# 14415

## Ascorbic Acid in Sweat.

E. R. KIRCH, T. CORNBLEET, AND O. BERGEIM.

*From the Department of Chemistry, College of Pharmacy, Department of Physiological Chemistry, and Department of Dermatology, College of Medicine of the University of Illinois, Chicago, Illinois.*

Some of the values reported in the literature on concentrations of ascorbic acid in sweat indicate that important losses might occur in heavy sweating while other results suggest that losses are negligible. Cornbleet, Klein, and Pace[1] found from 0.55 to 0.64 mg % and Bernstein[2] from 0.5 to 1.1 mg % of ascorbic acid in human sweat. Wright and MacLenathen[3] found 0.024 to 0.186 mg %, while Hardt and Still[4] report 0.18 mg % with higher values up to 0.47 mg % when subjects were given 750 mg of ascorbic acid. Zselyonka and Nánássy-Mégay[5] reported 0.1 to 0.2 mg %. Tennent and Silber[6] found no ascorbic acid but an average hourly loss of 0.23 mg % of dehydroascorbic acid. Mickelsen and Keys[7] report less than 0.1 mg % with an average of about 0.03 %.

In the past, much of the sweat was collected in rubber bags or rubber gloves.[1,7] Mickelsen and Keys[7] obtained results indicating that the type of rubber glove used might make a difference in results. Since the findings of different investigators have been so variable it seemed desirable to run controlled experiments in which the samples were collected in such a manner as to be free from possible interfering substances and also to make comparisons with the sweat collected in rubber bags.

*Procedure and Methods.* Samples of sweat were collected as follows: In one series of tests the subjects were encased in a rubber bag as far as their necks and seated in a cabinet. Incandescent lamps supplied heat sufficient to induce secretion of 100-150 ml of sweat in 20-30 minutes. In another series of tests the unclothed subjects were seated in the same cabinet, no bag being used. As soon as sweating started the sweat was collected directly into a beaker containing 2-3 g of metaphosphoric acid and thus did not come into contact with anything that could interfere with the ascorbic acid titration. All subjects received 100 mg ascorbic acid daily for 2 weeks prior to tests. To test effects of high vitamin C intake the subjects were given by mouth 500 mg of the vitamin 30-45 minutes before collecting samples.

Ascorbic acid was determined on 10 ml samples according to the method of Bessey and King[8] using 2,6-dichlorophenol indophenol. The oxidase procedure of Tauber and Kleiner[9] for true ascorbic acid was used as a check on interfering substances.

Dehydroascorbic acid was determined on samples of sweat containing 3% $HPO_3$, which were treated with hydrogen sulphide for one hour, allowed to stand for 30 minutes, the $H_2S$ then swept out with $CO_2$ or nitrogen and the dye titration carried out. In certain cases dehydroascorbic acid was also determined by the method of Roe and Kuether.[10] The re-

[1] Cornbleet, T., Klein, R. I., and Pace, E. R., *Arch. Dermat. and Syphilol.*, 1936, **34**, 253.

[2] Bernstein, R. E., *Nature*, 1937, **40**, 684.

[3] Wright, I. S., and MacLenathen, E., *J. Lab. Clin. Med.*, 1939, **24**, 804.

[4] Hardt, L. L., and Still, E. I., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 704.

[5] Zselyonka, L., and Nánássy-Mégay, K., *Orvosi Hetilap*, 1937, **81**, 800; *Chem. Abstr.*, 1937, **31**, 7098.

[6] Tennent, D. M., and Silber, R. H., *J. Biol. Chem.*, 1943, **148**, 359.

[7] Mickelson, O., and Keys, A., *J. Biol. Chem.*, 1943, **149**, 479.

[8] Bessey, O. A., and King, C. S., *J. Biol. Chem.*, 1933, **103**, 687.

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

[10] Roe, J. H., and Kuether, *J. Biol. Chem.*, 1943, **147**, 399.

TABLE I.
Indophenol Titrations of Sweat Collected in Rubber Bag.

| Volume of sweat, ml | Ascorbic acid added, mg | Oxidase added, ml | Dye solution used |
|---|---|---|---|
| — | 0.5 | — | 2.20 |
| — | 1.0 | 10 | 0.01 |
| 10 | — | — | 0.95 |
| 10 | — | 10 | 0.95 |
| 10 | 0.5 | — | 3.15 |
| 10 | 0.5 | 10 | 0.94 |

sults checked with those obtained by the dye titration procedure.

*Results and Discussion.* Table I gives results from tests in which the rubber bag was used in sweat collection. The usual dye titration indicated a rather high ascorbic acid content. However, after treatment with ascorbic acid oxidase, which would destroy any ascorbic acid present, the dye titrations remained the same indicating that the reducing substance present was not ascorbic acid. That this reducing substance was not a constituent of sweat but was derived from the rubber bag was indicated by the fact that the sweat collected directly from the skin in a beaker containing $HPO_3$ gave no such titration values. As a further check on this, 500 ml of distilled water was placed in the same rubber bag (previously thoroughly washed with soap and rinsed with distilled water) and placed in the same heating cabinet for 30 minutes. Ten ml portions of this water were removed from the bag, 2% $HPO_3$ added and the dye titration carried out. 0.4 ml of dye were required, equivalent to 0.08 mg ascorbic acid, thus indicating further that the bag was the source of the interfering substances.

TABLE II.
Ascorbic and Dehydroascorbic Acid in Sweat. Subjects on regular diet plus 100 mg ascorbic acid daily. Values expressed in mg per 100 ml.

| Subject | Volume of sweat | Ascorbic acid | Dehydro-ascorbic acid |
|---|---|---|---|
| 1 | 150 | 0 | 0.05 |
| 2 | 75 | 0 | 0.07 |
| 3 | 110 | 0 | 0.00 |
| 4 | 100 | 0 | 0.08 |
| 5 | 125 | 0 | 0.15 |
| 6 | 110 | 0 | 0.00 |
| 7 | 105 | 0.015 | 0.09 |
| 8 | 95 | 0 | 0.06 |

Table II summarizes the results on the ascorbic acid content of sweat collected directly into metaphosphoric acid solution from subjects on their regular diet supplemented with 100 mg of ascorbic acid per day. It will be noted that while no ascorbic acid was found, small amounts of dehydroascorbic acid appeared to be present. Even if all of the material titrating as dehydroascorbic acid is actually that substance, the amounts present in the sweat were very small.

TABLE III.
Ascorbic and Dehydroascorbic Acids in Sweat. 500 mg ascorbic acid ingested 30-45 minutes before sweating. Values are mg per 100 ml.

| Subject | Volume of sweat | Ascorbic acid | Dehydro-ascorbic acid |
|---|---|---|---|
| 1 | 75 | 0 | 1.74 |
| 2 | 100 | 0 | 1.88 |
| 3 | 95 | 0 | 2.13 |
| 4 | 80 | 0 | 1.36 |
| 5 | 105 | 0.054 | 2.48 |
| 6 | 75 | 0 | 1.92 |

Table III gives some results with subjects ingesting 500 mg ascorbic acid 30-45 minutes before sweating. Some increase in the dehydroascorbic acid concentration was noted but the total elimination was still very low.

*Conclusions.* Of the samples of human thermal sweat investigated by us only two showed any ascorbic acid present and in these cases the amounts found could be considered practically negligible. Dehydroascorbic acid determinations indicated the presence of this acid in most samples. The amounts found were not sufficient to suggest the induction of vitamin C deficiency through excessive sweating. The use of rubber bags in the collection of sweat may lead to high titration values and appears responsible for some of the high values for ascorbic acid in sweat that have been reported.