypervitaminosis C practically very seldom occurs in life or not at all (see note), and is observed only in clinics or in fanatical vitamin C eaters, who consume the vitamin as such. Theoretically, however, this hypervitaminosis C is of very great interest.

(Note: We can only think of the following. Grape and other fruit cures always have a favorable effect on the digestion, in the sense of a purgative or laxative effect. It is quite possible that here also the high vitamin C content could have a concurrent effect, in the same way as in infants who suffer more readily from diarrhea when the milk has a high

vitamin C content (Baumann). De Rudder, during the discussion of my paper, pointed out that he intends to investigate whether any people exhibit, during such fruit cures, also other symptoms of a hypervitaminosis C).

Widenbauer assumes that the tonus of the vagus is increased by vitamin C, and such an interpretation has a lot in its favor. It is true that insomnia indicates rather a sympathicotonic influence. Tislowitz (Klin. Wochenschr. 1935, 1641) has also observed in dogs, given large amounts of vitamin C, similar symptoms, which suggest the presence of an increased vagotonia (temperature drop, slowing of the heart beat, increased diuresis, tonus increase of the vascular wall, reduction in the amount of circulating blood, fatigue). Schade also mentions vagotonia after high vitamin doses (slower pulse, increased intestinal peristalsis) (Klin. Wochschr. 1935, 60).

Finally, this relation between vitamin C and blood platelets provides an explanation for many therapeutic successes achieved with vitamin C. It has been clearly established today that true thrombopenia (Werlhof's disease) is not an avitaminosis C and cannot be therapeutically affected by vitamin C. Nevertheless, reports continue to be found in the literature, indicating that a therapeutic success can be achieved with vitamin C in thrombopenic states. We believe rather that in those cases where a definite increase of thrombocytes was noted after ascorbic acid, the actual disease noxa of the thrombocytopenia was subsiding and that then ascorbic acid contributed in a supporting rôle to the increase in the number of blood platelets. Whether ascorbic acid acts as a stimulant for an increased regeneration (neoformation) or only for an elimination (flooding out) of thrombocytes is still a matter open to discussion. We believe, however, that ascorbic acid can definitely be used in such cases, but it should be clear that <u>no hypovitaminosis A or C</u> is present in all such states. With such a concept in mind, the use of vitamin C would be brought on an entirely wrong track.

A broad field of study opens up here for the question of vitamin C, which requires further careful clinical studies, since here also we stand at the beginning of our knowledge.

Summary

Large unphysiological doses of synthetic ascorbic acid can cause symptoms of a pathological reaction in infants and adults. In all infants, an increase in the number of blood platelets was found, which is in agreement with the studies performed by Papayanopulos and Schroeder. In some isolated cases, both among infants and adults, dyspeptic symptoms, great restlessness, insomnia, etc. were noted. The question of a hypervitaminosis C is therefore given an affirmative answer. Practically, however, the problem is of very little importance, but attention must be given to the fact that such pathological reactions should not occur more frequently during the administration of vitamin C, which has recently become fashionable. It appears probably that vitamin C leads to an increase in the rate of catalytic processes, which, under certain conditions, can be useful from a therapeutic standpoint. Such an indication, however, has nothing to do with an avitaminosis C or a hypovitaminosis C.

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GIBT ES EINE C-HYPERVITAMINOSE?*

Von

H. RIETSCHEL.

Aus der Universitäts-Kinderklinik Würzburg.

Eine C-Hypervitaminose wird im allgemeinen von allen Autoren, die sich darüber geäußert haben, abgelehnt (SCHROEDER, DEMOLE, KAHLER und LA CROIX, WILLSTAEDT u. a.). STEPP erwähnt in seinem neuesten Buch „Ernährungslehre" eine C-Hypervitaminose überhaupt nicht. Nur WIDENBAUER hat in 2 Arbeiten in der Klin. Wschr. 1936, 815 u. 1158 darauf hingewiesen, daß hohe unphysiologische C-Vitamingaben in einzelnen Fällen bei Erwachsenen und Säuglingen „toxische Nebenwirkungen" zum Vorschein brachten. Er hat bei Säuglingen flüchtige Erytheme, erhöhte Peristaltik des Darms wahrgenommen und glaubt, diese Erscheinungen auf einen erhöhten Tonus des Vagus zurückzuführen. Er will aber damit noch nicht eine sichere C-Hypervitaminose annehmen, sondern meint, daß „einzelne besonders vegetativ stigmatisierte Menschen vielleicht eine gewisse C-Vitaminempfindlichkeit" besäßen. Prinzipiell ist zunächst folgendes zu sagen:

Jeder Nahrungsstoff — und dazu gehört natürlich auch das C-Vitamin —, hat sein Minimum, das nicht auf die Dauer unterschritten werden darf, ferner ein Optimum, dessen Spannweite verschieden groß ist, und schließlich können wir dies Optimum überschreiten, und dann müssen wir eigentlich erwarten, daß auch schädliche Wirkungen auftreten, vorausgesetzt, daß dieser Nahrungsstoff in das ganze Stoffwechselgetriebe eingeschaltet ist. Selbstverständlich verfügt der Organismus über ausgiebige Regulationen, um sowohl bei zu geringer oder fehlender Zufuhr wie bei dauernd allzu großer Zufuhr jedes Nahrungsstoffes, also auch des C-Vitamins, nicht sofort zu erkranken. Wir sehen beim C-Vitamin diese Selbsthilfe daran, daß er einen großen Teil des C-Vitamins verbrennt und das überschüssige C-Vitamin möglichst schnell im Urin ausscheidet, aber es ist durchaus wahrscheinlich, ja eigentlich biologisch notwendig, daß eine dauernd erhöhte Zufuhr von C-Vitamin, die weit über dem Optimum liegt, für den Organismus nicht gleichgültig sein kann. Man könnte hier Parallelen mit anorganischen Stoffen (z. B. erhöhte Kochsalz- oder Kaliumzufuhr) anführen. Für das D-Vitamin ist sichergestellt, daß übergroße Zufuhr selbst reinen D-Vitamins Schaden verursachen kann. Auf die Hormone brauche ich gar nicht einzugehen, da hier die optimale Breite im allgemeinen sehr gering ist, aber der Organismus versteht es, bei den Hormonen stets in gesundem Zustand in dieser geringen optimalen Breite zu bleiben.

Ich habe kürzlich in einer Arbeit darauf hingewiesen (diese Wschr. 1939, Nr 5), daß wir nach hohen C-Vitamingaben** (peroral) bei *allen* Säuglingen, die wir daraufhin untersuchten, eine Steigerung der Blutplättchen fanden und PAPAYANOPULOS und SCHROEDER haben kürzlich ebenfalls diese Tatsache bei Erwachsenen beschrieben. Ich lasse hier unsere Untersuchungen bei Säuglingen folgen, die von Frl. LEONHARDT und Frl. IMHOF durchgeführt wurden.

Wir betrachten mit PAPAYANOPULOS und SCHROEDER diese Steigerung der Thrombocyten als eine Folge der hohen C-Vitamindarreichung. Die Münchener Autoren sprechen hier von einer „Heilstoffwirkung", richtiger wäre, von einer „pharmakologischen Wirkung" zu reden. Ob die Steigerung etwas Günstiges oder Ungünstiges vorstellt, ist zunächst nicht erkennbar. PAPAYANOPULOS und SCHROEDER haben an verschiedenen erkrankten Menschen die Befunde erhoben, und

Nr.	Patient	Tägliche Vitamin C-Dosis mg	Thrombocyten in ccm (nach Fonio ausgezählt)	Zeit der Wertung
1	Otto V., 6 Mon.		124000	vor dem Versuch
		100	118000	5—6 Tage
		200	194000	10—14 = 5 Tage lang
		100	160000	15—17 = 3 Tage lang
		200	202000	18—23 = 6 Tage lang
			132000	nach 3 Tagen
2	Irmgard R., $3^1/_2$ Mon.		120000	vor dem Versuch
		100	127000	3—9 = 7 Tage lang
		200	216000	10—14 = 5 Tage lang
		100	160000 bis 180000	15—17 = 3 Tage lang
		200	224000	18—23 = 6 Tage lang
			140000	nach 3 Tagen
3	Gerlinde D., 3 Mon.		265000	vor dem Versuch
		100	220000 bis 268000	8—15 = 8 Tage lang
		200	328000 bis 346000	16—18 = 3 Tage lang
			196000	nach 2 Tagen
4	Ingrid F., 3 Mon.		185000	vor dem Versuch
		100	199000	8 Tage lang
		200	472000	3 Tage lang
			191000	
5	Harald Schrb. 2 Mon.		114000	vor dem Versuch
		100	124000	8 Tage lang
		200	280000	3 Tage lang
			112000	
6	Adelheid G., 6 Mon.		140000	vor dem Versuch
		200	252000	nach 12 Tagen
7	Helga Sch., 4 Mon.		204000	vor dem Versuch
		200	312000	nach 12 Tagen
8	Hans Kn., 4 Mon.		193000	vor dem Versuch
		200	320000	nach 12 Tagen
9	Peter Kn., 7 Mon.		122740	vor dem Versuch
		200	252000	nach 12 Tagen
10	Klaus S., $3^1/_2$ Mon.		182000	vor dem Versuch
		200	289000	nach 12 Tagen
11	Heinr. M., 15 Mon.		297000	vor dem Versuch
		150	478000	nach 3 Tagen
12	Ferdinand L., 6 Mon.		244000	vor dem Versuch
		250	460000 bis 570000	nach 6 Tagen
13	Georg R., 6 Mon.		241000	vor dem Versuch
		250	294000 bis 396000	nach 6 Tagen

so wäre vielleicht die Auffassung, daß diese Blutplättchensteigerung eine „Heilstoffwirkung" darstelle, allenfalls vertretbar. Wir haben aber an ganz gesunden Säuglingen diese Steigerung der Thrombocyten beobachtet, und da muß man sie eigentlich als etwas Anormales, also Pathologisches, betrachten, denn normalerweise hat eben der Säugling keine so hohe Blutplättchenzahl, und dazu kommt ein zweites. Manche Kinder zeigten ganz ebenso, wie WIDENBAUER das hervorhebt, eine auffallende Unruhe und Schlaflosigkeit oder auch durchfällige Stühle. Bei Ingrid F. blieb seit der täglichen Zugabe von 200 mg das Gewicht stehen, das Kind wurde appetitlos und so unruhig, daß wir nach 3 Tagen das Vitamin C absetzten und sofort hörten alle diese Symptome auf. Ich muß aber ausdrücklich betonen, daß die Mehrzahl der Säuglinge keine weiteren krankhaften Symptome boten; aber neben der Unruhe boten andere, z. B. Harald Schramm, auch zerfahrene Stühle. Auch WIDENBAUER hat solche Symptome beobachtet, die in der Tat dafür sprechen, daß hier das C-Vitamin schädlich gewirkt hat, und BAUMANN gibt sogar

* Vortrag, gehalten bei der Südwestdeutschen Tagung der Kinderärzte in Heidelberg am 7. Mai 1939.

** Für die Bereitstellung der Ascorbinsäuremengen danke ich der Firma Hoffmann-La Roche und der I. G. Farbenindustrie.

an, daß ein besonders hoher C-Vitamingehalt der Frauenmilch und Kuhmilch auf längere Dauer gegeben leichter dyspeptische Stühle bewirkt (Z. Vit. 6,23)*.

Wir haben auch Versuche an Erwachsenen gemacht und ebenso wie die Münchener Forscher, aber unabhängig von ihnen, einen Anstieg der Thrombocyten gefunden, daneben auch Allgemeinerscheinungen, die durchaus dafür sprechen, daß hohe C-Vitamindosen schädigend wirken können. Ich selbst habe nach 5tägiger Einnahme von je 500 mg C-Vitamin neben einer Steigerung der Thrombocyten eine außerordentliche Verschlechterung des Schlafens bemerkt, daß ich den Versuch abbrach; und dasselbe bestätigten mir andere Herren und Damen meiner Klinik, die im Selbstversuch 500 mg täglich nahmen. Ich führe folgendes Protokoll an:

Frl. A. (27 Jahre), gesund, Blutplättchen 110000, nach 8 Tagen bei tägl. Zugabe von 500 mg C-Vitamin Steigerung auf 202000. In den letzten Tagen erschwertes Einschlafen, neben geringer Schlaftiefe, die sich allmählich bis zur Schlaflosigkeit steigert. Nach Absetzen der Ascorbinsäure verschwinden sehr bald die Beschwerden.

Ähnlich erging es 5 anderen Versuchspersonen. WÖRDEHOFF hat an der Grafeschen Klinik ausgedehnte Erfahrungen über hohe C-Vitaminzufuhr an Erwachsenen und an sich selbst gemacht und hat dabei ganz ähnliche Symptome beobachtet. Einzelne Menschen wurden unruhig und erregt, sie schliefen schlecht ein, der Schlaf war oft unterbrochen, und bei einzelnen Personen stellten sich Durchfälle ein, die mit dem Absetzen der C-Vitaminmengen sofort verschwanden**. Es unterliegt daher keinem Zweifel, daß hohe (unphysiologische) C-Vitamingaben im Organismus Störungen verursachen können.

Es erhebt sich hierbei die Frage: Sind diese Störungen der Ausdruck einer C-Hypervitaminose? WIDENBAUER will diesen Ausdruck vorsichtigerweise vermeiden und spricht nur „von toxischen Nebenwirkungen bei vegetativ stigmatisierten Personen". *Wir glauben*, daß wir *diese Symptome mit dem Ausdruck C-Hypervitaminose bezeichnen können.* Wir sind überzeugt, daß wir diese Erscheinungen bei viel mehr Menschen hätten auslösen können, wenn wir noch größere Dosen längere Zeit gegeben hätten. Daß die Menschen eine verschiedene Toleranz haben und der eine früher, der andere später mit diesen Symptomen reagiert, ist nichts Auffälliges und für alle Stoffe bekannt. Da aber all diese Symptome immer wieder nach großen C-Dosen auftreten, so sind sie als Folge der hohen C-Vitamindosen anzusprechen, und wir betrachten auch die erhöhte Blutplättchenzahl als die Folge eines spezifischen Reizes des C-Vitamins auf das Knochenmark, insbesondere auf die Thrombocytengenese. Wir können also in dieser Reaktion nichts Wünschenswertes beim *gesunden* Menschen erblicken. Da der gesunde Mensch (Säugling) normalerweise weniger Thrombocyten zeigt als nach hoher C-Vitamindarreichung und schließlich sich weitere sichere pathologische Symptome anschließen könnten (Unruhe, Schlaflosigkeit, Durchfälle usw.), so bejahen wir die Frage der C-Hypervitaminose, allerdings tritt sie *nur bei längerem Gebrauch unphysiologisch hoher C-Dosen auf*, weil der Mensch sich sehr gut dagegen schützen kann (Verbrennungssteigerung der Ascorbinsäure und Ausscheidung durch den Urin).

Von sehr vielen Forschern wird immer wieder angegeben, daß höhere Dosen von C-Vitamin eine Leistungssteigerung hervorrufen, die Menschen fühlen sich frischer usw. Wir möchten diese Beobachtungen keinesfalls in Abrede stellen, denn jede Zufuhr von C steigert die katalytischen Vorgänge (oxydative und reduzierende) im Organismus*. Hier beginnt aber jener Zustand, den wir C-Hypervitaminose nennen, ohne daß wir natürlich von Krankheit reden können. Diese Zustände einer Steigerung der katalytischen Vorgänge durch C sind unter Umständen sogar zweckdienlich und therapeutisch erwünscht, aber auf lange Zeit unter dem Einfluß einer hohen C-Vitamingabe zu stehen, hat natürlich auch seine Gefahren.

Wir befinden uns aber mit allen Autoren in Übereinstimmung, daß bei einer gewöhnlichen Kost, selbst wenn sie gemüse- und obstreich ist, diese Gefahr einer Hypervitaminose nicht besteht, denn so erhebliche Mengen von C-Vitamin können in Form von Gemüse und Obst wohl kaum zugeführt werden. Anders liegen schon die Dinge, wenn synthetische Ascorbinsäure täglich in größeren Mengen unserer Diät zugesetzt wird. Auch hier halten wir die Gefahr für nicht sehr erheblich, denn bei Infektionskrankheiten, in der Gravidität, nach Muskelanstrengungen ist der Bedarf an C-Vitamin gesteigert, und hier ist gegen eine zusätzliche Gabe von C-Vitamin nichts zu sagen; aber dauernde Zufuhr von 200 bis 300—500 mg pro Tag längere Zeit fortgesetzt, halten wir nicht für gleichgültig, und wir möchten alle kritischen Ärzte bitten, hier auf ihre Patienten zu achten. Immerhin glauben wir, daß die C-Hypervitaminose praktisch im Leben sehr selten oder nicht vorkommt** und nur in Kliniken oder bei fanatischen C-Vitaminessern, die das C-Vitamin in Substanz essen, beobachtet werden kann. Theoretisch beansprucht aber diese C-Hypervitaminose ein sehr hohes Interesse.

WIDENBAUER nimmt einen erhöhten Tonus des Vagus durch das Vitamin C an, und eine solche Deutung hat viel für sich. Freilich spricht die Schlaflosigkeit mehr für eine sympathicotonische Beeinflussung. TISLOWITZ (Klin. Wschr. 1935, 1641) hat an Hunden nach reichlichen C-Gaben auch derartige Erscheinungen beobachtet, die an einen erhöhten Vagotonus denken lassen (Temperatursenkung, Verlangsamung des Herzschlages, Zunahme der Diurese, Tonuszunahme der Gefäßwand, Verminderung der zirkulierenden Blutmenge, Müdigkeit). Auch SCHADE spricht von Vagotonie nach hohen Vitamindosen (langsamer Puls, gesteigerte Darmperistaltik) (Klin. Wschr. 1935, 60).

Endlich läßt dieser Zusammenhang zwischen C-Vitamin und Blutplättchen manchen therapeutischen Erfolg mit C-Vitamin verstehen. Es steht heute wohl fest, daß die echte Thrombopenie (Morbus Werlhof) keine C-Avitaminose ist und nicht durch C-Vitamin therapeutisch beeinflußt wird. Dennoch finden sich in der Literatur wiederholt Angaben, aus

*Hier ist vielleicht der Ort, ganz kurz STEPP und SCHROEDER auf ihren Aufsatz in der Klin. Wschr. 1939, 414 zu antworten. Ich bemerke, daß ich dabei zunächst absehe, ausführlich auf alle Angriffe einzugehen, um eine unfruchtbare Polemik zu vermeiden. Nur ein Punkt sei hier erwähnt:

STEPP und SCHROEDER sehen nämlich ein Argument für den hohen C-Vitaminbedarf des Menschen darin, daß die Muttermilch 4—6 mg% C-Vitamin enthält, der Brustsäugling also bei 1 l Frauenmilch Trinkmenge etwa 40—60 mg C-Vitamin zu sich nimmt. Diese Zahlen beziehen sich besonders auf eine Arbeit NEUBAUERS, sowie ältere Angaben von HARRIS, BLEYER u. a. BAUMANN und WIDENBAUER haben aber auch den C-Gehalt der Frauenmilch bestimmt und sind zu ganz anderen Resultaten gekommen. BAUMANN fand bei Ammen der Basler Klinik Werte von 0,9—1—1,5—2,0 mg%, also erheblich weniger, und WIDENBAUER teilt aus Danzig mit, daß bei sämtlichen stillenden Frauen der C-Gehalt der Milch bei 0,5—2,2 mg%, also „weit unter den Normalzahlen der Literatur" lag. Das ist nicht verwunderlich, denn die Menge C-Vitamin in der Milch ist, wie alle Autoren übereinstimmend betonen und was selbstverständlich ist, völlig abhängig von der Vitamin-C-Zufuhr. *Man kann also nicht von einem Normalgehalt der Frauenmilch an Vitamin C sprechen, denn der Gehalt von C-Vitamin in Frauenmilch ist eine völlig veränderliche Größe und ganz von der exogenen Zufuhr von C abhängig.* BAUMANN, der stillende Mütter der Stadt Basel auf ihren C-Gehalt untersuchte, fand dort bei einzelnen Frauen in den verschiedenen Monaten meist niedrige Werte.

Im Sommer boten verschiedene einzelne Frauen Schwankungen von 1,19—2, von 1,25—2,19, von 0,79—2,23, von 0,83—1,58, von 0,42—0,91 (im Dezember!). Einige Frauen zeigten im Dezember und Januar (erstmalig) Mengen von 0,5, 0,6, 0,92, 0,8. Nur ein Wert erreichte im Sommer 4,2 mg%, und nur einer sogar im Winter 5,4 mg%. Die letztere Frau war eine Italienerin, die täglich relativ große Mengen Orangen zu sich nahm. Die Ammen unserer Klinik boten auch relativ niedrige Werte. Wir fanden an verschiedenen Tagen bei 5 Ammen (mit Dichlorphenolindophenol) folgende Werte (im Mai-Juni untersucht und stets der niedrigste und höchste Wert angegeben) Amme B. K. 1,76—2,15 mg%, Amme L. K. 0,55—1,13 mg%, Amme M. F. 1,83—2,39 mg%, Amme G. N. 1,51—2,86 mg%, Amme K. M. 1,22—3,27 mg%. Wir glauben sogar, daß die meisten Säuglinge an der Brust der Mutter, besonders im Winter und Frühling, nicht mehr, öfter auch weniger C-Vitamin zu sich nehmen als bei Kuhmilchernährung, denn der Gehalt von C-Vitamin in der Kuhmilch beträgt etwa 2 mg und ist natürlich viel gleichmäßiger. Der Satz, daß der *Säugling an der Brust der Mutter eine Milch von 4—6 mg% C-Vitamingehalt trinkt, ist in dieser Form nicht richtig und muß aus der Literatur verschwinden.*

** WÖRDEHOFF wird darüber in einer Arbeit noch berichten.

* STRIECK fand bei Hunden in langfristigen Kammerversuchen eine Steigerung des Gesamtsauerstoffverbrauchs nach Zufuhr von C-Vitamin. Bei gesunden Menschen war nicht stets eine Wirkung auf den Grundumsatz durch C-Vitamin zu erzielen, doch kamen bei einzelnen Personen nach STRIECK Steigerungen bis zu 37% vor [Biochem. Z. 277, 279 (1935)].

** Nur an folgendes könnte man denken. Trauben- und andere Obstkuren haben stets einen günstigen Einfluß auf die Verdauung im Sinne einer abführenden Wirkung. Es wäre durchaus möglich, daß auch hier der hohe C-Vitamingehalt mit wirksam sein könnte, genau wie bei den Säuglingen, die bei hohem C-Vitamingehalt der Milch leichter Durchfälle bekommen (BAUMANN). DE RUDDER wies in der Diskussion zu meinem Vortrag hin, zu untersuchen, ob nicht manche Personen bei solchen Obstkuren auch andere Symptome der C-Hypervitaminose zeigten.

denen ein therapeutischer Erfolg bei thrombopenischen Zuständen hervorgeht. Wir glauben vielmehr, daß dort, wo man ein deutliches Ansteigen der Thrombocyten nach Ascorbinsäure sah, die eigentliche Krankheitsnoxe der Thrombopenie im Abklingen war und daß dann die Ascorbinsäure unterstützend zur Vermehrung der Blutplättchen mithalf. Ob die Ascorbinsäure zu einer erhöhten Neubildung oder nur zu einer Ausschwemmung von Thrombocyten anregt, steht wohl noch zur Diskussion. Wir glauben aber, daß man durchaus in solchen Fällen von der Ascorbinsäure Gebrauch machen soll, nur sei man sich klar, daß bei all diesen Zuständen keine *A- oder Hypovitaminose von Vitamin C* vorliegt. Mit einer solchen Vorstellung würde die C-Vitaminanwendung in ein völlig falsches Geleise gebracht.

Hier tut sich ein großes Arbeitsgebiet für die C-Vitaminfrage auf, das weiterer sorgfältigster klinischer Bearbeitung bedarf, denn auch hier stehen wir erst am Anfang unserer Erkenntnisse.

Zusammenfassung: Große unphysiologische Dosen synthetischer Ascorbinsäure können bei Säuglingen und Erwachsenen Symptome pathologischer Reaktion hervorrufen. Bei allen Säuglingen fand sich ein Ansteigen der Blutplättchen, was im Einklang mit den Untersuchungen von Papayanopulos und Schroeder steht. Bei Säuglingen und Erwachsenen traten auch in einzelnen Fällen dyspeptische Erscheinungen, sowie starke Unruhe, Schlaflosigkeit usw. auf. Die Frage der C-Hypervitaminose wird daher bejaht. Praktisch hat sie wohl sehr geringe Bedeutung, doch ist darauf zu achten, ob bei der neuerdings in Mode gekommenen Zufuhr von C-Vitamin nicht doch häufiger solche pathologische Reaktionen auftreten. Es ist wahrscheinlich, daß das C-Vitamin zu einer Steigerung der katalytischen Vorgänge führt, was unter Umständen therapeutisch von Nutzen sein kann. Eine solche Indikation hat aber mit einer C-Avitaminose oder C-Hypovitaminose nichts zu tun.

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Enzymes of Ascorbic Acid Metabolism in Rat Liver: Effect of Fasting Followed by Repletion

N. SASMAL, N. C. KAR, D. MUKHERJEE & G. C. CHATTERJEE

Department of Biochemistry, University College of Science Calcutta 19

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Young adult male rats (60-75 g) on fasting for 4 days showed 19% loss in body weight, with disproportionately greater loss in liver weight and reduced ascorbic acid content of the liver tissues. Repletion for 1 week with diet containing either casein or groundnut protein as the source of protein restored liver weight, body weight and liver ascorbic acid content. Sequential studies of the enzymes related to L-ascorbic acid synthesis and breakdown, and the synthesis of L-xylulose showed decrease in the activities of L-gulonolactone oxidase (EC 1.1.3.8, L-gulono-γ-lactone: O_2 oxidoreductase), ascorbate oxidase (EC 1.10.3.3, L-ascorbate: oxygen oxidoreductase), uronolactonase (EC 3.1.1.19, D-glucurono-δ-lactone hydrolase) and most strikingly of D-glucuronolactone reductase (EC 1.1.1.20, L-gulono-γ-lactone: NADP oxidoreductase), L-gulonate dehydrogenase (EC 1.1.1.45, L-gulonate NAD oxidoreductase) and 3-oxo-L-gulonate decarboxylase (EC 4.1.1.34, 3-oxo-L-gulonate carboxy-lyase). After repletion, 4.1.1.34, the original levels of all the enzymes were restored. Substitution of groundnut protein in place of casein in the repletion diet resulted in slightly greater activities of all the enzymes studied.

IT is known that fasting or an alteration in diet markedly affects the composition of liver and brings about alterations in effective enzyme concentrations in animal tissues which are subsequently changed almost to normalcy during fasting followed by repletion[1-5]. Stirpe and Comporti[6] studied only the effect of fasting on the biosynthesis of L-ascorbic acid and L-xylulose by rat liver tissues. It has been observed by Mukherjee *et al*[7] that both the biosynthesis of L-ascorbic acid and of L-xylulose and the catabolism of L-ascorbic acid are greatly affected by the dietary intake of proteins. Since casein and groundnut proteins are qualitatively different, it was thought worthwhile to study the alterations in the activities of the enzymes involved in the biosynthesis and catabolism of ascorbic acid, biosynthesis of xylulose, and also the alteration in the hepatic protein and ascorbic acid contents on fasting followed by repletion with diet containing either casein or groundnut protein as the source of protein.

The estimation of total ascorbic acid in liver, the preparation of rat liver homogenate, the incubation media for studying the biosynthesis of L-ascorbic acid and L-xylulose, the test system for studying the catabolism of L-ascorbic acid, and determination of uronolactonase activity and protein were carried out as described earlier[7,8].

Male albino rats (60-75 g) which had been maintained on a stock diet were divided into several groups of 4 animals each. The animals were given for 3 days a nutritionally adequate diet of the following composition (%): corn starch, 73; casein, 18; groundnut oil, 5; and Hegsted salts IV (ref 9), 4. In addition, the animals were given 0·1 ml of a solution of B-vitamins (0·1 ml contained: thiamine, 100 μg; riboflavin, 100 μg; niacin, 200 μg; Ca-pantothenate, 400 μg; pyridoxine hydrochloride, 50 μg; biotin, 2 μg; folic acid, 4 μg; vitamin B_{12}, 0·4 μg; inositol, 2 mg; and choline chloride, 30 mg) daily and 0·05 ml of adexoline (12,000 IU of vitamin A and 2000 IU of vitamin D per ml) per rat twice a week.

The animals were then fasted for 96 hr. Water was supplied to the animals *ad libitum*. The fasted animals were refed for one week with either a diet containing 18% casein or with a diet containing 18% groundnut protein as the source of protein, other constituents being the same as in the casein containing diet. One control group of animals was fed on the casein containing diet and another control group was maintained on diet containing groundnut protein for a period of eleven days. The animals in the control groups were not subjected to fasting and were weighed daily.

During the period of fasting, 4 animals were killed by decapitation every 24 hr. For repletion studies, the animals, after being maintained on repletion diets for one week, were killed in the same way.

Young adult male rats (60-75 g) on fasting for 4 days showed 19% loss in body weight, with disproportionately greater loss in liver weight and a marked decrease in their liver ascorbic acid content (Table 1). Repletion for one week with diet containing either 18% casein or groundnut protein as the source of protein restored liver weight, liver ascorbic acid content and to some extent body weight.

Sequential studies of the liver enzymes related to L-ascorbic acid synthesis and its breakdown and the synthesis of L-xylulose showed significant decrease in the activities of L-gulonolactone oxidase (EC 1.1.3.8, L-gulono-γ-lactone: O_2 oxidoreductase), ascorbate oxidase (EC 1.10.3.3, L-ascorbate: oxygen

TABLE 1—CHANGES IN BODY WEIGHT, LIVER WEIGHT, LIVER PROTEIN AND ASCORBIC ACID CONTENTS OF LIVER DURING FASTING FOLLOWED BY REPLETION IN RATS

(Each result is expressed as the mean ± SD of four experiments, each on a different animal. Where the differences between the two means are significant, the P values are given)

Experimental conditions	Body wt % increase and decrease	Liver wt g	Liver protein g	Ascorbic acid mg/liver
Unstarved (18% casein diet)	(+) 18 ±3·3	2·6 ±0·24	0·75 ±0·09	0·84±0·12
Unstarved (18% groundnut protein)	(+) 13 ±4·9	—	—	—
Starved				
24 hr	(−) 1·2 ±1·6	2·6 ±0·26	0·71 ±0·14	0·45±0·18 $P<0·01$
48 hr	(−) 10 ±1·4	2·42 ±0·38	0·74 ±0·31	0·35±0·24 $P<0·01$
72 hr	(−) 16 ±3·1	2·25 ±0·12	0·71 ±0·14	0·33±0·18 $P<0·01$
96 hr	(−) 19 ±2·7	1·35 ±0·58	0·42 ±0·04	0·31±0·38 $0·01<P<0·02$
96 hr starved +18% casein diet (1 week)	(−) 2·5 ±2·1	2·70 ±0·24	0·73 ±0·08	0·77±0·08 NS at 10% level
96 hr starved+ 18% groundnut protein diet (1 week)	(−) 3·0 ±1·3	2·60 ±0·26	0·67 ±0·07	0·69±0·20 $0·01<P<0·02$

NS, not significant.

oxidoreductase), uronolactonase (EC 3.1.1.19, D-glucurono-δ-lactone hydrolase) and most strikingly of D-glucuronolactone reductase (EC 1.1.1.20, L-gulono-γ-lactone: NADP oxidoreductase), L-gulonate dehydrogenase (EC 1.1.1.45, L-gulonate: NAD oxidoreductase) and 3-oxo-L-gulonate decarboxylase (EC 4.1.1.34, 3-oxo-L-gulonate carboxy-lyase), especially with rats fasted for 4 days (Table 2). After repletion with casein diet, the activities of all the enzymes were restored to the original levels. Substitution of groundnut protein in the repletion diet resulted in significantly higher activities of L-gulonolactone oxidase ($P<0·001$), L-gulonate dehydrogenase, 3-oxo-L-gulonate decarboxylase ($P<0·01$) and uronolactonase.

The data on the loss of body weight, liver weight and liver protein content are in agreement with those reported earlier by Soberon and Sanchez[5] in respect of studies on certain liver enzymes. The observation that marked decrease occurs in liver weight, with an accentuated loss of hepatic solids on the 4th day of fasting, has also been confirmed. The results further indicate that starvation brings about more severe impairment of the activity of D-glucuronolactone reductase as compared to that of L-gulonolactone oxidase. The observation that the synthesis of L-xylulose is greatly affected during starvation contradicts the findings and also the postulation by Stirpe and Comporti[6] that increased xylulose biosynthesis accounts for the decreased synthesis of ascorbic acid during starvation. That starvation does not affect the activity of rat liver uronolactonase as reported by these workers is also

TABLE 2—VARIATIONS IN L-ASCORBIC ACID AND L-XYLULOSE SYNTHESIS AND IN 2,3-DIOXO-L-GULONIC ACID FORMATION AND IN HYDROLYTIC BREAKDOWN OF D-GLUCURONOLACTONE TO ITS FREE ACIDS BY RAT LIVER ON FASTING FOLLOWED BY REPLETION

(Each result is expressed as the mean ± SD of four experiments, each on a different animal. Where the differences between the two means are statistically significant, P values are given)

Experimental conditions	L-Ascorbic acid synthesized (μmoles/liver)		2,3-Dioxo-L-gulonic acid formed (μmoles/liver)	L-Xylulose synthesized (μmoles/liver)	D-Glucurono-lactone hydrolysed (μmoles/liver)
	From D-glucurono-lactone	From L-gulono-lactone			
Unstarved (18% casein)	9·0±2·94	8·3±0·84	226±3·72	45·24±3·08	143±7·62
Starved					
24 hr	7·09±1·06 NS	7·8±0·72 NS	177±8·76 HS	22·68±4·08 HS	106±3·62 HS
48 hr	6·6±1·44 NS	7·9±1·08 NS	147·8±9·62 HS	13·30±2·06 HS	103±3·24 HS
72 hr	4·8±1·12 $P<0·02$	7·3±0·60 NS	148±6·20 HS	14·10±1·10 HS	98·7±2·38 HS
96 hr	1·2±0·28 $P<0·001$	3·3±0·46 HS	91·3±5·92 HS	6·64±1·24 HS	54·0±2·16 HS
96 hr starved+18% casein diet (1 week)	8·9±0·88 NS	8·6±0·40 NS	211·7±9·40 $P<0·02$	42·34±2·14 NS	160±3·10 $P<0·001$
96 hr starved+18% groundnut protein diet (1 week)	9·5±0·92* NS	10·0±0·46† $P<0·01$	215·6±6·52* $P<0·02$	47·18±2·04‡ NS	182±2·94† $P<0·001$

NS, not significant.
HS, highly significant ($t>5·96$ for 6 degrees of freedom)[11].
*Mean values are not statistically different from the group of rats fed 18% casein diet after 96 hr starvation.
†‡Mean values are statistically different from the group of rats fed 18% casein diet after 96 hr starvation. P values are $<0·001$† and $<0·01$‡.

not in agreement with the results presented in this paper.

Further it is noted that during starvation all the enzymes involved in ascorbic acid metabolism in rats manifest lowered activities, the most affected being D-glucuronolactone reductase, L-gulonate dehydrogenase and 3-oxo-L-gulonate decarboxylase.

In rats fed repletion diet containing groundnut protein as source of protein, the activities of all the enzymes studied are not only restored to the original levels but also their levels are slightly higher than those observed in rats fed repletion diet containing casein. The observation that groundnut protein is better than casein with respect to tissue retention of ascorbic acid cannot be easily explained by assuming inadequate utilization as suggested by Esh *et al*[10], since the enzyme levels of both biosynthesis and also catabolism of L-ascorbic acid by rat liver tissues after repletion with groundnut protein are higher as compared to that with casein.

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THE ROLE OF ASCORBIC ACID IN THE PREVENTION OF BLADDER TUMOR FORMATION

J. U. SCHLEGEL, G. E. PIPKIN, R. NISHIMURA* AND G. N. SHULTZ*

From the Section of Urology, Department of Surgery, Tulane University School of Medicine, New Orleans, Louisiana

Studies in our laboratories have raised serious doubts about the suggested direct carcinogenic role of orthoaminophenolic compounds. The incidence of bladder cancer resulting from exposure to some aromatic amines, notably B-naphthylamine, appears rather convincing.[1] Bladder implantation in mice with pellets containing orthoaminophenolic metabolites of dietary tryptophan certainly lends support to the suggestion that spontaneous bladder tumor formation may be a result of faulty tryptophan metabolism.[2–4] However, it appears that a number of other possibilities are equally or even more likely.[5] The ease with which 3-hydroxyanthranilic acid (3-HOA) and similar orthoaminophenols are oxidized raises serious questions as to which are the primary carcinogens. Since the presence of ascorbic acid in a solution containing 3-HOA will prevent oxidation of the latter,[6] it was considered appropriate to compare the carcinogenicity of 3-HOA pellets implanted into mice bladders with the variant being the ascorbate level in the urine. If a significant decrease in incidence of bladder tumor formation resulted from oral administration of ascorbic acid in amounts which would increase the urinary level of ascorbate significantly, one could assume that 3-HOA is not a primary carcinogen and that the presence of ascorbic acid in the urine prevented the oxidative decomposition of 3-HOA.

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TECHNIQUE AND MATERIALS

There were 310, 60 to 120-day-old Swiss Albino female mice anesthetized with pentobarbital and ether. Pellets were prepared as described by Bryan and associates[3] and inserted into bladders of mice by the technique of Jull[7] as modified by Allen and associates.[2] Groups 1 and 2 received pellets containing cholesterol only, while groups 3 to 6 received cholesterol pellets containing 3-hydroxy-5-carboxy-benzoquinone-(2-hydroxy-6-carboxy-anil)-(1) imide-(4), (compound 3) and 3-HOA respectively (table 1). Compound 3 was synthesized by the method described by Butenandt and associates.[8] After the operation the mice were returned to their cages and fed purina mice pellets ad libitum. The mice in groups 2, 4 and 6 were fed L-ascorbate (250 mg. per cent) in their drinking water ad libitum.

The concentration of ascorbate in the urine excreted by all groups of mice was analyzed by the method of Hughes.[9] Mice which received vitamin C in their drinking water showed an average urinary concentration of ascorbate of 400 mg. per cent compared to approximately 16 mg. per cent in the groups of mice not receiving vitamin C in their drinking water.

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TABLE 1. *Data summary on the effects of ascorbic acid, compound 3 and 3-hydroxyanthranilic acid and their interactions on the course and development of bladder tumors in mice*

Group	Chemicals Implanted In Cholesterol Pellets Into Mice Bladders	Ascorbate Fed In Drinking Water	No. Mice Treated Originally	No. Mice Which Survived 40 Weeks	Tumors Observed In 40 Weeks		
					Pre-Malignant	Malignant	Total
1	Cholesterol alone	None	58	49	3	2	5
2	Cholesterol alone	250 mg.%	33	23	1	2	3
3	Compound 3*	None	56	39	0	2	2
4	Compound 3*	250 mg.%	42	28	1	2	3
5	3-HOA†	None	70	46	1	8	9
6	3-HOA†	250 mg.%	51	37	1	2	3

* 3-hydroxy-5-carboxy-benzoquinone-(2-hydroxy 6-carboxy-anil)-(1) imide-(4).
† 3-hydroxyanthranilic acid.

All surviving mice were killed 40 weeks postoperatively. They were examined at postmortem in a manner previously described by Jull.[7] The bladders were distended by the injection of Bouin's solution and fixed for 24 hours before they were opened and examined with a hand lens. Selected material from each mouse bladder was then removed for microscopic examination. Since it was particularly important to evaluate any possible difference of incidence of bladder tumor formation between groups 5 and 6, we asked for an authoritative, unbiased opinion and Dr. Charles E. Dunlap, professor and chairman of the Department of Pathology, Tulane University School of Medicine, most graciously consented to review all of these slides. The rest of the slides were reviewed by us using the criteria applied in groups 5 and 6.

RESULTS

In table 1 is shown the number of mice which survived 40 weeks and the number of pre-malignant and malignant tumors found at autopsy. Mice implanted with 3-HOA (group 5) have a significantly higher incidence of bladder tumor formation than any of the remaining groups. The administration of ascorbic acid prevented the 3-HOA from exerting any specific carcinogenic effect. Consequently one is forced to conclude that 3-HOA by itself is not carcinogenic and it appears that an anti-oxidant in the urine in amounts sufficient apparently to prevent the oxidation of 3-HOA will also prevent bladder tumor formation beyond that seen in the control animals. Animals with compound 3 implanted showed no higher incidence of bladder tumor than those implanted with cholesterol pellets and thus it appears that compound 3 which is an oxidative product of 3-HOA is not carcinogenic for the mouse bladder. Various examples of the changes found in the bladders implanted with pellets containing 3-HOA are illustrated—parts *A* shows a pre-malignant lesion while parts *B* to *D* show definite malignant tumors.

Our initial purpose was to prevent oxidation of 3-HOA in the urine since it was our feeling that the proved carcinogenicity of 3-HOA may be a result of its oxidation rather than carcinogenicity by this compound in itself. The presence of ascorbate, an anti-oxidant, in the urine has been shown in vitro to prevent the oxidation of 3-HOA and these experiments seem to indicate that if 3-HOA is not oxidized it is not carcinogenic.

DISCUSSION

We previously suggested the administration of oral ascorbic acid since it was our feeling that it might prevent spontaneous bladder tumor formation and at least not be harmful to the patient.[5] In this connection it may be of interest to review some pertinent data concerning the relationship of ascorbic acid consumption and excretion to age, tobacco consumption and sex, as well as information available concerning the toxic levels of ascorbic acid administration.

From the abundant literature concerning ascorbic acid it appears that normal individuals show sex difference inasmuch as men have a significantly lower plasma and leukocyte vitamin C concentration than that of women.[10] It is also apparent that non-smokers show a significant decline in plasma vitamin C concentration with increasing age, which is true for men as well as women, while the leukocyte content of vitamin C does not change with age.[11-31] Cigarette smoking

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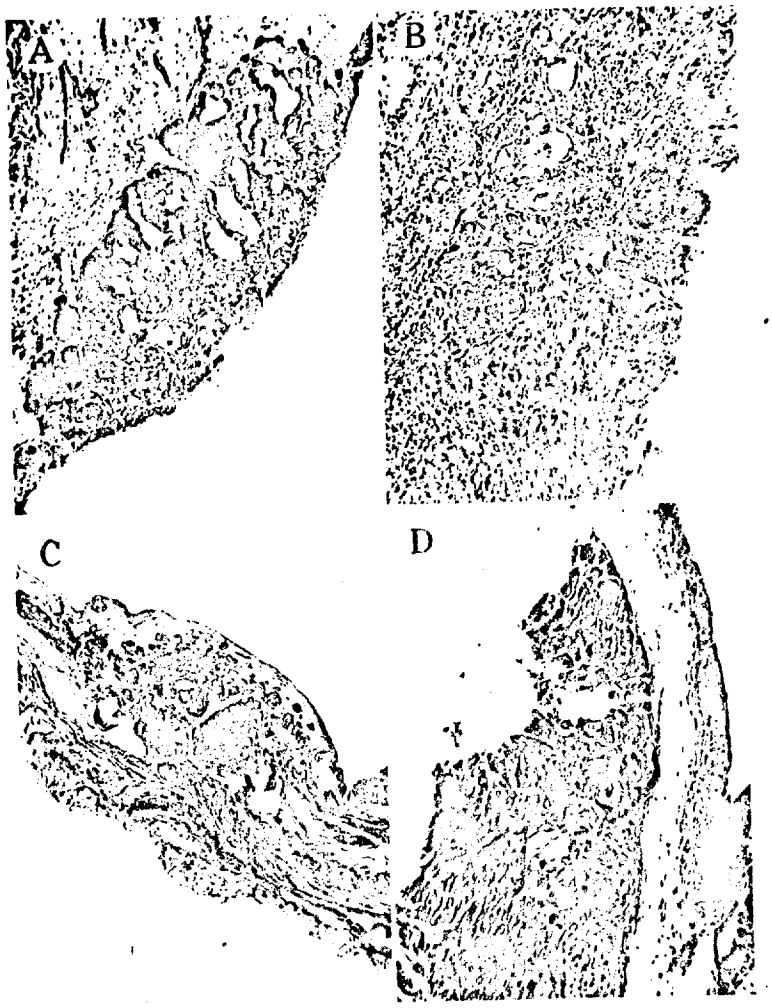

A, adeno-squamous pre-malignant lesion. H & E 80×. *B*, epidermoid carcinoma. H & E 125×. *C*, low grade carcinoma invading muscularis. H & E 43×. *D*, poorly differentiated malignant tumor. H & E 125×.

lowers significantly both the plasma and leukocyte vitamin C concentration.[10, 12] Chronic disease apparently results in low ascorbic acid concentration in plasma and white cells and this applies to patients with cancer as well as chronic non-cancerous disease.[13] Concerning the apparent increased need for vitamin C in smok[illegible] vestigator claims that the smoking of 1 cigarett[illegible] by the ordinary inhalation method results in the neutralization of 25 mg. vitamin C in the body [illegible] the equivalent of the vitamin C content of [illegible] average size orange.[14] In 1940 Longenecker and associates demonstrated that narcotics such a[illegible]

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TABLE 2. *Mean ascorbic acid concentration in urine of patients (mg. %)*

Age	Non-Smoker	Smoker	Bladder Tumor
0-50	1.22 (24)*	0.65 (24)*	
50 up	0.40 (9)*	0.18 (11)*	0.23 (13)*

* No. patients in each group.

chloretone raise the excretion of ascorbic acid in rats.[15] Boyland and Grover suggested that foreign compounds can increase ascorbic acid synthesis in animals.[16] However, as is well known, man, primates and guinea pigs cannot synthesize ascorbic acid and require a dietary intake. One cannot help but feel in reviewing the literature that individuals who have low serum ascorbic acid levels are the ones with the highest probability of forming bladder tumors. It also appears obvious that ascorbic acid excretion by the kidney relates to its level in the serum and thus a low serum level will result in a low urinary ascorbic acid excretion.[17, 18] Our own studies confirm this theory (table 2). There is an apparent decrease in ascorbic acid concentration in the urine of smokers and bladder tumor patients in both age groups but this is not statistically significant. However, the decrease in concentration between the 2 age groups is statistically significant with a P value of less than .05.

Administration of ascorbic acid in amounts which will give a significant level in the urine in patients is well below toxic levels. The possible toxic effect of ascorbic acid could result from its metabolism inasmuch as some is excreted in the urine as oxalic acid.[19, 20] However, several investigations show that only when the daily ingestion of ascorbic acid exceeds 3 to 4 gm. does the urinary level of oxalic acid increase.[21, 22] It thus can be considered safe to administer 1½ gm. per day as we have advocated and this will in most individuals produce significant urinary levels to exert its effect as an anti-oxidant.

Finally, we call attention to investigations that seem to indicate that ascorbic acid may have beneficial effect upon the aging process of atherosclerosis[23, 24] and although this has been shown more clearly in animals it certainly should at least encourage the intake of ascorbic acid in individuals who due to age, cigarette smoking or other factors may be prone to bladder tumor formation. We thus repeat what has been previously stated, that there seems to be reason to consider the beneficial effects of an adequate ascorbic acid level in the urine as a possible preventive measure in regard to bladder tumor formation and recurrence. The administration of 500 mg. ascorbate 3 times a day should have no harmful consequences. However, the experiments described in this study are to our knowledge the first biological demonstrations of the possible preventive effect of administration of ascorbic acid upon the formation of bladder tumor by pellet implantation of a proved bladder carcinogen.

SUMMARY

Bladder pellet implantation in mice with one of the tryptophan metabolites, 3-hydroxyanthranilic acid (3-HOA), has confirmed its carcinogenicity.

Oral administration of large amounts of ascorbic acid which resulted in high urinary levels of ascorbate prevented this carcinogenic effect presumably because of the competitive affinity for oxygen which prevented the oxidation of 3-HOA.

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It appears that oral administration of ascorbic acid prevented the carcinogenic effect of 3-HOA and that 3-HOA, in itself, is not a carcinogen but rather the oxidation of this compound is of importance in carcinogenesis induced by this ortho-aminophenol. On the basis of these results we believe our recommendation for oral administration of ascorbic acid as a possible preventive measure of spontaneous bladder tumor formation and recurrence in man is warranted, a suggestion which is further supported by the evidence of lack of toxicity of the dosage required to maintain an adequate urinary level.

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ACQUIRED HYPEROXALURIA, NEPHROLITHIASIS, AND INTESTINAL DISEASE

Description of a Syndrome

LYNWOOD H. SMITH, M.D., HANS FROMM, M.D., AND ALAN F. HOFMANN, M.D.

Abstract In seven patients with resection of the distal ileum, calcium oxalate nephrolithiasis and increased urinary excretion of oxalate (64 to 135 mg per 24 hours) developed. Cholestyramine therapy (4 g four times a day) lowered the 24-hour urinary excretion of oxalate to normal in the four patients in whom it was used. Urinary excretion of oxalate was measured in an additional 42 patients with various types of intestinal disorders without evidence of nephrolithiasis. Five of 18 patients with ileal resection, two of seven with bacterial overgrowth, and six of 15 with miscellaneous conditions, including nontropical sprue and cirrhosis, had hyperoxaluria.

THE occurrence of nephrolithiasis as a complication of bowel disease, with or without bowel resection, has frequently been described.[1-10] Gelzayd et al.[1] reviewed 885 cases of inflammatory bowel disease and reported nephrolithiasis in 7.2 per cent. This figure was much greater than the overall estimated prevalence of renal calculi for hospitalized patients – 1:1,000 – in the United States.[11] Although complete data on the chemical analysis of the renal stones were not available, the stones contained calcium and were radiopaque in 46 patients (72 per cent) and contained uric acid and were radiolucent in 12 patients (19 per cent). The renal stones could not be classified in six of the patients.

In 1970 we reported hyperoxaluria and nephrolithiasis in two patients with previous small-bowel resection.[12,13] An additional case,[14] described as an unusual example of primary hyperoxaluria, in retrospect now appears also to be acquired hyperoxaluria. Dowling et al.[15] studied 11 patients with ileal dysfunction, eight of whom had hyperoxaluria; three had calcium oxalate nephrolithiasis. Admirand et al.[16] have studied a similar group of cases.

None of our seven patients with hyperoxaluria, nephrolithiasis, and resection of the lower small intestine had other known causes of secondary hyperoxaluria such as excessive intake of ascorbic acid or rhubarb. Tryptophan loading to rule out pyridoxine deficiency was only done in Case 1. Of an additional group of patients with intestinal disease but no nephrolithiasis 13 had hyperoxaluria.

CASE REPORTS

CASE 1. A 61-year-old woman was seen at the Mayo Clinic in January, 1967, for evaluation of rapidly growing stones in both kidneys (case previously reported).[14] In 1960, 275 cm of infarcted small bowel was removed; she had been given anticoagulant therapy for 8 months afterward. Four months later, another 183 cm of infarcted small bowel was removed. Subsequently, she was maintained on anticoagulant therapy. A roentgenogram of the abdomen showed no stone formation in 1964 (Fig. 1, upper portion). In November, 1966, because of moderate abdominal pain and hematuria, excretory urography was carried out and showed bilateral nephrolithiasis. There was no family history of kidney stones.

When seen by us, the patient was having 5 to 7 loose stools per 24 hours. Urine volume ranged from 1080 to 1810 ml per 24 hours. The hemoglobin concentration was 13 g per 100 ml; erythrocyte morphology was normal. Serum protein electrophoresis, calcium, phosphorus, and uric acid were also normal, as were blood acid-base factors. The serum carotene was 21 μg per 100 ml. Thromboplastin generation was accelerated. The urinary pH was 5.4, and large numbers of oxalate crystals were present in the urinary sediment. Urine cultures were negative on 2 occasions. The 24-hour urinary clearance of endogenous creatinine was 34 ml per minute per 1.73 m^2 (serum creatinine was 1.6 mg per 100 ml). The urinary excretion of calcium was 32 mg per 24 hours. The urinary excretion of oxalate was 108, 97.5, and 116 mg per 24 hours (normal value, 10 to 40 mg per 24 hours[17]). The excretory urogram showed decreased kidney size bilaterally. There was a dense, homogeneous stone in the right renal pelvis, without obstruction, and multiple smaller stones bilaterally (Fig. 1, lower portion).

The patient was hospitalized in the metabolic unit in July, 1967, for investigation of the role of possible pyridoxine deficiency, secondary to the small-bowel resection, in the hyperoxaluria and rapid renal-stone formation. No serious deficiency in pyridoxine was noted with tryptophan loading, and the intramuscular administration of pyridoxine had no appreciable effect on the excretion of oxalate and glycolate in the urine.

CASE 2. A 68-year-old man was seen at the Mayo Clinic in April, 1969, for evaluation of recurrent bladder stones. In 1963, 45 cm of terminal ileum and 12 cm of cecum had been removed; there was gross and microscopical evidence of severe, diffuse, regional enteritis of the distal 35 cm of the ileum. After operation he had 4 to 6 watery stools per

From the Mayo Clinic and Mayo Foundation, Rochester, Minn. (address reprint requests to the Section of Publications, Mayo Clinic, Rochester, Minn. 55901).

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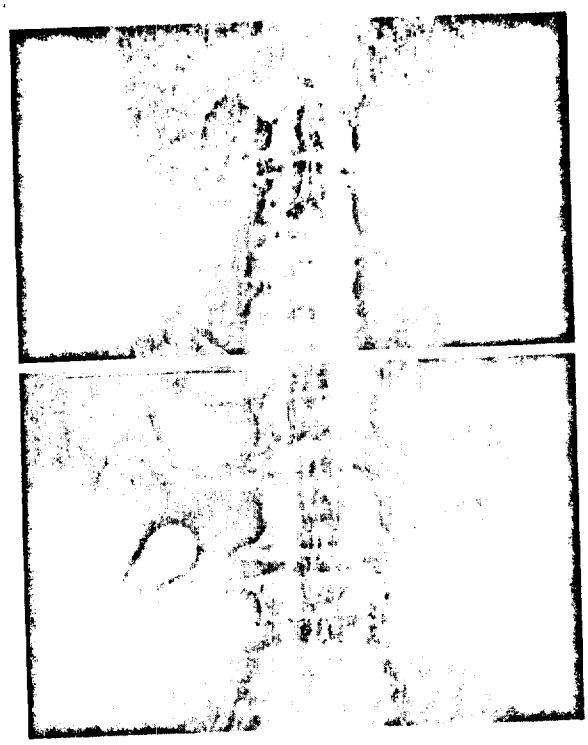

Figure 1. Roentgenogram Taken in January, 1964 (Upper), Showing No Stones (Reproduced from Smith et Al.,[12] with the Permission of the Publisher), Compared with Roentgenogram Taken in January, 1967 (Lower), Showing Radiopaque Stones Bilaterally.

day. Bladder stones were first noted in the fall of 1966; he had hypercalcemia (serum calcium of 10.8, 11.7, and 10.9 mg per 100 ml) and a urinary calcium excretion of 416 mg per 24 hours. In January, 1967, a cystic chief-cell parathyroid adenoma was removed (weight, 3.4 g). The 3 remaining parathyroid glands were identified and appeared normal. After that the serum calcium and phosphorus and urine calcium values remained normal. In March, 1967, new bladder stones were found and removed at cystoscopy. During the summer and fall of 1968 he passed 3 additional stones without colic or hematuria. There was no family history of kidney stones.

In April, 1969, multiple bladder stones were removed by cystoscopy. Stones within the kidney were never visualized. The hemoglobin was 14.8 g per 100 ml; erythrocyte morphology was normal, as were serum protein electrophoresis, calcium, phosphorus, and uric acid. Urine culture showed *Streptococcus faecalis* on 2 occasions, with a colony count greater than 100,000 per milliliter. The urine pH was 5.4. The 24-hour clearance of endogenous creatinine was 100 ml per minute per 1.73 m^2 (serum creatinine was 1.0 mg per 100 ml). The urinary excretion of calcium was 113 and 150 mg per 24 hours. The urinary excretion of oxalate was 121, 169, and 115 mg per 24 hours. Excretory urography showed no kidney stones. The bladder stones had calcium oxalate as the major constituent, with a small amount of carbonate and hydroxyapatite.

CASE 3. A 55-year-old woman was seen at the Mayo Clinic in March, 1969, for evaluation of recurrent renal colic with passage of stones and gravel. Between 1934 and 1952 she had had a total of 12 abdominal surgical procedures, 8 of which were for recurrent symptoms of bowel obstruction. In January, 1952, 152 cm of terminal ileum had been removed. She subsequently had 8 to 14 stools per day, usually watery, with mucus. Her 1st kidney stone had been passed in 1958 and was reported to be uric acid. She continued to pass 5 to 8 uric acid stones per year until 1964, when she passed her 1st calcium oxalate stone. After that she averaged 6 to 12 calcium oxalate stones per year, with the passage of gravel between episodes of renal colic and stone passage. There was no family history of kidney stones.

In March, 1969, the hemoglobin was 13.3 g per 100 ml, and erythrocyte morphology and serum calcium, phosphorus, uric acid, carotene, and protein electrophoresis were normal. The prothrombin time was also normal. Urine culture was negative on 3 occasions. The 24-hour clearance of endogenous creatinine was 63 ml per minute per 1.73 m^2 (serum creatinine was 0.95 mg per 100 ml). The urinary excretion of calcium was 41 and 54 mg per 24 hours. The urinary excretion of oxalate was 89 and 118 mg per 24 hours. The urine volume ranged between 425 and 842 ml per 24 hours. Excretory urography showed small radiopaque stones in both kidneys. A recently passed stone had calcium oxalate as the major constituent, with a small amount of carbonate and hydroxyapatite.

CASE 4. A 50-year-old man was seen at the Mayo Clinic in June, 1969, for evaluation of recurrent kidney-stone formation. He had had regional enteritis in 1949. In January, 1950, jejunoileostomy and ileotransverse colostomy were done without resection of bowel. In March, 1951, a jejunotransverse colostomy was done, with resection of the distal jejunum, ileum, cecum, ascending colon, hepatic flexure, and a portion of the transverse colon. There was extensive involvement by regional enteritis. In December, 1961, the disease recurred in the jejunum, and an additional 20 cm of jejunum and 10 cm of transverse colon were resected. It was estimated that 76 cm of jejunum remained from the ligament of Treitz to the jejunocolostomy. After this operation he had averaged 3 to 5 loose to watery bowel movements per day. There was no evidence of further recurrence of his regional enteritis.

In October, 1950, he passed his 1st kidney stone (not analyzed), with ureteral colic. Renal colic recurred in 1966, and a radiopaque calculus was noted in the left kidney; 2 weeks later he passed the stone (not analyzed). After 1968, he passed intermittent gravel from the urinary tract, and in October, 1968, he passed 2 stones, which were calcium oxalate. There was no family history of kidney stones.

In June, 1969, the hemoglobin was 14.1 g per 100 ml; erythrocyte morphology was normal as were the prothrombin time and serum calcium, bilirubin, and uric acid. The serum phosphorus was 1.9 and 2.2 mg, the serum creatinine 1.85 mg, and the serum carotene 11 μg per 100 ml. Serum protein electrophoresis showed an albumin of 2.98 g per 100 ml, with α_1 globulin of 0.34, α_2 globulin of 0.52, β-globulin of 1.03 and γ-globulin of 1.89. Sulfobromophthalein retention was 6 per cent in 1 hour. Urine culture was negative. Urinary excretion of calcium was 25 mg per 24 hours. Urinary excretion of oxalate was 59, 68, and 69 mg per 24 hours. The urine volume ranged between 625 and 800 ml per 24 hours. Excretory urography showed small radiopaque stones in both kidneys.

CASE 5. A 25-year-old woman was seen at the Mayo Clinic in June, 1971, for evaluation of renal calculi and re-evaluation of regional enteritis. She had been seen here in September, 1968, for recurrent symptoms of diarrhea and abdominal pain since 1957. An ileosigmoidal fistula was resected, and 40 cm of terminal ileum and 20 cm of cecum and ascending colon were removed; regional enteritis involved the distal 10 cm of the ileum. Since that operation she had averaged 2 to 3 soft stools per day.

She had her 1st episode of renal colic in March, 1971, with passage of a stone; she passed a 2d stone 2 weeks later and a 3d stone in May. Analysis of 2 of these stones showed approximately equal amounts of calcium oxalate and ammonium urate. There was no family history of kidney stones.

In June, 1971, the hemoglobin was 11.7 g per 100 ml; erythrocyte morphology was consistent with generalized macrocytosis. The serum vitamin B_{12} was 302 pg, and the folic acid 2.6 ng per milliliter, and the serum calcium, phosphorus, creatinine and uric acid were normal. Urine culture was negative. The urinary pH was 5.8. Urinary excretion of calcium was 32 mg per 24 hours. Urinary excretion of oxalate was 49 and 86 mg per 24 hours. The urine volume ranged between 1025 and 1265 ml per 24 hours. Excretory urography showed 2 small, radiopaque calculi in the lower pole of the right kidney.

CASE 6. A 28-year-old woman was seen at the Mayo Clinic in May, 1971, for evaluation of a history of regional ileocolitis and renal colic. The onset of diarrhea and abdominal pain was in 1956, and in 1969, an ileostomy was done, with resection of 457 cm of terminal ileum and the entire colon. One sister has regional ileocolitis, with an ileocolostomy, and has had recurrent uric acid stones. This sister's oxalate excretion is normal. Another sister and her maternal grandfather were said to have "colitis." In February, 1971, the patient had had renal pain with chills and fever and had passed gravel and stone material (not analyzed). She had had several episodes of renal pain without chills or fever since that time, with no apparent passage of stones or gravel.

In May, 1971, the hemoglobin was 14.9 g per 100 ml; erythrocyte morphology was normal, as were serum calcium, phosphorus, creatinine, uric acid, protein electrophoresis, folic acid, and vitamin B_{12}. Urine culture was negative. Urinary excretion of uric acid was 702, and that of oxalate 133 mg per 24 hours. Excretory urography gave no evidence of renal calculi.

CASE 7. A 49-year-old man was seen at the Mayo Clinic in April, 1971, for evaluation of recurrent regional enteritis. The symptoms of his regional enteritis had begun in 1949 as fever, chills, diarrhea, and weight loss. In 1953 an ileotransverse colostomy was done; an additional section of ileum was resected in 1960. It was estimated that approximately ⅔ of the distal small bowel and the entire ascending colon had been resected. Since the operation in 1960 he had averaged 5 to 6 loose stools per day. Symptomatic renal lithiasis had begun in 1968. A right ureteral stone was removed at cystoscopy, and he passed 2 additional small stones (said to be calcium stones but not analyzed). In 1969 the right renal colic recurred, and he passed stones and gravel at intervals of approximately a month with little difficulty. His mother had passed 1 kidney stone at the age of 70 years. There was no other family history of nephrolithiasis.

In April, 1971, the hemoglobin was 15.3 g per 100 ml, and the white-cell count 14,900, with 82 per cent segmented neutrophils, 9 per cent lymphocytes, and 9 per cent monocytes. The serum calcium, phosphorus, alkaline phosphatase, uric acid, iron, and folic acid were normal; the serum vitamin B_{12} was 206 pg per milliliter. The prothrombin time was 21 seconds. Urine culture was negative. Urinary excretion of calcium was 6 mg per 24 hours. Urinary excretion of uric acid was 277, and that of oxalate 95 mg per 24 hours. Excretory urography showed bilateral radiopaque nephrolithiasis.

URINARY EXCRETION OF OXALATE BY PATIENTS WITH INTESTINAL DISEASE WITHOUT NEPHROLITHIASIS*

Five of the 18 patients with ileal resection had urinary excretion of oxalate greater than 40 mg per 24 hours (range, 53 to 102 mg per 24 hours). Comparison of these five patients with the seven who passed kidney stones revealed no differences in the urinary volume, amount of ileum resected or duration since ileal resection. In the two patients with regional enteritis without ileal resection normal values for urinary oxalate excretion were found. Two of the seven patients with bacterial overgrowth had abnormal values (71 mg per 24 hours in both). Six of the 15 patients with the miscellaneous conditions of the alimentary tract or hepatobiliary system had abnormal values (range, 48 to 146 mg per 24 hours), the highest being found in a patient with alcoholic cirrhosis. Thus the amount of oxalate excreted per 24 hours tended to be less than that excreted by the patients with nephrolithiasis, but the difference was slight.

The urinary excretion of oxalate was measured in 42 patients with various types of gastrointestinal disease. None of these patients had evidence of nephrolithiasis. Eighteen patients had had ileal resection, nine for regional enteritis and nine for other conditions. There were two patients with regional enteritis but no resection, seven with bacterial overgrowth of the small intestine, and 15 with miscellaneous conditions involving the intestinal tract and liver.

SPECIAL STUDIES

Cholestyramine, 4 g four times per day, was given to four of the patients with hyperoxaluria and nephrolithiasis (Cases 1 through 4) in the hope of increasing urinary volume by reducing water loss from the gut.[18] There was an increase in the urinary volume in these patients, and also the urinary excretion of oxalate returned to normal (Table 1). In none of the patients have new kidney stones formed on cholestyramine therapy, and one patient lost two of her three stones (Case 3). The effect of cholestyramine persisted as long as therapy continued (up to one year). In one patient the cholestyramine had to be discontinued because of aggravation of diarrhea.

DISCUSSION

Increased urinary excretion of oxalate has been thought to be due primarily to a rare genetic disorder of glyoxylic acid metabolism, primary hyperoxaluria.[19] Two enzyme defects now recognized in this metabolic pathway result in the accumulation of glyoxylic acid and its conversion to the end product, oxalic acid. Type I hyperoxaluria, glycolic aciduria, is characterized by increased urinary excretion of oxalic acid, glyoxylic acid, and glycolic acid and is

Table 1. Effect of Cholestyramine on Oxalate Excretion in Patients with Acquired Hyperoxaluria.

CASE No.	MEAN URINARY OXALATE*	
	BEFORE THERAPY	WITH CHOLESTYRAMINE†
	mg/24 hr	
1	101	17
2	135	37
3	104	23
4	64	35

*Mean of 3 or more determinations over a period of >1 mo.
† 4 g 4 times/day

*For more detailed information order NAPS Document 01817 from National Auxiliary Publications Service, c/o CCM Information Corporation, 866 3d Ave., New York, N.Y. 10022; remitting $2 for each microfiche-copy reproduction or $5 for each photocopy. Checks or money orders should be made payable to CCM Information Corporation–National Auxiliary Publications Service.

due to a deficiency of a soluble 2-oxoglutarate: glyoxylate carboligase.[20] This is the most common form of the inherited disorder. In Type II hyperoxaluria, *L*-glyceric aciduria, urinary excretion of glycolic acid is normal, and there is a marked increase in the excretion of *L*-glyceric acid. This condition is due to a deficiency in *D*-glyceric dehydrogenase.[21] The disease caused by these enzyme defects has been characterized by nephrolithiasis, nephrocalcinosis, renal failure, and early death in many cases. Hockaday et al.[22] summarized the clinical course of 63 patients with typical primary hyperoxaluria. Symptoms began before the age of 30 years in all patients, and only one of the group had lived beyond the age of 40. Twenty-eight of the patients died before the age of 20 years.

Acquired hyperoxaluria has been recognized in several conditions. The ingestion of ethylene glycol, which is metabolized through the glyoxylic acid pathway, causes increased urinary excretion of oxalate.[23,24] Rhubarb gluttony has been reported to cause hyperoxaluria in one case on the basis of high dietary intake of oxalate,[25] and very large doses of ascorbic acid may lead to hyperoxaluria by degradation of the ascorbic acid to oxalate. Pyridoxine is a coenzyme in the conversion of glyoxylic acid to glycine, and hyperoxaluria has been reported[26,27] in states of pyridoxine deficiency. In addition, Dempsey et al.[28] observed increased oxalate excretion in several miscellaneous conditions, including cirrhosis, renal tubular acidosis, and sarcoidosis; the mechanism is unknown. The two patients with cirrhosis and hyperoxaluria in our study did not have diarrhea or malabsorption, and the mechanism is not clear.

The etiology of nephrolithiasis in patients with intestinal disease is obscure. When bowel resection had been done, this complication was more frequent.[1,4] Decreased urinary volume due to decreased absorptive surface in the bowel has been considered a major cause in the formation of stones.[29,30] Loss of water, bicarbonate, and sodium in patients with ileostomies results in very acidic urine of low volume, a situation ideal for the formation of uric acid stones. Yet, where information is available about the types of stones found in patients with intestinal disease, calcium-containing stones have been more common.[1,4] Infection of the urinary tract, with formation of magnesium ammonium phosphate (struvite) stones, has been suggested as the cause of at least some of the radiopaque stones.[7] However, it is doubtful whether infection alone, in the absence of obstruction and stasis or a primary cause of metabolic stone formation with stones present, ever causes nephrolithiasis de novo.[31] Calcium mobilization as a result of steroid therapy and immobilization, with resultant hypercalciuria, may have some role in nephrolithiasis.[1] Against the development of hypercalciuria is the fact that fecal loss of calcium and phosphorus is often increased in diarrheal states and after bowel resections.[32,33] The finding of marked hyperoxaluria in the patients with intestinal disorders and nephrolithiasis, described above and confirmed by others,[15,16] offers a new explanation for this complication.

In seven of our 12 patients with ileal resection and hyperoxaluria kidney stones have formed. In comparing the groups with and without nephrolithiasis, we observed no important differences in quantity of oxalate excreted or urinary volume between the two groups, though our data do not eliminate the possibility that there were marked differences before study. It has been suggested that this lack of difference, which is common in all types of metabolic stone disease, may be related to variations in urinary inhibitors of mineralization, at least in calcium-stone formation.[34-38] Even in cystinuria it is not uncommon to find siblings of cystine-stone formers with cystinuria but no stones, and it appears that there are factors other than solute load that are important in the formation of a kidney stone. It may be important that at least three of the seven patients with nephrolithiasis had had uric acid stones before the formation of calcium oxalate stones. The role of preformed crystal aggregates (micro stones) in collecting tubules and papillary tips in the nucleation and growth of any potential crystal system has been stressed.[39]

The etiology of hyperoxaluria in these patients is not settled. The evidence, although incomplete, suggests that hyperoxaluria results, at least in part, from the profound disturbance in bile acid glycine metabolism known to be present in patients with intestinal resection or malabsorption in whom the bile acid pool is exposed to deconjugating bacteria.[40-42] It has recently been shown that bile acid glycine turnover rates measured by isotope dilution technics correlate highly (r equal to 0.95) with cumulative $^{14}CO_2$ excretion rates in healthy persons.[43] In the patients with hyperoxaluria there was an increased $^{14}CO_2$ excretion rate — i.e., greatly increased bile acid glycine degradation, as compared to the patients without hyperoxaluria; thus, in the steady state, patients with hyperoxaluria had a greatly increased bile acid glycine synthesis. The precursor of bile acid glycine is not known, but could be glyoxylic acid, the precursor of oxalic acid; indeed, Admirand et al. have suggested that the patients in whom hyperoxaluria develops have a latent enzyme defect in glyoxylic acid metabolism.[16] Pyridoxine deficiency was ruled out only in the first case, but there were none of the physical or laboratory signs of this disorder in the patients with hyperoxaluria.

The reduction in oxalate excretion caused by cholestyramine in this syndrome appears paradoxic since cholestyramine increases bile acid loss, causing a still further increase in bile acid synthesis and conjugation.[11] Yet, if cholestyramine simultaneously decreased the amount of unconjugated bile acid returning to the liver, it would reduce the amount of glycine required for bile acid conjugation. The magnitude of those two opposing effects in patients with this syndrome is unknown, but it seems likely that cholestyramine could reduce bile acid glycine production in some cases. Taurine has also been reported to decrease urinary oxalate in these patients by correcting a proposed taurine deficiency and decreasing the fraction of glycine conjugated bile acids; however, this rationale was based on the

view that bile acid glycine was metabolized by intestinal bacteria to glyoxalate, a hypothesis now known to be incorrect in the patients whom we have studied.[15,16] In our hands taurine has had no effect on urinary oxalate in the four patients with this syndrome in whom it has been used.

The diagnosis of this syndrome is based on the demonstration of hyperoxaluria with calcium oxalate nephrolithiasis in patients with intestinal disorders associated with increased bile acid deconjugation. The response to administration of cholestyramine or taurine,[15,16] which appears to be of considerable therapeutic benefit, excludes coexisting primary hyperoxaluria. It seems desirable to measure oxalate excretion in all patients with intestinal disease and nephrolithiasis, since effective therapy for prevention of this complication is available.

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)WTH AND REPRODUCTION OF GUINEA PIGS
) THREE LEVELS OF ASCORBIC ACID

der No. 69-8863)

rilyn Lorna Steel, Ph.D.
nell University, 1968

The purpose of this study was to investigate the growth and
roduction of guinea pigs fed an ascorbic acid deficient but
erwise adequate diet. Fourteen day old females were fed
of three levels of ascorbic acid: 0.4, 1.0, or 10.0 mg per
gm of body weight, until they had given birth to and weaned
ee litters. The young males were sacrificed at fourteen
s of age. The young females were fed the same level of
orbic acid as the parent animal, and were kept for breeding
rposes. The criteria were growth, matings, pregnancies,
rtions, offspring mortality, and tissue ascorbic acid levels.
Growth results showed that first generation offspring were
avier at both 14 and 92 days of age, and gained more weight
ring the 78 day growth period, than the parent animals. Dif-
erences in weight gain among the three parent groups for the
rowth period were not significant, but differences between
arents and offspring were significant.

The data showed that animals fed 0.4 mg of ascorbic acid
er 100 gm of body weight had more difficulty in starting a
regnancy, pregnancy was more likely to end unsuccessfully,
e abortion rate was higher, the death rate was higher, and
bortions and deaths occurred in earlier pregnancies than in
e groups fed 1.0 or 10.0 mg of ascorbic acid per 100 gm of
ody weight. The highest level of ascorbic acid appeared to
rotect the parent animal from obvious malfunctions of the
reproductive faculty.

The survival rates of the offspring were highest when the
arent animal was fed 0.4 mg of ascorbic acid per 100 gm of
ody weight, and lowest when it was fed 10.0 mg of ascorbic
cid per 100 gm of body weight. Adrenal ascorbic acid values
14 day old males increased significantly with the ascorbic
cid level fed the parent animal.

The results presented here support the hypothesis that the
arent animal does not show obvious signs of malfunction on a
igh ascorbic acid regimen, but that such a regimen is detri-
ental to the survival of the offspring. It does not support the
ypothesis that such offspring develop scurvy in utero, although
eir ascorbic acid requirements may be increased by such
reatment.

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ASCORBIC ACID AS AN ANTISLUDGING AGENT IN EXTRACORPOREAL CIRCULATION

TAKASHI TANAKA, M.D., LESLIE R. BENNETT, M.D., AND JAMES V. MALONEY, JR., M.D., F.A.C.S.

Many investigators have confirmed the existence of intravascular sludging of erythrocytes in animals and man undergoing extracorporeal circulation. Previous studies from this laboratory identified certain structural alterations in plasma proteins (denaturation) as a cause of this sludging. With an understanding of the mechanism of sludging, it becomes possible on the basis of chemical logic to select "antidotes" to prevent the phenomenon. Sachs and associates have pointed out 4 general classes of compounds which might be expected to influence sludging favorably. In preliminary screening tests 3 agents in addition to low molecular weight dextran were demon-

From the Departments of Surgery and Radiology, University of California Medical Center, Los Angeles, California. *Aided by United States Public Health Service Grants H-2812 and HTS-5357.*

strated to be effective in the treatment of postperfusion intravascular sludging in the dog. The present study is a follow-up evaluation of ascorbic acid as an antisludging agent. It was selected for test because it is a nontoxic biological reducing agent, a class of compounds known to obviate some of the biophysical effects of protein denaturation.

METHODS

The evaluation of sludging by microscopic observation of the capillary circulation is subjective and not quantitative. A modification of Shoemaker's method was used to quantitate erythrocyte sequestration. Radioactivity of whole blood and hematocrit were determined by successive sampling for as long as 90 min. after the injection of Cr^{51}-tagged erythrocytes. The equilibration of tagged cells with the total body erythrocyte mass is normally completed within 10 min. after the injection; it is delayed when sequestration is present. The volume of erythrocytes sequestered was obtained from the difference in the red blood cell volumes calculated on the basis of the 10 min. and 90 min. samples. Control observations on erythrocyte mixing were made in 40 splenectomized dogs anesthetized with sodium pentobarbital. Nineteen splenectomized dogs were subjected to 1 hr. perfusion (at normal body temperature) with a disposable bubble oxygenator. In 14 of the animals undergoing perfusion, 3.0 gm. of ascorbic acid were administered (1 gm. intravenously prior to perfusion, 1 gm. added to the homologous blood used to prime the

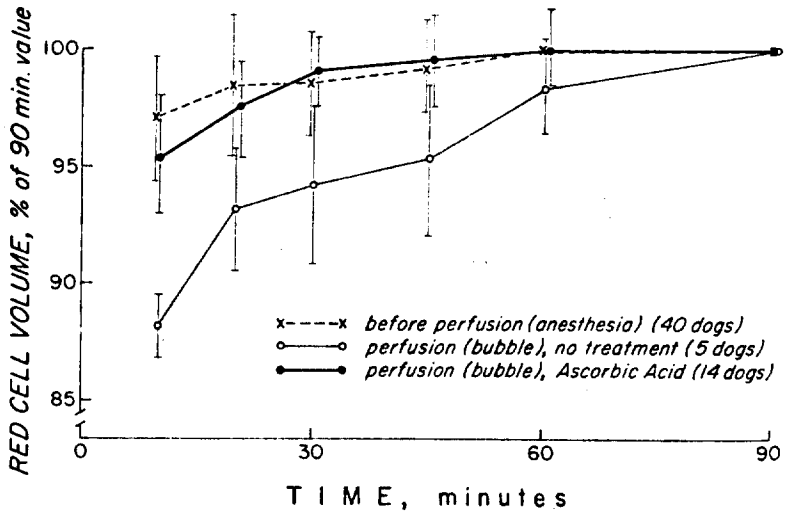

Fig. 1

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oxygenator, and 1 gm. during perfusion). The other 5 animals received no special treatment.

RESULTS

Prior to perfusion (control period) 3.0% ± 2.7 (S.D.) ± 0.1 (S.E.) of the total circulating red blood cell mass was sequestered, presumably as the result of the pentobarbital anesthesia (Fig. 1). Following the perfusion 11.8% ± 1.4 (S.D.) ± 0.6 (S.E.) of the total body erythrocyte mass was demonstrated to be sequestered and some erythrocytes remained sequestered in the peripheral microcirculation for as long as 60 to 90 min. When ascorbic acid was administered, only 4.6% ± 2.1 (S.D.) ± 0.7 (S.E.) of the total circulating erythrocyte mass was found to be sequestered. Sequestration was strikingly diminished in the animals treated with ascorbic acid ($p < 0.001$) and was not statistically different from the sequestration due to anesthesia alone.

SUMMARY

By means of a radioisotope method which quantitates erythrocyte sequestration, the effect of ascorbic acid on erythrocyte sequestration in the microcirculation was evaluated. The study demonstrated a striking beneficial effect of ascorbic acid in the prevention of sequestration following extracorporeal circulation in dogs.

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TOXIC SIDE EFFECTS OF ASCORBIC ACID - HYPERVITAMINOSIS C?

F. Widenbauer (From the Children's Clinic of the State Academy for Practical Medicine in Danzig - Director: Prof. Dr. med. habil. A. Adam)

The possibility of the occurence of hypervitaminosis C is generally denied (Stepp), namely particularly in view of the fact that vitamin C is stored in the body only up to a certain extent and additional amounts administered are excreted in the urine. v. Jeney, Gagyi and Baranyi have observed a fatty degeneration of the liver in guinea pigs after daily administration of 2 mg ascorbic acid, while Demole, using extremely high doses of ascorbic acid on cold-and warm-blooded animals, was unable to note any changes which would indicate the presence of a hypervitaminosis C. Only in the mouse did an oral administration of 5 g ascorbic acid exert a temporary (transient) laxative effect. In man, only Schade has described a mild hypervitaminosis in adults, which expressed itself in a tendency towards vagotonia (slowing of the pulse rate, hyperperistalsis, changes in the blood picture).

In loading tests carried out according to Harris and Ray on 29 infants, 93 young and school-age children and 20 adults during a period of over 1400 days with the administration of 100 to 6000 mg ascorbic acid, it was possible to observe toxic phenomena in 5 adults and 4 infants (Widenbauer). The symptoms were always clearly expressed and included, in healthy adults, nausea, retching, a sensation of heat, hot flushes in the head, a reddening of the face, headache, fatigue, exhaustion, and disturbed sleep, including diarrhea with watery stools and increased peristalsis. In addition, a slowing of the pulse rate by 6-10 beats per minute and increased diuresis was noted. These symptoms, which are caused in part by an increased vagotonia, occur by no means only after saturation of the body at the ascorbic acid loading level, but already after oral administration of 25-100 mg ascorbic acid. These symptoms vanished after administration was discontinued, or did not occur at all or only in a reduced form during simultaneous intake of ephetonin tablets. A slight or moderate vitamin C deficiency was noted in the test subjects, which received a dose of 750 to 1600 mg ascorbic acid in the loading tests.

Do these observations of definite toxic effects of ascorbic acid entitle us to speak about a hypervitaminosis C? I would like to give a negative answer to this question, because these toxic symptoms were already present at a dosage corresponding to the daily consumption, because a several times greater dose was tolerated by the great majority of people without any kind of unfavorable side effects, and because the oral and parenteral administration to these sensitive people of 100 mg of the sodium salt of ascorbic acid (Redoxon, supplied by the F. Hoffmann-LaRoche firm in Basel) did not produce any toxic reaction, but, on the contrary, had a restorative effect according to consistent judgement. In addition, in one case, none of these toxic symptoms could be noted 2 months later during a further oral administration of 1000 mg ascorbic acid.

For the above reasons, it is no longer possible to speak about a hypervitaminosis C; rather, we must assume that we are dealing here with a particular sensitivity of certain persons towards orally administered free ascorbic acid. This appears to be all the more probable, since in 2 cases a pathological sensitivity to other substances was observed, namely in one case an idiosyncrasy towards quinine, and in another case towards atropine, strawberries and crayfish (crabs) could be established from case history details. All of these cases, in addition, involved highly differentiated and autonomically unstable persons with characteristics (traits) pointing in a vagotonic direction. It is understandable that a substance acting on the vagus can occasionally cause more violent symptoms of this nature in such persons. Since the oral and parenteral administration of the sodium salt did not cause such side effects, the thought occurs in our mind that these side effects are perhaps induced by the free acid group and are caused by variations (shifts) in the pH of the blood.

Observations of toxic side effects in infants due to oral administration of ascorbic acid have already been reported by me elsewhere, and consisted in increased dermography, skin hyperemia, rubella-like, morbilliform or scarlatiniform erythemas, especially on the chest and abdomen, and increased peristalsis. In one case, urticaria and edema were noted. In general, these symptoms disappeared several hours after administration of ascorbic acid was discontinued. Only in one case, in a puffy 6-months old infant with rickets and spasmophilia, did erythema extend further after administration was stopped and an itching urticaria set in, accompanied by edemata which led to a

weight increase of 480 g within the 10 days of medication. After 2 days, all these symptoms had again disappeared. In some other cases, daily doses of 50 mg ascorbic acid sometimes caused thin, watery stools without any mucus or blood admixtures, which disappeared immediately after ascorbic acid was discontinued. These toxic side effects of ascorbic acid have never resulted in more severe injuries, and must be considered as pathological increased rates (excesses) of actual therapeutically desirable processes (better blood supply of the skin, stimulation of peristalsis, water binding) in particularly sensitive subjects. Edema formation in the puffy infant represents a paradoxical reaction to ascorbic acid, since otherwise an elimination of subcutaneous tissue water with increased diuresis has always been achieved in puffy subjects. Water storage (infiltration) under ascorbic acid has otherwise been observed only in water-deficient dystrophic persons.

Because these toxic symptoms occurred in infants only after administration of high (650-850 mg) doses of ascorbic acid, which were close to the saturation peak, and because smaller amounts were well tolerated, the term "hypervitaminosis" was used to designate this phenomenon, as I have done myself a short time ago. This should be understood, however, with the limitation (reservation) that a hypervitaminosis C can arise only in certain particularly sensitive infants. It would appear more correct, however, to assume, both for adults and infants, not the presence of a hypervitaminosis, but rather a particular (special) sensitivity to orally administered ascorbic acid in especially predisposed subjects, since the majority of the infants examined did not exhibit any such toxic symptoms. The reason why the toxic dose of ascorbic acid is higher in the infant having a special predisposition to it than in the adult with special vegetative characteristics can be explained by the fact that the infant is also able to tolerate higher doses of other drugs (e.g. atropine, luminal, spirocid) than the adult.

These toxic side effects of orally administered free ascorbic acid can perhaps also be avoided in the infant by using the sodium salt, although sofar I have no experimental proof of this fact. In such cases, it is possible also that the correlation of vitamins has been disturbed (disrupted) by the increased administration of <u>one</u> vitamin. The nutritional case history in 3 of these cases would appear to suggest a deficiency of B vitamins, which could have facilitated the disruption of vitamin correlation. The

infants presently 5, 6 and 7 months old have namely received, after a nursing period of 3, 2 1/2, and 2 months, only half-strength cow's milk or half-strength cow's milk and gruel. The loading test has established a lack of vitamin C (loading or functional dose of 550, 750 and 1000 mg ascorbic acid).

Disorders caused by ascorbic acid were never observed in small and school-age children.

Summary

Oral administration of small amounts of ascorbic acid ocassionally caused some toxic side effects in individual adults with special vegetative characteristics, which should not be considered as hypervitaminosis C, but as a special sensitivity to vitamin C. These toxic side effects did not occur after oral or parenteral administration of the sodium salt of ascorbic acid, which resulted rather in an improvement of the subjective general state of health.

In individual infants, high doses of ascorbic acid led to toxic side effects, which quickly disappeared without leaving any injuries and are also based on a special sensitivity.

However, the prescription of ascorbic acid should not be restricted in view of these facts; when toxic side effects occur in isolated cases, the medication should be discontinued.

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TOXISCHE NEBENWIRKUNGEN VON ASCORBINSÄURE — C-HYPERVITAMINOSE?

Von

F. WIDENBAUER.

Aus der Kinderklinik der Staatlichen Akademie für praktische Medizin zu Danzig (Direktor: Professor Dr. med. habil. A. ADAM).

Die Möglichkeit einer C-Hypervitaminose wird allgemein abgelehnt (STEPP), und zwar insbesondere deswegen, weil Vitamin C nur bis zu einem gewissen Grade im Körper gespeichert wird und darüber hinaus zugeführte Mengen im Urin ausgeschieden werden. v. JENEY, GAGYI und BARANYI haben bei Meerschweinchen nach täglicher Zufuhr von 2 mg Ascorbinsäure Verfettung der Leber gesehen, während DEMOLE bei Kalt- und Warmblütern mit außerordentlich hohen Dosen keine Veränderungen feststellen konnte, welche auf Hypervitaminose hätten schließen lassen. Nur bei der Maus riefen perorale Gaben von 5 g Ascorbinsäure einen vorübergehenden laxativen Effekt hervor. Am Menschen hat nur SCHADE eine milde Hypervitaminose bei Erwachsenen beschrieben, welche sich in Neigung zur Vagotonie (Pulsverlangsamung, Peristaltiksteigerung, Änderungen im Blutbild) geltend machte.

Bei an 29 Säuglingen, 93 Klein- und Schulkindern und 20 Erwachsenen während mehr als 1400 Versuchstagen durchgeführten Belastungsprüfungen nach HARRIS und RAY mit Gaben von 100 bis 6000 mg Ascorbinsäure war Gelegenheit gegeben, toxische Erscheinungen bei 5 Erwachsenen und 4 Säuglingen (WIDENBAUER) zu beobachten. Die Symptome waren stets deutlich ausgeprägt und bestanden bei den gesunden Erwachsenen in Übelkeit, Brechreiz, Hitzegefühl, heißen Wallungen zum Kopfe, Rötung des Gesichtes, Kopfschmerzen, Müdigkeit, Abgeschlagenheit, gestörtem Schlaf, bis zu Durchfällen mit wässerigen Stühlen gesteigerter Peristaltik. Außerdem trat Verlangsamung des Pulses um 6 bis 10 Schläge in der Minute und gesteigerte Diurese auf. Diese Erscheinungen, die zum Teil auf einer Steigerung des Vagotonus beruhen, setzen durchaus nicht erst nach Sättigung des Körpers auf der Höhe der Belastung mit Ascorbinsäure ein, sondern schon nach peroraler Zufuhr von 25–100 mg. Sie verschwanden nach Absetzen oder traten nicht oder vermindert auf bei gleichzeitiger Einnahme von Ephetonintabletten. Bei den Personen bestand leichter oder mittlerer Vitamin C-Mangel (Belastungsdosis 750 bis 1600 mg Ascorbinsäure im Belastungstest).

Berechtigen uns nun diese Beobachtungen solcher zweifelsohne toxischer Wirkungen der Ascorbinsäure dazu, von C-Hypervitaminose zu sprechen? Ich möchte die Frage verneinen, weil diese toxischen Symptome schon bei einer Dosierung, wie sie dem täglichen Verbrauch entspricht, vorhanden waren, weil von der überwiegenden Mehrzahl der Personen eine vielfache Dosis ohne jede ungünstige Nebenwirkung vertragen wurde und weil bei diesen empfindlichen Personen perorale und parenterale Verabreichung von 100 mg des Natriumsalzes der Ascorbinsäure (Redoxon*) keine toxische Reaktion auslöste, sondern im Gegenteil nach übereinstimmendem Urteil erfrischend wirkte. Überdies konnte in einem Fall 2 Monate später bei einer neuerdings durchgeführten Belastung mit 1000 mg Ascorbinsäure per os keines dieser toxischen Zeichen mehr beobachtet werden.

Aus den angeführten Gründen ist es daher wohl nicht angängig, von einer C-Hypervitaminose zu sprechen; vielmehr muß angenommen werden, daß es sich hier um eine besondere Empfindlichkeit gewisser Personen gegen peroral zugeführte freie Ascorbinsäure handelt. Dies erscheint um so wahrscheinlicher, als in 2 Fällen krankhafte Empfindlichkeit gegen andere Substanzen, in einem Fall Idiosyncrasie gegen Chinin und in einem anderen gegen Atropin, Erdbeeren und Krebse anamnestisch festgestellt werden konnte. Zudem handelte es sich in allen diesen Fällen um sehr differenzierte, vegetativ labile Personen mit Stigmata nach der vagotonischen Seite hin. Es mag verständlich sein, daß eine Substanz, die auf den Vagus wirkt, bei solchen Personen mitunter zu derartigen heftigeren Erscheinungen führen kann. Da das peroral und parenteral zugeführte Natriumsalz keine solchen Nebenwirkungen auslöste, wäre daran zu denken, ob diese vielleicht von der freien Säuregruppe ausgehen und durch Verschiebungen im p_H des Blutes bedingt sind.

Über Beobachtungen von toxischen Nebenwirkungen peroral zugeführter Ascorbinsäure bei Säuglingen habe ich bereits an anderer Stelle berichtet. Sie bestanden in verstärktem Dermographismus, Hyperämie der Haut, rubeoli-, morbilli- oder scarlatiniformen Erythemen, besonders an Brust und Bauch, gesteigerter Peristaltik. In einem Fall trat Urticaria und Ödem auf. Im allgemeinen verschwanden diese Erscheinungen einige Stunden nach Absetzen der Ascorbinsäure. Nur in einem Fall, bei einem pastösen sechs Monate alten Säugling mit Rachitis und Spasmophilie breitete sich nach Absetzen das Erythem noch weiter aus und trat unter Begleitung von Ödemen, die innerhalb der 10 Medikationstage zu einer Gewichtszunahme von 480 g geführt hatten, juckende Urticaria auf. In 2 Tagen war alles wieder abgeklungen. In einigen anderen Fällen verursachten täglich 50 mg Ascorbinsäure bisweilen dünne, wässerige Stühle ohne Schleim- oder Blutbeimengung, welche sofort nach Absetzen wieder verschwanden. Diese durch Ascorbinsäure hervorgerufenen toxischen Nebenwirkungen haben in den beobachteten Fällen nie zu einer ernsteren Gefährdung geführt und sind als pathologische Übersteigerungen an sich therapeutisch erwünschter Vorgänge (bessere Hautdurchblutung, Anregung der Peristaltik, Wasserbindung) bei besonders Empfindlichen aufzufassen. Die Ödembildung bei dem pastösen Säugling stellt eine paradoxe Reaktion auf Ascorbinsäure dar, denn anderweitig wurde bei Pastösen immer eine Ausschwemmung des Unterhautgewebewassers unter Diuresesteigerung erzielt. Wassereinlagerung unter Ascorbinsäure wurde sonst nur bei wasserverarmten Dystrophikern beobachtet.

Weil diese toxischen Erscheinungen bei Säuglingen erst nach Verabreichung hoher Dosen von Ascorbinsäure, 650 bis 850 mg, nahe dem Höhepunkte der Sättigung auftraten und geringere Mengen vertragen wurden, könnte dafür die Bezeichnung „Hypervitaminose" angewendet werden, wie ich das kürzlich getan habe. Dies müßte aber mit der Einschränkung verstanden werden, daß C-Hypervitaminose nur bei bestimmten, in besonderer Weise empfindlichen Säuglingen auftreten kann. Es mag jedoch richtiger sein, nicht Hypervitaminose, sondern ebenso wie beim Erwachsenen auch beim Säugling nur eine besondere Empfindlichkeit gegen peroral zugeführte Ascorbinsäure bei dafür besonders Disponierten anzunehmen. Denn die Mehrzahl der untersuchten Säuglinge zeigte keine derartigen toxischen Erscheinungen. Daß die toxische Dosis von Ascorbinsäure bei dem besonders dafür disponierten Säugling größer ist als bei dem vegetativ stigmatisierten Erwachsenen, kann darin begründet sein, daß der Säugling überhaupt auch von anderen Medikamenten (z. B. Atropin, Luminal, Spirocid) größere Dosen als der Erwachsene vertragen kann.

Vielleicht sind diese toxischen Nebenwirkungen von peroral zugeführter freier Ascorbinsäure auch beim Säugling zu vermeiden durch Verwendung des Natriumsalzes; Erfah-

* Von der Firma F. Hoffmann-La Roche, Basel, in freundlicher Weise zur Verfügung gestellt.

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rungen darüber fehlen mir noch. Möglicherweise kann es sich in diesen Fällen auch um eine Störung der Korrelation der Vitamine durch die vermehrte Zufuhr *eines* Vitamins gehandelt haben. Die Ernährungsanamnese ließe in 3 von diesen Fällen an einen Mangel an den B-Vitaminen denken, der die Korrelationsstörung erleichtert haben kann. Die zur Zeit 5, 6 und 7 Monate alten Säuglinge haben nämlich nach einer Stillzeit von 3, $2^1/_2$ und 2 Monaten nur Halbmilch oder Halbmilch und Brei erhalten. Ein Mangel an C-Vitamin konnte mit der Belastungsprüfung nachgewiesen werden (Belastungsdosis 550, 750, 1000 mg Ascorbinsäure).

Bei Klein- und Schulkindern konnten niemals durch Ascorbinsäure ausgelöste Störungen beobachtet werden.

Zusammenfassung: Perorale Verabreichung geringer Mengen von Ascorbinsäure hatte bei einzelnen vegetativ stigmatisierten Erwachsenen zeitweise toxische Nebenwirkungen, die nicht als C-Hypervitaminose, sondern als besondere Vitamin C-Empfindlichkeit aufzufassen sind. Sie traten nicht auf nach peroralen oder parenteralen Gaben des Natriumsalzes der Ascorbinsäure; diese hatten vielmehr eine Hebung des subjektiven Allgemeinbefindens zur Folge.

Bei einzelnen Säuglingen führten hohe Dosen von Ascorbinsäure zu toxischen Nebenwirkungen, welche rasch vorübergingen, niemals Schädigungen hinterließen und ebenfalls auf einer besonderen Empfindlichkeit beruhen.

Die Verordnung von Ascorbinsäure erfährt damit jedoch keine Einschränkungen. Sollten toxische Nebenwirkungen in Einzelfällen auftreten, so ist die Medikation abzusetzen.

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Vitamin C makes colds less complex

Results from a recent clinical trial—which avoided severe design limitations of previous tests—suggest that daily vitamin C reduces the complexity of cold symptoms. Furthermore, women appear to be better equipped metabolically to cope with colds than are men

Cedric Wilson
is professor of pharmacology at Trinity College, University of Dublin

Since 1971 Linus Pauling has been proclaiming the benefits of vitamin C (ascorbic acid) as a prophylactic against the common cold. He concluded that the vitamin has considerable anti-cold effects after analysing collected data on the subject presented in the journal Nutrition Reviews in 1967. Pauling's view was in direct opposition to an editorial in the same issue of the journal which concluded that "there is no conclusive evidence that ascorbic acid has any protective effect against, or any therapeutic effect on the course of, the common cold in healthy people not depleted of ascorbic acid". Since 1967 there have been many reports on the anti-cold efficacy of vitamin C, some of which support Pauling's hypothesis, some of which do not. A drawback of many of these trials is that they take no account of such factors as sex, age, and vitamin C status of the subjects studied. My colleagues and I in Dublin are soon to publish results of extensive investigations on vitamin C—in both humans and animals—in which we report that the vitamin does indeed have marked prophylactic effects against the common cold. We also find a significant difference between men and women in their metabolic utilisation of vitamin C.

Investigations into the relationship between the common cold and vitamin C have provided questionable results in the past because it is difficult to precisely define the common cold and because people have not taken account of the fact that vitamin C is a normal constituent of human tissues. We have now taken care to define these symptoms and their relationship to one another. The previous studies reported in Nutrition Reviews, describe the effect of vitamin C on the numbers of colds occurring in groups given vitamin C or dummy tablets, but other characteristics of the symptomatology of the common cold are not discussed. In our trial on boarding school children in Dublin we asked the pupils to record daily over a nine month period the occurrence and severity of the symptoms listed below.

Toxic symptoms	Catarrhal symptoms
Headache	Cold in head
Feverish	Cough
Out of sorts	Nasal obstruction
Sore throat	Nasal discharge

During this period we gave the children in two male and two female boarding schools either dummy or 200 mg tablets of vitamin C, or 200 or 500 mg tablets of vitamin C on a double blind clinical trial basis. We confirmed that the children absorbed the doses by looking at the level of vitamin C in their blood. We discovered, somewhat to our surprise, that the girls attained higher blood levels than males and utilised the vitamin differently. When they were given dummy tablets the girls tended to get more intense colds of longer duration than boys, but the boys' symptoms tended to be more complex than the girls. In the groups receiving 200 mg vitamin C daily we found that severity and intensity of catarrhal colds became significantly reduced in girls, and it fell still further when we gave 500 mg. The complexity of the girls' colds was also reduced. On the other hand even the larger doses of vitamin C did not alter the intensity of symptoms in the boys. In fact the dose of 200 mg appeared to exacerbate them. With the dose of 500 mg, symptom intensity again reverted to that in the untreated boys given dummy tablets. Vitamin C did, however, reduce the complexity of boys' colds and so produced some beneficial effect in them.

Two animals that need vitamin C

Because of a genetic mutation, guinea pigs and man are unable to synthesise the enzyme gulonolactone oxidase—which is used to manufacture vitamin C—so they depend on vitamin C in their food. Scurvy and ultimate death result from a dietary absence of vitamin C in man and his metabolic companion the guinea pig. There is, however, considerable variability in guinea-pig requirements for exogenous vitamin C to prevent the development of scurvy. Researchers, using both experimental investigations in which volunteers are given scorbutogenic (scurvy-forming) diets, and clinical observations, find that humans also vary in their need for the vitamin.

Irwin Stone has suggested that vitamin C really does act as an essential food factor in guinea pigs and human beings, it should be relatively easy to determine the extent of the defect in genetic inheritance by observing the development of scorbutic symptoms, and measuring ability to synthesise ascorbic acid while subjects are on a scorbutogenic diet. We have now completed such an experiment on guinea pigs. We found that all male guinea pigs on the diet died of scurvy after 30 days. In contrast, the majority of female guinea pigs died after 36 days; moreover, in a number of these experiments some females lived for as long as 150 days. It turns out that these females activate a small amount of gulonolactone oxidase—the enzyme needed to manufacture C—in their livers.

We have administered gulonolactone—the precursor of vitamin C—to female guinea pigs and we discover that they can convert it to vitamin C and therefore survive longer. We conclude from these results that in conditions when excessive demands are being made for vitamin C female guinea pigs can

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activate their gulonolactone oxidase and synthesise limited quantities of the vitamin. They can therefore survive the critical 27 to 36th days of the diet when vitamin C deficient males normally die.

Vitamin C, in addition to iron and folic acid is necessary for formation of haemoglobin the iron-containing protein in red blood cells. When iron is given to humans for the treatment of iron deficiency anaemia the tissues also require folic acid and vitamin C to produce haemoglobin. Folic acid comes for this purpose from the tissue stores. Vitamin C is withdrawn from the stores in the white cells and platelets (the buffy coat of the blood). When iron is given daily to elderly male patients, haemoglobin rises to a value of five per cent above its initial level after 16 weeks. Their vitamin C stores fall consistently to 86 per cent of their initial value over the same period. When elderly females are given the same dose of iron their haemoglobin rises to 110 per cent of its initial value after 16 weeks Their vitamin C stores fall sharply to 63 per cent after six weeks, then rise to 133 per cent of their initial value at the end of the trial. It seems, therefore, that women also react to a tissue demand for vitamin C by instituting a metabolic readjustment which activates vitamin C synthesis, and that males do not have this capacity. This suggestion is supported by the clinical evidence that women show less pronounced symptoms of scurvy than men, and have a lower incidence of the disease.

Vitamin C remains as a labile store in the buffy coat of the blood, and can be rapidly transferred into the plasma from whence it passes into the tissues for metabolic use. A small stable pool is maintained in the buffy coat, even when an animal is dying from scurvy. This stable pool is used by the white cells to maintain their viability and sustain their defensive action against invading organisms. When a loading dose of 500 mg vitamin C is administered during a cold, the plasma vitamin C rises but not to the same extent as after recovery from the cold. This indicates that the loading dose is being absorbed from the gut. However, it is excreted in the urine less during the cold than after recovery from the cold symptoms. We have demonstrated that during a cold the vitamin C labile store in the buffy coat is significantly reduced. A loading dose of vitamin C does not go into the buffy coat in either males or females, but metabolic turnover of vitamin C in the tissues is more pronounced in females. When a loading dose of 1000 mg is given during a cold, females can replete their labile stores for up to six hours, but males' vitamin C stores remain at a diminished level. This indicates that vitamin C utilisation is enhanced while cold symptoms are present. We find that there is a direct correlation between utilisation of ascorbic acid and severity of cold symptoms. It also demonstrates that the larger supplement is incapable of compensating for the increased vitamin C utilisation in males during colds, although this dose can compensate for a period of about six hours in females.

When catarrhal symptoms predominate, vitamin C is absorbed chiefly into the inflamed naso-pharyngeal tissues. When toxic symptoms predominate, vitamin C passes into the body cells to combat toxic effects of the virus. In females, vitamic C primarily combats the damage associated with catarrhal symptoms and excess vitamin C is utilised during the control of toxic symptoms. Of a dose of 1000 mg of vitamin C during a cold, a small amount of the vitamin is stored for a maximum of four hours in males but for six hours or more in females. Our results suggest that 2000 mg of vitamin C six-hourly will maintain a normal metabolic turnover rate of the vitamin during a cold, and that 2000 mg daily should have a beneficial prophylactic effect against the common cold in about 90 per cent of females, but that males will require about 2500 mg daily. We conclude from our investigations that vitamic C utilisation increases during a cold because the vitamin is required to enhance immunological defence mechanisms, maintain phagocytosis, and promote tissue healing. Females require smaller doses than males because of their more efficient metabolic readjustment to tissue demand for ascorbic acid during a cold.

Inadequate recommendations

When the United Kingdom Panel on Recommended Allowances of Nutrients (1969) discussed the possibility that human beings should be maintained in a constant state of tissue saturation with vitamin C, it said that there is no evidence that man derives any benefit from a daily dose of the vitamin adequate to maintain such a state. The panel therefore recommended that an amount of the vitamin sufficient to prevent signs of deficiency (scurvy), and having a safety margin to allow for individual variation and for stresses of everyday life, is adequate as a dietary intake. They recommended 30 mg daily for adults of both sexes of 15 years or more. Pregnant women should take 60 mg a day. These levels must now be seen as inadequate.

Our investigations demonstrate that cold symptomatology can be assessed in terms of symptom association and that this method of analysis appears to be more sensitive than measurement of the syndrome in its entirety. It provides a method of relating the cold syndrome to concurrent changes in ascorbic acid utilisation. The therapeutic effect of vitamin C on the common cold cannot be assessed by standard clinical trial methods because vitamin C is a normal constituent of the tissues and plays an essential role in maintaining the viability and normal health of the tissues. This must be taken into account in any clinical trial dosing. To maintain this healthy state the tissues must be saturated with ascorbic acid at all times. We have shown that females have an innate ability enabling them to maintain this state of saturation more efficiently than males during conditions of stress. This type of sex differential has previously been reported to characterise certain types of drug metabolism, but it has never previously been demonstrated to be characteristic of a vitamin.

UNIVERSITY OF DUBLIN

Professor C. W. M. Wilson

DEPARTMENT OF PHARMACOLOGY
TRINITY COLLEGE
DUBLIN 2
IRELAND

Tel. 772941
Ext. 563

CWMW/CAB

15th May, 1974.

Dr. Humphrey F. Sassoon,
Tracor Jitco, Inc.,
1300 East Gude Drive,
Rockville,
Maryland 20851,
U.S.A.

Dear Humphrey,

Thank you for your letter of May 8th. I am enclosing a reprint of the paper from the Practitioner which has just appeared. With reference to your question on the saturation of leucocytes and immunological efficiency, I have been doing some investigations on this. You may already have obtained the abstract of our paper on the antigen challenge test, in case you have not found it I am enclosing a photostat of it. We are now treating asthmatic patients and patients suffering from hay fever with doses of Vitamin C ranging from 500-1000 mg daily. I have some evidence that the symptoms improve to a limited extent after about two months. This is the time when our previous investigations have shown that tissue saturation has probably been achieved. We have already established that administration of supplementary Vitamin C significantly increases the FEV. This has already been shown by Zuskin et al in the United States. In relation to the work of tissue saturation in cell tissues it does appear that raising the degree of saturation by high supplementation does improve immunological efficiency both from the point of view from increasing respiratory volume and also by beneficially altering immunological mechanisms. I will let you have further reprints as soon as possible. In the meantime you can take the above information as being certain as a pilot investigation.

With kind regards.

Yours sincerely,

C.W.M. Wilson, M.D., Sc.D.,
FRCPE, FRSE.

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VITAMIN C

TISSUE SATURATION, METABOLISM AND DESATURATION

C. W. M. WILSON, M.D., SC.D., F.R.C.P.ED., F.R.S.ED.,
Professor of Pharmacology, University of Dublin, Trinity College, Dublin

IN 1753 Lind showed that scurvy could be prevented in his sailors by administration of oranges and lemons. Since Lind's first control trial in 1747, other investigators have induced scurvy deliberately in volunteer subjects and confirmed Lind's findings (e.g. Bartley, Krebs and O'Brien, 1953; Hodges *et al.*, 1969, 1971).

TISSUE SATURATION WITH VITAMIN C

The United Kingdom Panel on Recommended Allowances of Nutrients (HMSO, 1969) based its present recommendation of a daily allowance of 30 mg of vitamin C for adult men, and non-pregnant women, on the investigations of Bartley and his colleagues. The Panel proposed that sufficient of the vitamin to prevent signs of deficiency can be recommended as an adequate dietary intake. It pointed out, however, that animal species which can synthesize their own ascorbic acid (that is all except primates, guinea-pigs and the fruit-bat) maintain a high level of tissue saturation. The Panel stated that, for man, tissue saturation requires an intake of at least 60 mg/day. It affirmed that there is no evidence showing that man derives any benefit from such a high intake of vitamin C.

That British opinion in this matter is not accepted on an international basis can be seen from figure 1. The range of recommended doses varies from 30 mg in the United Kingdom to 125 mg in the USSR. This fourfold range can be explained in terms of the desirability for an individual either to take sufficient vitamin C to prevent the development of scurvy, or for the individual continuously to maintain full saturation of the tissues with ascorbic acid. The former explanation is a negative concept in which the desirable dose is that which prevents deficiency. The latter is the positive concept that vitamin C can improve tissue efficiency and integrity when it is available in sufficient quantity. Critics have claimed that the so-called large doses of vitamin C necessary to maintain tissue saturation may cause adverse effects, but the critics seem to have forgotten that vitamin C differs from any exogenous drug: it is a vitamin. By definition its presence in adequate quantity is necessary for normal metabolic function of the tissues. Physiological requirements differ between individuals, and requirements are greatly affected in pathological conditions. In any particular circumstance a small dose for one individual may be a large dose for another, just as the dose of vitamin B_{12} necessary to maintain an optimal haemopoietic and central nervous condition varies between patients with pernicious anaemia. General tissue metabolism is stimulated by a supply of the vitamin adequate to maintain tissue saturation (Evans and Hughes, 1963;

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Williams and Hughes, 1972). In such circumstances, factors increasing tissue demand for vitamin C may produce relative tissue desaturation at what in other individuals, who are less saturated, would be a high tissue saturation of ascorbic acid (Rhead and Schranzer, 1971; Briggs, 1973). Reduction in efficiency and integrity of the desaturated tissues then takes

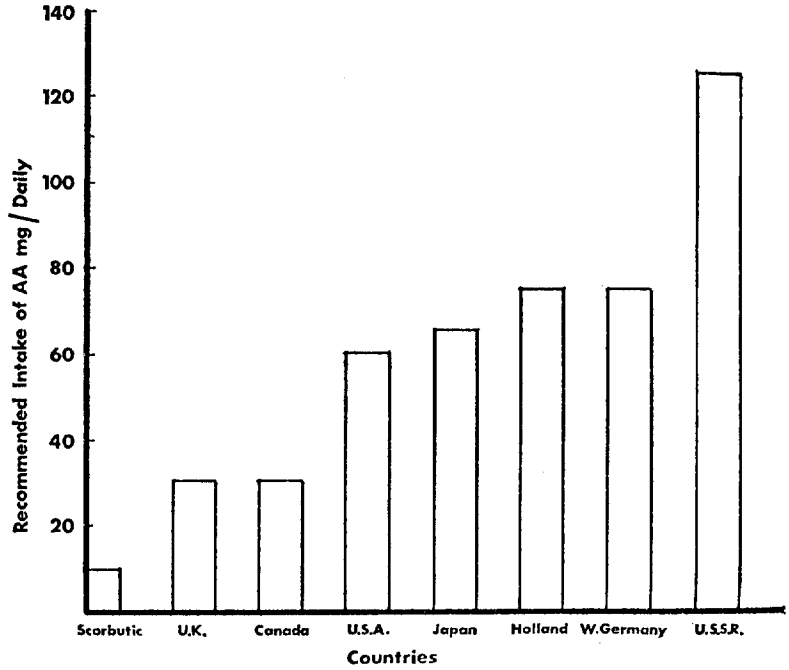

FIG. 1.—The minimum daily intake of vitamin C (AA) which will prevent frank clinical scurvy, compared with the official recommended daily intake of vitamin C in various countries.

place, while other tissues maintain their normal efficiency and integrity by virtue of their high ascorbic acid concentrations.

THE METABOLISM OF VITAMIN C

(1) ABSORPTION, UTILIZATION AND EXCRETION

Vitamin C is absorbed through the buccal mucous membrane, and the mucous membranes of the stomach and the upper part of the small intestine, whence it passes into the plasma (Spencer *et al.*, 1963). It is absorbed passively and actively (Odumosu and Wilson, 1971; Stewart and Booth, 1964). The active process becomes inhibited when the mucosal cells are damaged either by disease or as a result of surgical interference with the bowel (table I). The reduced blood concentration in these conditions is attributable to impaired absorption of the vitamin, particularly when there is extensive destruction of the mucous membrane as in malabsorption or gastroenteritis. Blood levels are reduced in peptic ulceration. This suggests that another factor involved in absorption or utilization of the

State of health	Leucocyte concentrations (Mean and standard deviations)		Reference
	Male	Female	
Healthy controls (age in years)			
4 to 12	56·4 ± 21·9	—	Loh and Wilson, 1971
11 to 18	36·6 ± 9·1	42·8 ± 12·6	
22 to 49	30·4 ± 8·7	34·0 ± 10·9	
56 to 87	24·4 ± 13·6	23·4 ± 11·3	
Smoking			
Non-smokers	24·6 ± 1·3	30·7 ± 1·4	Brook and Grimshaw, 1968
Moderate	19·6 ± 1·7	25·6 ± 1·6	
Heavy	17·4 ± 3·5	27·8 ± 1·4	
Petechiae and purpura in aged			
Sublingual petechiae (range and mean)	5·1 – 21·3	10·23	Andrews and Brook, 1966
Senile purpura	6·4 – 31·7	16·0	
Positive Hess test	15·2 ± 1·2	-27·9 ± 2·3	Eddy, 1972
Common cold			
During cold—3rd day	24·7 ± 9·7	27·6 ± 5·8	Wilson and Loh, 1974
3 weeks post-cold	32·9 ± 8·6	34·1 ± 11·6	
During cold—1st day	10·3 ± 0·3		Hume and Weyers, 1973
10 days after onset	24·0 ± 6·5		
Peptic ulceration			
Controls	17·9 ± 0·9		Russell *et al.*, 1968
Gastrointestinal haemorrhage	14·2 ± 0·9		
Aspirin or alcohol ingestion	12·6 ± 0·9		
Without history	18·2 ± 0·8		
Duodenal ulceration			
Controls	28·7 ± 8·1		Dymock *et al.*, 1968
Stenosis	11·7 ± 4·5		
Gastric surgery:			
without symptoms	10·1 ± 4·5		Cohen and Duncan, 1967
with symptoms	20·4 ± 8·4		
Duodenal ulcer:			
before surgery	8·7 ± 3·1		
after surgery	12·8 ± 4·3		
Controls	22·9 ± 5·9		
Duodenal ulcer general	10·6 ± 4·9		Esposito and Valentine, 1968
Following gastric surgery	12·7 ± 6·8		Williamson *et al.*, 1967
Controls	22·1 ± 6·4		Esposito and Valentine, 1968
Gastrointestinal disease			
Gastroduodenal disorders	11·0 ± 4·4		Cohen and Duncan, 1967
Intestinal malabsorption	10·7 ± 5·2		Williamson *et al.*, 1967
Controls	20·8 ± 10·5		

TABLE I.—Leucocyte ascorbic acid concentrations. Values related to age in healthy control subjects, in smokers, and related to petechiae and purpura in the elderly. Values four hours after a loading dose of 500 mg of vitamin C during and following common cold, and mean value during and 10 days after onset of cold symptoms. Reported values in gastrointestinal disorders.

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vitamin may also be deranged. Following surgical trauma, ascorbic acid enables tissue healing to take place.

Plasma and leucocyte ascorbic acid concentrations attain their maximum values four hours after oral administration of a loading dose of 500 mg of vitamin C to a normal individual (Wilson and Loh, 1974). The plasma concentration exceeds the renal threshold and ascorbic acid appears in the urine. Ascorbic acid passes actively and passively into the leucocytes, where it is stored. The maximum leucocyte ascorbic acid concentration rarely exceeds 60 $\mu g/10^8$ cells whatever the size or duration of oral administration. Ascorbic acid is a very labile compound passing into or out of the leucocyte stores into the plasma so that plasma and leucocyte concentrations simultaneously follow circadian (Loh and Wilson, 1973), monthly (Loh and Wilson, 1971a), and annual rhythms. The annual rhythm shows peak plasma and leucocyte levels in March and July, and a trough in October, suggesting that ascorbic acid undergoes an intrinsic metabolic rhythm in man which is independent of dietary intake of vitamin C (Loh, 1973).

Ascorbic acid goes from the plasma into the tissue where it plays an essential metabolic role in ovarian function, haemopoiesis, brain metabolism, corticosteroid release, and liver metabolism. It is a powerful reducing agent. It is involved in cholesterol synthesis and fatty acid metabolism, synthesis of lipoproteins, plasma and tissue proteins. It influences carbohydrate metabolism by affecting the action of insulin (Sigal and King, 1936; Roller, 1936; Stoicesio and Gingold, 1936). The more rapid rate of growth which follows administration of supplementary vitamin C is attributable to an energy-dependent process associated with greater retention of ascorbic acid by the tissues. Ascorbic acid is responsible for the formation of hydroxyproline from proline and this enables normal collagen formation to occur and tissue healing to take place.

Ascorbic acid is excreted by glomerular filtration and active tubular reabsorption, which is limited by a maximal rate. At low glomerular filtration rates, 97 to 99 per cent. is reabsorbed; 20 to 60 per cent. of a daily intake of 40 to 100 mg of vitamin C is excreted. When more than 100 mg is administered daily, 60 to 80 per cent. is excreted in healthy individuals (Friedman *et al.*, 1940; Ralli and Sherry, 1941). Due to these dynamic variations in its renal excretion, aspirin-induced depletion of ascorbic acid in normal individuals initially gives rise to a high excretion of ascorbic acid. This, however, does not result in scurvy on account of its large tubular reabsorption at low plasma levels (Loh *et al.*, 1973a). It is normally excreted unchanged, as reduced (dehydro) ascorbic acid, 2-3 diketogulonic acid, and as oxalate. About 40 per cent. of the urinary oxalate is derived from ascorbic acid but the mechanism of its formation is unknown (Atkins *et al.*, 1964). When leucocyte stores are fully saturated with ascorbic acid, and in consequence plasma levels are abnormally elevated in an individual following unjustified overdosage with vitamin C, oxaluria and formation of oxalic acid stones could occur (Wilson and Loh, 1973a). In deficiency

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the vitamin is excreted wholly as metabolites. When the body pool of ascorbic acid resumes a level of 1·5 G at a plasma level of about 0·2 mg/100 ml, ascorbic acid reappears in the urine.

(2) AGE AND SEX

An inverse relationship exists between age, and plasma and leucocyte ascorbic acid concentrations (Brook and Grimshaw, 1968; Loh and Wilson, 1971) so that lower levels occur in older subjects. In the youngest group, the leucocyte ascorbic acid concentrations were 36·6 $\mu g/10^8$ cells in boys and 42·8 $\mu g/10^8$ cells in girls (table I). Plasma and leucocyte ascorbic acid concentrations are even higher in children aged 4 to 12 years (plasma $0{\cdot}95 \pm 0{\cdot}25$; leucocyte $56{\cdot}4 \pm 21{\cdot}9$). Transfer of ascorbic acid into the plasma tends to be greater in adolescence. During the ageing process, leucocyte storage of ascorbic acid falls: metabolic demands for ascorbic acid become less and plasma levels diminish, particularly at the end of the reproductive period in women.

Females have a lower intake of vitamin C than males (Morgan *et al.*, 1955). In spite of this women have higher plasma and leucocyte concentrations of ascorbic acid (table I). In both sexes increased intake of the vitamin results in elevation of blood ascorbic acid levels, but the difference between the sexes in their blood concentrations of ascorbic acid is maintained (Woodhill, 1970; Loh, 1972). The sexes differ in their utilization of ascorbic acid (Loh *et al.*, 1974). This difference is greater during adolescence and disappears in old age when ascorbic acid metabolism tends to be unstable. In adults ascorbic acid concentrations tend to be more stable since an equilibrium has been attained between dietary intake, metabolic turnover and storage of the vitamin (Loh and Wilson, 1971b; Loh, 1971; Wilson, 1973). The sex difference does not manifest itself so clearly when the metabolic needs of the vitamin have reached equilibrium. It can then be detected in adults during the female reproductive cycle (Loh and Wilson, 1971), during deficiency of the vitamin, and in disease.

ASCORBIC ACID DESATURATION

(1) DEFICIENCY OF THE VITAMIN IN SCURVY

Reduction in the tissue saturation to which an individual is accustomed results in a variety of clinical manifestations (table II) (Hodges *et al.*, 1971). Their order of appearance depends upon metabolic demands for ascorbic acid by individual tissues, and the circadian availability of ascorbic acid from plasma. When the body pool of ascorbic acid is below 1·5 grammes, 6·5 mg of vitamin C daily does not uniformly relieve the features of scurvy (Bartley *et al.*, 1953; Hodges *et al.*, 1971). Scorbutic manifestations may appear in conditions of relative desaturation in subjects with high metabolic requirements for ascorbic acid in particular tissues. Petechial haemorrhages are characteristic of subclinical scurvy (Eddy, 1972). The metabolic state and initial saturation of the tissues will determine the appearance of this manifestation of desaturation, just as it does of other early manifest-

ations of subclinical scurvy such as aching limbs, swollen gums, or nose-bleeds. Any of these symptoms can appear to a minor degree one to two months after daily administration of 1G is stopped because relative de-saturation of the tissues occurs. The tissues then become adjusted to the altered metabolic state and symptoms recede.

Human studies in scurvy support the laboratory evidence regarding the capacity of females to readjust their ascorbic acid metabolism during deficiency of vitamin C. Historical evidence from the 9th Crusade suggests

Capillary and vascular changes Petechial tongue lesions Petechial haemorrhages Conjunctival haemorrhages Bleeding from the gums Nose-bleeds Ecchymoses Bruises Haematuria	*Respiratory changes* Dyspnoea Tonsillitis Tenderness of buccal mucosa Dental caries Xerostomia Colds
Changes in the skin Coiled hairs Congested follicles Loss of hair Acne Hyperkeratosis Scleroderma Pruritus Skin scaling Pigmentation: brown or purple Sjögren's syndrome	*Changes in peripheral nerves* Femoral neuropathy Skin ulceration *Mental changes* Physical fatigue Subjective fatigue Lassitude Drowsiness and lethargy Depression Hypochondriasis Hysteria and anxiety
Changes in joints, bones and muscles Arthralgia Joint effusions Bone tenderness Aching of limbs Pain and discoloration of legs	*Metabolic changes* No change in appetite Weight loss Oedema

TABLE II.—Clinical features of scurvy appearing between day 26 and day 180 in human subjects on scorbutogenic diets. Descriptions of the features are derived from four studies in which experimental scurvy was produced by administration of vitamin C free diets for up to 180 days, and from five accounts of clinical scurvy in Britain in which the diagnosis was confirmed by regression of lesions after administration of vitamin C. (References are obtainable from the author.)

that women can produce babies while men are suffering from severe scurvy (Wilson, 1973), and a living baby was born while scurvy was affecting the men of the Mayflower on its voyage to America. Women can reproduce, and bear living children, while men living under the same conditions are suffering from severe scurvy. This indicates that radical readjustments take place in the female utilization of ascorbic acid during periods of deficiency and stress. Scurvy is less common in women than in men (Cut-forth, 1958; Walker, 1968) and the scorbutic signs and symptoms are less severe than those in men (Brown, 1951; Bartley *et al.*, 1953; Cut-forth, 1958; Shafar, 1965). It has been suggested that the metabolic path

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for ascorbic acid is abnormal in women who develop scurvy because the disease has occurred in women living on a normal dietary intake of the vitamin (Mitra, 1969). Griffiths and his colleagues (1967) have carried out a survey of thiamine and ascorbic acid blood levels of geriatric patients admitted to hospital and of elderly people in their own homes. The majority of the patients showed at least one sign of classical vitamin deficiency on admission to hospital (Brocklehurst *et al.*, 1968). There was no evidence to suggest that dietary intake differed between the two sexes. Thiamine deficiency was evenly distributed between the sexes. Ascorbic acid deficiency was found to be significantly more common in the males. It can therefore be concluded that the even distribution of thiamine deficiency between the sexes was attributable to the deficient dietary intake of elderly people (Brin *et al.*, 1965; Hejda, 1969; Wilson and Nolan, 1970). In contrast it appears that the ascorbic acid deficiency and any associated lesions were metabolically determined characteristics of the sexes which were more pronounced in the males. Smokers have reduced leucocyte stores of ascorbic acid and lower plasma values than non-smokers, the male levels being significantly lower than those of females (Brook and Grimshaw, 1968). Smokers do not develop frank scurvy, due to renal conservation of their ascorbic acid. Smokers, however, have a higher incidence of respiratory infection than do non-smokers.

(2) ASCORBIC ACID DESATURATION DURING COLDS

During the past five years considerable information has become available about the metabolism of ascorbic acid during colds, and the mechanisms responsible for its desaturation while symptoms are present. During the first three days of the common cold, stores of ascorbic acid in the leucocytes are significantly reduced in males and females (Wilson and Loh, 1969; Hume and Weyers, 1973). Plasma ascorbic acid also tends to be reduced. As the viral infection develops, ascorbic acid passes out of the leucocyte stores into the plasma. It then passes into the tissues during the toxic phase of the infection to maintain cellular defence, and specifically into the inflamed respiratory tissues during the catarrhal phase (Wilson and Loh, 1973a, b; Wilson and Loh *et al.*, 1973a, b; Wilson and Loh, 1973; Wilson *et al.*, 1974). Administration of a single loading dose of 500 mg of vitamin C during the first three days of the cold symptoms causes a rise in plasma ascorbic acid. Absorption is therefore occurring from the alimentary canal. Urinary excretion of ascorbic acid is greatly diminished during a cold, less ascorbic acid appearing in the urine after administration of the loading dose. Leucocyte ascorbic acid concentrations do not increase in males or females during six hours following the loading dose, whereas increased plasma and leucocyte levels occur in both sexes three weeks after the last symptom has disappeared. Transfer of ascorbic acid through the plasma from the leucocytes into the tissues is more pronounced in males than in females during colds. When the loading dose of vitamin C is administered

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(fig. 2) three weeks after symptoms have disappeared, the passage of ascorbic acid from the plasma into the leucocyctes is significantly greater in the females. This indicates that the ascorbic acid tissue desaturation which occurred during the cold had become reversed in females. In contrast less ascorbic acid was transferred from the plasma into the leucocytes in males, indicating that tissue desaturation was still present. During colds predominance of catarrhal symptoms is associated with a greater flow of ascorbic acid from the labile store of the leucocytes into the plasma (Wilson and Loh, 1974).

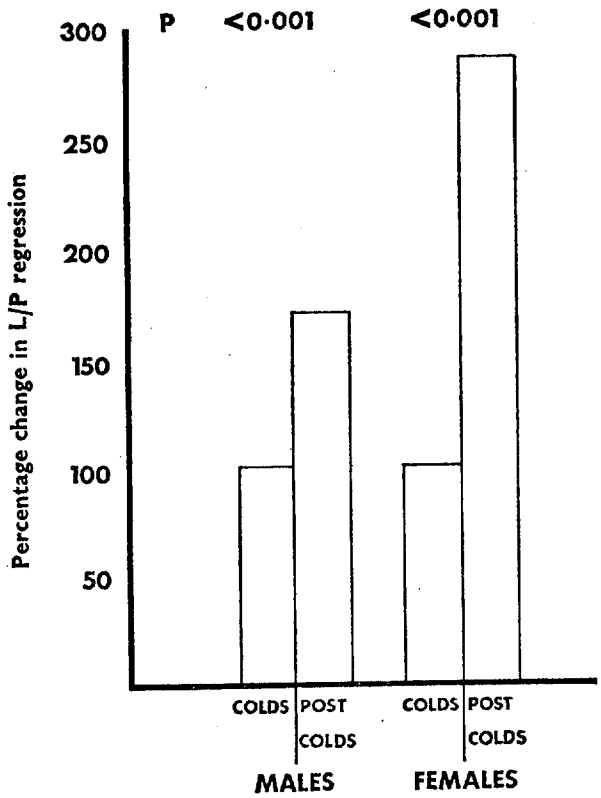

FIG. 2.—Analysis of the pooled regression coefficients relating leucocyte and plasma ascorbic acid values (L/P regressions) during the ascorbic acid blood-response curves after a loading dose of 500 mg of ascorbic acid in male and female subjects during and following recovery from the common cold. The L/P regressions provide a measure of the transfer of ascorbic acid from the plasma into the leucocytes following the loading dose.

In women a loading dose of 2000 mg of vitamin C during a cold results in significant elevation of leucocyte ascorbic acid concentrations in four hours, but in men this dose raises stores only a slight amount. Maintenance of tissue saturation by this therapeutic dose six-hourly is associated with reduction in severity of cold symptoms in the acute stage of a cold. Prophylactic administration of 500 mg of vitamin C daily can significantly reduce the intensity of upper respiratory infections in females but it is not effective in males. It was proposed by Wilson and Loh (1973a) that a prophylactic daily dose of 2000 mg of vitamin C would prove affective against the common cold in about 80 per cent. of the population. The dose-response curve relating oral dosage of vitamin C resistance to the common cold provides the evidence for this suggestion (Loh, Odumosu and Wilson, 1974). Anderson

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and his co-workers (1972) and Coulehan and his colleagues (1974) have provided confirmatory evidence for the proposition made by Wilson and his colleagues.

Tissue saturation with ascorbic acid must be maintained during upper respiratory inflammation so that ascorbic acid is present in adequate concentration to carry out its normal physiological functions, maintain tissue defensive mechanisms and integrity, and enable tissue replacement and healing to take place. Administration of vitamin C causes pulmonary bronchodilatation; desaturation of pulmonary tissues would therefore give rise to diminished pulmonary function. Urinary excretion of ascorbic acid during acute episodes of asthma is significantly reduced, and increases again when the wheezing disappears (MacNally, 1953). This provides further evidence that ascorbic acid plays an important role in maintaining pulmonary function. Upper respiratory symptoms of allergic origin resemble those of the common cold. Leucocyte uptake of ascorbic acid is reduced in atopic allergy (Loh, Watters and Wilson, 1973b). There is a significant inverse correlation between the size of skin reactions to specific allergens and the uptake of ascorbic acid by the leucocytes in the presence of the same allergen (Wilson, 1974). An alteration in ascorbic acid metabolism therefore occurs in the leucocytes in association with the antigen-antibody reaction. It is probable that deficiency of ascorbic acid further reduces immunological reactivity of the leucocytes and production of antibody during the viral attack (Cate *et al.*, 1964; Pereira *et al.*, 1972). Furthermore, an adequate concentration of ascorbic acid is essential for the leucocytes in order to ensure phagocytosis (Collingham and Mills, 1943; Nungester and Ames, 1948), which is a most important mechanism in the body's defence against infection (Stewart, 1968). Destruction of respiratory mucous membranes takes place in the common cold and in atopic allergy. Formation and growth of epithelial basement membrane is diminished when the tissue concentration of ascorbic acid is reduced (Priest, 1970), so that administration of supplementary vitamin C, by increasing the integrity and thickness of the mucous membranes, may prevent penetration and spread of the rhinovirus. Although it has been stated that ascorbic acid does not have viricidal effects on strains of viruses commonly known to cause colds (Walker *et al.*, 1967), it does have non-specific antibacterial, antifungal and antiviral effects (Ericsson and Lundbeck, 1955). More recently it has been demonstrated that ascorbic acid can prevent replication of RNA and DNA bacteriophage viruses (Murata *et al.*, 1972a, b).

Blood often appears in the nasal secretion during the later stages of a cold; aches and pains in the joints and muscles during the toxic stage of a cold or during the acute stage of an allergic reaction are common; patients may complain of dyspnoea or rashes during summer and winter colds; depression is a common occurrence during the terminal phase of, or early recovery from, colds. These scorbutic symptoms can be attributed to the acute, relative, ascorbic acid tissue desaturation which takes place in tissues

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exposed to viral attack or immunological reaction. Administration of adequate supplementary vitamin C is essential to maintain the normal degree of tissue saturation, and thus reduce severity of the cold symptoms. Such therapeutic doses do not produce abnormal elevation of blood ascorbic acid. In consequence they are not associated with production of undesirable side-effects.

(3) ASCORBIC ACID DESATURATION AND TISSUE NUTRITION

When discussing the action of ascorbic acid on the growth of guinea-pigs, Evans and Hughes (1963) suggested that primarily it exerts a metabolic effect on tissue formation. Ascorbic acid desaturation of the tissues is associated with nitrogen loss, interference with cholesterol and triglyceride metabolism, and inhibition of the action of insulin on tissue uptake of glucose. Experimental findings in guinea-pigs suggest that Fenfluramine ('Ponderax') exerts its anti-obesity action by promoting the catabolism and possibly tissue release of ascorbic acid, thus leading to tissue desaturation (Odumosu and Wilson, 1972, 1973a, b).

Significant loss of weight has been reported during the development of experimental scurvy in a male (Crandon, Lund and Dill, 1940). Smaller degrees of weight loss have been reported in the terminal phase (Bartley *et al.*, 1953) and also early during the repletion phase in a male by Hodges and his colleagues (1971). Preliminary investigations in human beings indicate that the action of Fenfluramine may be potentiated by a vitamin C deficient diet and that its administration is associated with loss of ascorbic acid (Wilson, 1974). In modern weight-reducing diets, fat, carbohydrate and protein intake are controlled to variable degrees but little attempt is made to control intake of vitamin C (Spence, 1966; Silverstone, 1970). The action of Fenfluramine as an anti-obesity agent is unpredictable. In obese patients, when vitamin C intake is strictly controlled in addition to control of fat, carbohydrate and protein intake, more effective weight control may be achieved. If Fenfluramine is used to accelerate weight loss, catabolic loss of weight may be delayed until tissue ascorbic acid concentrations begin to fall.

CONCLUSION

Blood levels and tissue concentrations of ascorbic acid are affected by normal physiological and biochemical factors throughout the whole life cycle. When dietary intake of the vitamin is reduced for any reason, the normal demand for ascorbic acid by specific tissues may not be satisfied during troughs in the ascorbic acid cycle. When acute tissue desaturation occurs in response to pathophysiological demands for ascorbic acid, leucocyte stores become desaturated. Signs of subclinical scurvy become obvious and can be identified by increased demand for ascorbic acid in specific tissues. In such circumstances appropriate doses of supplementary vitamin C resaturate the tissues, maintain normal leucocyte and plasma levels of ascorbic acid, and the subclinical scorbutic signs regress. Further administra-

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tion of supplementary vitamin C is unjustified when the leucocytes are saturated: plasma levels are unnecessarily elevated and it has been stated that adverse effects may appear (Briggs, 1973; Wilson and Loh, 1973b).

The negative concept that individuals require only sufficient vitamin C to prevent clinical signs of scurvy is unjustified because it makes no allowance for the lability of ascorbic acid in the body, spontaneous physiological fluctuations in its availability, or pathophysiological tissue demands which can produce acute desaturation and subclinical scurvy.

The positive concept of maintained tissue saturation with ascorbic acid increases tissue efficiency and integrity, and enables body stores to meet sudden pathophysiological demands for ascorbic acid by the tissues. Tissue response to stress, infection and disease is thus improved.

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The transfer and utilization of vitamin C in human tissues. By C. W. M. Wilson and M. Greene, *Department of Pharmacology, University of Dublin, Trinity College, Dublin 2, Ireland*

Ascorbic acid (vitamin C) is stored in the leucocytes and undergoes a circadian rhythm (Wilson, 1973). It is a physiologically active compound which is transferred rapidly backwards and forwards between the leucocytes and plasma, and between the plasma and tissues. Its utilization is affected by ageing (Wilson, 1974) and pathological conditions. The leucocyte-plasma transfer can be investigated after a loading dose of 2000 mg ascorbic acid by measuring plasma and leucocyte levels during a 4 h period, so as to assess physiological and pathological states. Normal female leucocytes absorb more ascorbic acid than those of males. Absorption continues until 4 h after the loading dose in females. It ceases in males after 2 h. During colds 2000 mg ascorbic acid causes a significant rise in the level in female leucocytes. It is greater than the post-cold rise. This dose does not raise male leucocyte levels. Plasma levels are raised more in the post-cold test in both sexes. Males and females do not differ in maximum leucocyte ascorbic acid concentrations after receiving adequate supplementation (Loh & Wilson, 1971). Females have a lower intake of ascorbic acid than males (Morgan, Gillum & Williams, 1955). Increased intake raises leucocyte concentrations; but the female sex differential is maintained (Woodhill, 1970). The sex difference in response to dietary intake, or loading doses, of ascorbic acid is a metabolic characteristic. Normal guinea-pigs and human beings cannot synthesize ascorbic acid because they do not possess gulonolactone oxidase, which performs the synthesis of ascorbic acid from gulonolactone (Roy & Guha, 1958). Female guinea-pigs can survive for prolonged periods on scorbutogenic diets, and gulonolactone administration prolongs life during vitamin C deficiency (Odumosu & Wilson, 1973). D-Glucuronolactone is converted into ascorbic acid in man (Baker, Sauberlich, Wolfskill, Wallace & Dean, 1962). Thus humans may also synthesize the vitamin from suitable precursors (Atkins, Dean, Griffin & Watts, 1964). Women develop scurvy less than men during vitamin C deficiency (Wilson, 1974). Both species may therefore have the capacity to react to stress, or deficiency, by more economic utilization, and by synthesis, of ascorbic acid.

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