and a known amount of ascorbic acid solution added to each. At the end of the boiling period, an equal volume of 6-per cent metaphosphoric acid was added to each solution. The solutions were cooled and their ascorbic acid content determined by the titration method.

The inhibition produced by a given amount of extract was found not to be affected by the length of the boiling period; the percentage inhibition produced in triplicate reaction mixtures, boiled for 5-, 10-, and 15-minute periods, was constant. It was influenced, however, by the amount of ascorbic acid present. In buffered (pH 5.7) reaction mixtures containing 1.0 mg. of ascorbic acid, 2 ml. of extracts of lettuce, cabbage, and cauliflower produced 50.0, 79.2, and 91.7 per cent inhibition, respectively, while in similar reaction mixtures containing 8.0 mg. of ascorbic acid these extracts produced 16.0, 53.8, and 71.7 per cent inhibition, respectively.

In reaction mixtures containing a fixed amount of ascorbic acid, increasing amounts of an extract produced an increasing inhibition until a maximum was reached. The maximal inhibition produced by extracts of different vegetables varied: in unbuffered reaction mixtures containing 1.0 mg. of ascorbic acid the maximal inhibition produced by extracts of 10 vegetables ranged from 86 to 47 per cent. The vegetables, when listed according to their decreasing capacity to inhibit the oxidation of ascorbic acid, fall in the following order: Brussels sprouts, green beans, squash, Irish potatoes, broccoli, cauliflower, cabbage, spinach, sweet potatoes, and lettuce. Extracts of all but the last three vegetables produced an inhibition of 70 per cent or more. Two to 4 ml. (0.5–1.0 gram of fresh material) were required to produce maximal inhibition.

The inhibition produced by the extracts was closely associated with copper. In reaction mixtures containing fixed amounts of ascorbic acid and vegetable extract, the percentage inhibition was found to decrease as the amount of copper, added as $CuCl_2H_2O_3$, was increased. Conversely, in solutions containing fixed amounts of ascorbic acid and of added copper, increasing amounts of extract produced an inhibition which increased until a maximum was reached; the maximal inhibition produced varied with the level of added copper.

Results of experiments carried out with extracts at room temperature have confirmed those obtained during boiling. For example, unbuffered reaction mixtures of equal volume (50 ml.) containing 5 mg. of ascorbic acid and 2.0 ml. of extracts of cauliflower and cabbage retained 52.4 and 44.7 per cent, respectively, of their ascorbic acid content after standing at room temperature for 9 hours, whereas only 9.6 per cent of the vitamin remained in a control solution containing no vegetable extract. In similar solutions containing 20 μg. of added copper 16.6, 5.4, and 0 per cent, respectively, of the ascorbic acid was retained after 9 hours.

Of a number of substances examined, those containing either or both the –SS- and –SH groups have been found to exert an inhibitory effect during boiling similar to that exhibited by aqueous extracts of vegetables; these were cystine, cysteine, and an aqueous extract of papain (either fresh or previously boiled). It appears possible that the inhibition of oxidation of ascorbic acid by vegetable extracts may be attributed, in part, to the presence of these groups.

The Use of 2,4-Dinitrophenylhydrazine for the Determination of Ascorbic Acid [1]

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The assay of ascorbic acid by the method of Roe and Kuether (*6*) is based on the reaction of dehydroascorbic acid with 2,4-dinitrophenylhydrazine to form an osazone which, on treatment with sulfuric acid, results in a colored dehydration product. This procedure, when used for blood as recommended by Roe and Kuether, gives excellent results. When applied to certain freshly prepared synthetic or biologically derived ascorbic acid solutions, it gave results which generally conformed to the values obtained by using the oxidation-reduction indicator 2,6-dichlorobenzenone indophenol and those obtained by biological assay (*5*). Thus, so far, it appears reliable for blood and for certain freshly prepared biological materials.

This communication concerns itself with errors inherent in the procedure if applied to biological material or ascorbic acid solutions where unpredictable antecedent oxidation of the vitamin has occurred. We were surprised when a 10-day stock preparation of orange juice, which had been sufficiently aerated to oxidize most of the ascorbic acid (0.2 mg. per cent remaining unoxidized) but which contained 60–70 mg. per cent of "vitamin C" (as dehydroascorbic acid) as determined by the phenylhydrazine method (*6*), failed to prevent the occurrence of scurvy in guinea pigs on a vitamin C-free diet. It was the purpose of the original experiment to demonstrate that the vitamin C potency of a food was not dependent entirely on its ascorbic acid content. The animals received a daily intake of 1.5 mg. of presumed dehydroascorbic acid and yet developed the gross and microscopic lesions

[1] The statements and opinions set forth in this article are those of the authors and not necessarily those of the Navy Department.

of scurvy in four weeks, whereas the control group on a similar amount of the vitamin in fresh juice did not become diseased. The orange juice was then treated with H_2S (*2*) and found to contain only 4 mg. per cent of dehydroascorbic acid. It seemed unlikely, therefore, that the high value obtained by the phenylhydrazine method was due to vitamin C (to include ascorbic acid and dehydroascorbic acid). It was more likely that mutarotation had occurred with loss of lactone structure (*2*) and formation of diketogulonic acid. Similar results, obtained with synthetic ascorbic acid, are presented in Table 1.

TABLE 1

ASSAY VALUE OF SYNTHETIC ASCORBIC ACID
(Unbuffered in aqueous systems)
Water Bath Temperature 38° C.
Mg./100 ml.

Gas	Ascorbic acid (reductone)		Dehydroascorbic acid (by H_2S)		Total vitamin C content of fluid		Value obtained by dinitrophenylhydrazine		Final H-ion concentration pH	
Gas used	N_2	O_2	N_2	O_2	N_2	O_2	N_2	O_2	N_2	O_2
(Time)										
Initial	22.6	19.4	1.8	4.06	24.4	23.46	27.0	27.0	5.02	4.97
2 hours	12.8	0	4.4	7.0	17.2	7.0	26.0	23.0	5.05	5.05
4 hours	7.2	0	5.6	5.2	12.8	5.2	27.0	21.5	5.32	5.30
6 hours	5.2	0	6.8	7.0	12.0	7.0	26.0	20.0	5.50	5.50

H-ion concentration of glass distilled water = pH 6.22.
No extraordinary precautions were taken other than the meticulous cleansing of glassware throughout. However, catalytic oxidation and not autoxidation occurred, because at this hydrogen-ion concentration there should have been little or no change in ascorbic acid values when aerated with nitrogen (*1*).

From these data it is apparent that the phenylhydrazine reaction is not necessarily specific for dehydroascorbic acid. This is borne out by previous studies which show that 2,4-dinitrophenylhydrazine reacts with diketogulonic acid (*3*), possibly phenylpyruvic acid (*4*), and other alpha-keto acids (*7*).

Thus, the 2,4-dinitrophenylhydrazine method may give unreliable results for the antiscorbutic value of certain biological preparations if loss in lactone structure of dehydroascorbic acid has taken place. Its use for the laboratory evaluation of blood and possibly urine is warranted (*5*); other biological tissues must be assayed with caution in regard to oxidation products of ascorbic acid or other substances entering into the phenylhydrazine reaction.

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News and Notes

Dr. Margery C. Carlson, assistant professor of botany at Northwestern University, has been granted leave for three months to undertake an expedition collecting plants in the region of Ahuachapán, El Salvador, close to the Guatemalan border. The expedition is a joint research project sponsored by the Chicago Natural History Museum and Northwestern University. Mr. Paul C. Standley of the Museum suggested this area for study because of its accessibility and because its floristics are practically unknown. The plants collected will fill an important gap in our knowledge of the flora of Central America. Special attention will be paid to the flowering plants of the region. In addition, effort will be made to get representative samples of algae, fungi, and certain forms of beetles. Dr. Carlson was accompanied by Miss Kate Staley, who will assist her in the field work. Guides and native helpers will be secured locally. This expedition is of considerable interest, not only because the territory is practically unexplored botanically, but also because Dr. Carlson is perhaps the first woman to lead a natural scientific expedition to El Salvador.