antibodies and an experimental glomerulonephritis by injections of autolyzed homologous kidney was essentially negative in its results.

9003 C

Stability of Vitamin C, and Absence of Ascorbic Acid Oxidase in Citrous Fruits and Milk.

HENRY TAUBER.
Long Island City, N. Y.

It has been shown recently that in certain plants such as Hubbard Squash and Summer Squash, a powerful enzyme is present which oxidizes rapidly vitamin C[1, 2] although these plants contain almost none of this vitamin. Similar is the case with cucumbers. Statements have appeared more recently that "plant tissues which contain ascorbic acid apparently also contain an ascorbic acid oxidizing enzyme," and that the partial destruction of vitamin C in cow's milk is also brought about by ascorbic acid oxidase. The present writer could not find ascorbic acid oxidase in mammalian tissue,[3] and Roe and Barnum[4] found in human and rat blood cells and plasma an enzyme which reduces the reversibly oxidized form of ascorbic acid, thus having just the opposite effect from the ascorbic acid oxidase of plant tissues.

The aim of the present work was to find out whether the vitamin C content of juices of citrous fruits, which are excellent sources of the vitamin, is exposed to the destructive action of the ascorbic acid oxidase. In other words, whether these juices contain the oxidase.

Table I shows that the vitamin C content of the juices of oranges,

TABLE I.
Analysis of Vitamin C per cc. of Fruit Juice.

	After 5 hr. at 6° mg.	After 5 hr. at 38° mg.
Orange Juice (type *t*)	.51	.46
Lemon " (" *t*)	.56	.53
Tangerine " (" *a*)	.39	.33
Grapefruit " (" *a*)	.46	.44

1 Tauber, H., and Kleiner, I. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 577.

2 Tauber, H., Kleiner, I. S., and Mishkind, D., *J. Biol. Chem.*, 1935, **110**, 211.

3 Tauber, H., *Experimental Enzyme Chemistry*, Burgess Pub. Co., Minneapolis, 1936.

4 Roe, J. H., and Barnum, G. L., *J. Nutrition*, 1936, **11**, 359.

tangerines, lemons and grapefruits is not much affected when kept for 5 hours at 38° as compared to control samples which were placed in a refrigerator at 6° for the same amount of time. The slight decrease in reducing power does not necessarily indicate an irreversible oxidation. The results show, however, that there is no ascorbic oxidase in these fruit juices and that the vitamin keeps fairly well even at 38°. It is well known that the pH of these fruit juices is a stabilizing factor of vitamin C. Samples of orange juice which have been adjusted to pH 6.5 with ammonium hydroxide or $CaCO_3$, however, kept equally well and no evidence of enzyme action could be noticed at this pH either. 2,6-Dichlorobenzenone indophenol was used for the titration of vitamin C. It should be noted that oranges and tangerines contain a small amount of a substance other than vitamin C, which reduces the oxidation-reduction indicator.[5]

Since it was recently suggested that milk contains ascorbic acid oxidase[6] thus causing partial destruction of vitamin C, this point was also tested. To a 100 cc. sample of fresh raw milk, 25 mg. of ascorbic acid, which was dissolved in 10 cc. of distilled water, was added* and kept for 3 hours at 38°. To a second sample of 100 cc. of milk the same amount of ascorbic acid was added just before titration (zero time). Only about 3% of the added vitamin C was oxidized within 3 hours, indicating that at most there is only a trace of ascorbic acid oxidase in cow's milk. The pH of the milk was 7.0. The vitamin C was determined by titration, after coagulation of the milk proteins by adding 2 cc. 2/3 N H_2SO_4 to 20 cc. of milk, without filtration, so as to eliminate the error owing to slow filtration, as proposed by Sharp.[6] The partial destruction of vitamin C in cow's milk is probably due to traces of copper in the milk introduced by feeding or by technical means, but not to enzyme action.

[5] Tauber, H., and Kleiner, I. S., *J. Biol. Chem.*, 1935, **110**, 559.

[6] Sharp, P. F., *Science*, 1936, **84**, 461.

* Ascorbic acid was added because milk is a very poor source of vitamin C. The samples analyzed contained 17.5 mg. of vitamin C per 1000 cc. of milk.