treated controls. However, the variation was such that in the other two experiments the dogs receiving methionine showed a somewhat earlier and greater degree of liver damage.

This failure of supplementary methionine in a normal protein diet to protect against CCl_4 liver damage may be likened to the failure of supplementary thiamine to produce any added effect in the presence of an adequate vitamin intake. From the present experimental studies one would expect a beneficial effect of methionine or choline on an abnormal hepatic function only in the face of a previous history of protein deficiency. To date three studies have failed to find any beneficial effect of methionine or choline in homologous serum jaundice (*11*) or in infectious hepatitis (*3, 12*). On the other hand, choline, in combination with high protein and high vitamin diets, has been reported to be of value in the treatment of hepatic cirrhosis (*8, 10*), but not all are in agreement with this point (*10*). Experimentally, a protective effect of supplements of methionine added to a normal diet might later be demonstrated with another type of liver damage. The above experiments are fairly acute, and when larger amounts of methionine are available some effect may be demonstrated on a more chronic liver damage. Studies on hepatic repair are also needed as previous studies are all prophylactic in nature.

Summary. At the present time the experimental evidence demonstrates that supplements of methionine will decrease the degree of liver damage produced by toxic agents in protein-depleted animals. In animals receiving a normal protein intake of 20-per cent or 41-per cent casein, methionine supplements did not decrease the degree of hepatic damage produced by carbon tetrachloride as judged by serum phosphatase values or bromsulphalein retention.

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In the Laboratory

Inhibition of Oxidation of Ascorbic Acid by Certain Vegetable Extracts

Ruth Reder

Oklahoma Agricultural Experiment Station, Stillwater

Aqueous extracts of a number of vegetables exert an inhibitory effect on the oxidation of ascorbic acid. Evidence of this effect was first obtained when, to avoid errors in sampling, an attempt was made to use an extract of cabbage, rather than the vegetable itself, in a study of the effects of various compounds on the oxidation of ascorbic acid during boiling. When an aqueous extract of cabbage containing added ascorbic acid was boiled, it was observed that only a small percentage of the vitamin was oxidized. The inhibition of oxidation was found not to be caused by a hydrogen-ion concentration unfavorable to the oxidation, although it was influenced by this factor; nor was it an apparent effect resulting from the formation or release of ascorbic acid or other reducing substances during boiling. Similar aqueous extracts of other vegetables were found to differ widely in their capacity to inhibit oxidation.

Both fresh and previously boiled extracts produced inhibition; the latter were used in the present experiments. The extracts were prepared by grinding a weighed amount of the fresh vegetable with water in a Waring blendor; the filtrate from the blended mixture was boiled for 30 minutes, chilled, filtered, and made to volume. One milliliter of extract was equivalent to 0.25 gram of fresh material.

Inhibition of oxidation was determined by the difference between the amounts of ascorbic acid oxidized, during the same boiling period, in equal volumes (40 ml.) of two solutions (buffered or unbuffered) containing the same amount of ascorbic acid, but differing in that one solution contained a known amount of vegetable extract. The reaction mixtures, complete except for the ascorbic acid, were brought to boiling in uncovered 150-ml. beakers containing glass beads,

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]

M. Pijoan, Lt. Cdr. (MC), USNR, and H. J. Gerjovich, PhM2/c V6 SV, USNR
Naval Medical Research Institute, Bethesda, Maryland

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.