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Some Water-soluble Vitamins in the Sweat of Tropically Acclimatized European Men

BY J. W. H. LUGG

Department of Biochemistry, University of Malaya, Singapore

AND F. P. ELLIS

Medical Research Council's Royal Naval Tropical Research Unit, Singapore

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The earlier literature on the concentrations of ascorbic and dehydroascorbic acids in human sweat and on the dermal losses has been reviewed by Kirch, Cornbleet & Bergeim (1943), Mickelsen & Keys (1943), Sargent, Robinson & Johnson (1944), Shields, Johnson, Hamilton & Mitchell (1945) and Tennent & Silber (1943), who all, in turn, contributed their own findings. General reviews of dermal losses of ascorbic and dehydroascorbic acids and other water-soluble vitamins are given in *Nutrition Reviews* (Anonymous, 1946) and by List (1948).

The more recent work has not confirmed the earlier view that the loss of ascorbic acid in profuse sweating may be considerable. There is, however, appreciable conflict between some of the conclusions drawn from two of the more recent studies by Kirch *et al.* (1943) and by Shields *et al.* (1945). The latter encountered a decrease in the ascorbic-acid and an increase in the dehydroascorbic-acid content of sweat after an increase in the ascorbic-acid intake, instead of an increase in both, as encountered by the former workers: further, for subjects receiving large dietary supplements of L-ascorbic acid and sweating under rather similar conditions, they reported a dehydro-ascorbic-acid level in the sweat only about one-eighteenth as high as that encountered by the former workers. In these studies the subjects were exposed periodically to artificial climatic conditions, which usually simulated hot dry or hot humid climates, but they lived in much more temperate, natural climates when not taking part in the experiments. The subjects studied by Shields *et al.* (1945), however, spent a considerable part of their daily lives in the artificial climates.

An opportunity arose between programmes of work in progress at the Royal Naval

Tropical Research Unit, Singapore, of examining the sweat of tropically acclimatized European subjects. Observed rates of sweating in hot environments may be very high, for example, 8.5 l. during a 5 h shift in a coal mine (Moss, cited by Ladell, 1945) and 10 l. a day in a desert climate (Dill, Jones, Edwards & Oberg, 1933). The brief investigation described below was designed primarily to determine whether, in tropically acclimatized subjects who were working daily in environments which caused them to sweat profusely, the dermal excretions of ascorbic and dehydroascorbic acids might be so large that dietary supplements became advisable. The renal excretion of these substances was also examined, and the opportunity was taken of examining the dermal excretion of thiamine.

EXPERIMENTAL

Subjects

The subjects were healthy European men between 20 and 40 years of age, who had been living in a warm, moist, tropical environment for periods ranging from 1.5 to 4.5 years.

Plan of experiment

In each experiment the men carried out work in a climate considerably warmer than the normal Singapore climate to which they had become accustomed. The work consisted in stepping on and off a stool 12 in. in height, twelve times per min. They wore drill 'shorts', rubber-soled canvas shoes, and full-length oiled-silk arm bags*, to each of which was attached, at the dependent opening, a 50 ml. conical flask containing 2 ml. of 'stock' metaphosphoric-acid solution (160 g defined 'metaphosphoric acid' per litre, adjusted to a pH such that on dilution to one-fifth stock concentration the solution is of pH 1.45-1.55) for the collection of the arm sweat. In an experiment (Exp. 0) that was not one of the main series, the left arms only were encased in the arm bags. In the main experiments (1-4) both arms were so encased and the contents of the two flasks were pooled for each individual. The length of the work period required to furnish an adequately large sample ranged from 20 to 40 min, varying with the climate and the individual. The subjects were encouraged to drink freely during and after the exercise.

The main experiments comprised two pairs. In the first of each pair (1 and 3) the subjects had been living on a normal diet, essentially of European type and believed to be adequate in all respects. For the second of each pair (2 and 4), performed, respectively, approximately 48 h after the first of each, the subjects consumed, in addition to their normal diets, four doses of 125 mg L-ascorbic acid, the final dose being taken approximately 2 h before the exercise and the other three doses at equally spaced intervals during the preceding 24 h. In the second pair of experiments the subjects emptied their bladders before beginning the exercise; as soon as they were again able to urinate (after approximately 90 min) they furnished for examination all, or most, of the urine secreted during that period.

* Oiled-silk irrigation envelopes, full arm size, Ash/1 (Standard Irrigation Envelopes Ltd., London).

Sweat samples only were collected and examined in the first pair of experiments, as these were exploratory in nature. Much material was expended in ascertaining that the ascorbic- and dehydroascorbic-acid contents were indeed low, and pooled samples only were available for the more delicate estimations of ascorbic acid. The quantitative findings of the first pair of experiments were presumed, provisionally, to hold for the second pair, performed on a different group of subjects, and the individual samples were all submitted to the more delicate examination. The titrations from which the ascorbic- and dehydroascorbic-acid contents of the sweat and urine samples were calculated were of the A'_2 , A_3 , B_2 , B_3 types described by Lugg (1942), but other types of titration were made also to ascertain the possibility of interference by extraneous reducing material. With the sweat samples the titrations were so small as to leave an appreciable uncertainty that the still smaller differences (A'_2-A_3 and B_2-B_3), when greater than zero, were due entirely to the presence of ascorbic acid.

Analytical methods

Ascorbic-acid estimation. It seemed unlikely that the pronounced disagreement between the concentrations in sweat of dehydroascorbic acid reported by Kirch *et al.* (1943) and by Shields *et al.* (1945) could be due entirely to such deficiencies as may exist in the methods adopted. The latter authors employed the method of Roe & Kuether (1943), and, though Kirch *et al.* (1943) employed the method of Bessey & King (1933) in much of their work, they also used the method of Roe & Kuether (1943), obtaining with it results concordant with those by the other method.

The method of Roe & Kuether (1943) has been submitted to critical examination by Penney & Zilva (1945) and to comparison with the method of Lugg (1942), which was earlier examined critically by Snow & Zilva (1943, 1944). Zilva and his colleagues focused their attention upon the use of these methods for the estimation of ascorbic acid rather than of dehydroascorbic acid, and they concluded that the choice of method for maximum specificity would depend upon the nature of such contaminants as might be present.

The method of Lugg (1942) was employed by us for the estimations of ascorbic and dehydroascorbic acids, the 'drop-persistence' titration technique being followed, for, although this technique is not readily adaptable to the estimation of minute quantities of the substances concerned, there are reasons for believing it to be more specific than the alternative more delicate colorimetric technique. When the total reducing power was very low (the A_1 , A_2 and A_3 titrations (Lugg, 1942) of sweat samples), the adaptation consisted in titrating with dye solution of 0.25 standard concentration (namely only 0.125 mg dichlorophenolindophenol/ml.) and with the timing of 'persistence' suitably modified (e.g. with 0.0025 mg ascorbic acid initially present the period for 50% persistence of the coloration due to a 0.02 ml. drop of the dye solution would be 12 sec at 25°, calculated from the data of Lugg (1942)).

Thiamine estimations. The thiamine contents of sweat samples were estimated by the method of Friedemann & Kmiecik (1943). For purposes of checking the urinary outputs, creatinine contents of the urine samples were determined by the method of Folin (1914).

RESULTS

Table 1 records the quantities of arm sweat and urine collected from each subject in each experiment and, when estimated, the concentrations of ascorbic acid, dehydroascorbic acid calculated as ascorbic acid, the sum of these, thiamine and creatinine. Table 1 also records (as Exp. 0) some earlier observations on the thiamine contents of arm-sweat samples collected from a group of four subjects, two of whom subsequently participated in Exps. 1 and 2.

DISCUSSION

Neglecting, in some measure, the less precisely estimated ascorbic-acid and ascorbic-acid + dehydroascorbic-acid concentrations in the sweat samples obtained in Exp. 1, it is apparent that no significant changes followed the administration of the L-ascorbic-acid supplements. Overall, the concentrations of ascorbic acid + dehydroascorbic acid were, in no instances, greater than 0.05 mg/100 ml. and were usually much lower or undetectably small; the ascorbic-acid concentrations were undetectably small in all the more critically examined samples. Clearly the loss of even appreciably more than 10 l. per day of body sweat of similar general composition could not be expected to involve serious loss of ascorbic acid from subjects on normal intakes, but if dermal losses are maintained when intakes are low, certain subjects might become depleted.

The ascorbic-acid values (mean for Exps. 2-4, 0.00 mg/100 ml.) are in close conformity with those reported by Kirch *et al.* (1943) for (presumably) body sweat, obtained from unclad subjects under conditions of induced thermal sweating and receiving a supplement of 100 mg L-ascorbic acid per day or receiving this supplement and an additional 500 mg shortly before the experiment. They agree less closely with the values (mean 0.033 mg/100 ml.) reported by Shields *et al.* (1945) for the body sweat of subjects receiving no L-ascorbic-acid supplement and, incidentally, with those of Mickelsen & Keys (1943) for the arm sweat of subjects on normal diets, but closely with the values of Shields *et al.* (1945) for subjects receiving a supplement of 500 mg L-ascorbic acid per day for a week before the experiment.

The dehydroascorbic-acid values are in close conformity with those of Shields *et al.* (1945) for subjects receiving no supplementary L-ascorbic acid and are, on the average, about a third as great as those reported by Kirch *et al.* (1943) for subjects receiving a supplement of 100 mg L-ascorbic acid per day. They are much lower than the values reported by Shields *et al.* (1945) for subjects receiving a supplement of 500 mg L-ascorbic acid per day (mean 0.107 mg/100 ml.) and very much lower than those of Kirch *et al.* (1943) for subjects receiving an additional 500 mg L-ascorbic acid shortly before the experiment (mean 1.92 mg/100 ml.).

The possibility cannot be ignored that dehydroascorbic acid appearing in shed sweat may be secreted as ascorbic acid, this being oxidized in the sweat ducts or on the skin surface (see Sargent *et al.* 1944), but Shields *et al.* (1945) could not confirm the high rate of oxidation on the skin observed by Sargent *et al.* (1944). The pH of shed arm sweat is probably low enough usually (range for samples A, B, G and H in Exp. 0, 4.4-4.6) to preclude rapid decomposition of dehydroascorbic acid to diketogulonic acid, even in the absence of any added acid (destruction per minute at

Table 1. Ascorbic acid, dehydroascorbic acid and thiamine in arm sweat and creatinine, ascorbic acid and dehydroascorbic acid in urine of tropically acclimatized European men

Exp. no.	Climate*	Arm sweat				Urine				
		Supplementary ascorbic acid (mg)	Ascorbic acid† (mg/100 ml.)	Dehydro-ascorbic acid†‡ (mg/100 ml.)	Ascorbic acid and dehydro-ascorbic acid† (mg/100 ml.)	Volume (ml.)	Creatinine (mg/100 ml.)	Ascorbic acid (mg/100 ml.)	Dehydro-ascorbic acid§ (mg/100 ml.)	Ascorbic acid and dehydro-ascorbic acid (mg/100 ml.)
1	HmD	0	0.03	—	0.05	—	—	—	—	—
1	HmD	0	0.03	—	0.05	—	—	—	—	—
1	HmD	0	0.03	—	0.05	—	—	—	—	—
2	HmD	500	0.00	0.00	0.00	—	—	—	—	—
2	HmD	500	0.00	0.00	0.00	—	—	—	—	—
2	HmD	500	0.00	0.05	0.05	—	—	—	—	—
3	vHD	0	0.00	0.01	0.01	82	2.52	0.17	0.08	0.25
3	vHD	0	0.00	0.03	0.03	40	3.42	0.10	0.32†	0.42†
3	vHD	0	0.00	0.03	0.03	52	3.80	0.14	0.28†	0.42†
4	HH	500	0.00	0.04	0.04	100	1.60	0.49	0.00	0.49
4	HH	500	0.00	0.00	0.00	61	2.34	0.42	0.24	0.66
4	HH	500	0.00	0.02	0.02	119	1.70	0.70	0.28	0.98
0	vHD	0	—	—	—	—	—	—	—	—
0	vHD	0	—	—	—	—	—	—	—	—
0	vHD	0	—	—	—	—	—	—	—	—
0	vHD	0	—	—	—	—	—	—	—	—

* HmD=hot, moderately dry; air temperature 43-46°; mean radiant temperature of surroundings 52-54°; relative humidity 41-44%; air movement c. 150 ft./min. HH=hot, humid; air temperature 37°; mean radiant temperature of surroundings c. 37°; relative humidity 93%; air movement c. 150 ft./min. vHD=very hot, dry; air temperature 49°; mean radiant temperature of surroundings c. 49°; relative humidity 29%; air movement c. 150 ft./min.

† More especially in Exp. 1, the positive values should be regarded as upper limits rather than as precise estimates.

‡ The presence, after H₂S-treatment of the samples, of relatively large amounts of substances behaving like reductone, reductive acid, quinol or ferrous salts (Lugg, 1942; Snow & Zilva, 1943) reduces the reliability of these values.

§ By difference between values in columns on either side, dehydroascorbic acid being calculated as the corresponding quantity of ascorbic acid.

|| Mean values, for pooled samples from the three subjects.

approximately 30° calculated from data of Ball (1937) and of Lugg (1951) is 0.08% at pH 1.5, 0.1% at pH 5.2, 0.6% at pH 6.3, 10% at pH 7.24.

Sweat from the full-length arm may not be fully representative of that of the body as a whole in composition (see, for example, Mickelsen & Keys, 1943). In so far as it may be representative, however, the above comparisons invite comment. For reasons already given from the work of Kirch *et al.* (1943) it seems unlikely that the major parts of the disagreements can be attributed to deficiencies of the methods of estimation employed. This view is supported by the fact that with the methods of Bessey & King (1933) and Lugg (1942) we observed no significant increases in gross reducing capacities (B_1 titrations (Lugg, 1942)) of the H₂S-treated sweat samples after the administration of the L-ascorbic-acid supplement to our subjects. As they stand, and ignoring the differences of circumstances in which the 500 mg L-ascorbic-acid supplements were given, the various findings could be regarded as consistent with the hypothesis that the tendency to excrete dehydroascorbic acid (or, as a precursor, ascorbic acid) in sweat diminishes with increasing degrees of acclimatization to hot environments: our subjects were thoroughly acclimatized thereto, those of Shields *et al.* (1945) must surely have been so to an appreciable degree, to judge from the description of the programme of their investigation, and, by the same criterion, those of Kirch *et al.* (1943) must have been much less so. But high dehydroascorbic-acid levels, as reported by Kirch *et al.* (1943), were found after administration of the 500 mg L-ascorbic acid supplement just before sweat collection to subjects already receiving a daily supplement of 100 mg, whereas our 500 mg supplement was administered during the 26 h preceding sweat collection to subjects receiving no other supplement. It is possible that much longer or heavier dosing, or both, of our subjects might have occasioned appreciably higher levels of dehydroascorbic acid in their sweat samples.

The urinary concentrations of ascorbic acid were markedly elevated by the administration of the L-ascorbic-acid supplement. The group mean urinary output of ascorbic acid, calculated on the basis of an individual mean creatinine output in the two experiments (3 and 4) for each subject, was raised by administration of the L-ascorbic-acid supplement to almost seven times its former value, whereas the dehydroascorbic-acid 'apparent' output was not significantly raised (35%). But the destruction of urinary dehydroascorbic acid by spontaneous conversion to diketogulonic acid may be appreciable during the collection of urine in the bladder, especially if the pH is rather high. The urinary pH values for subjects D, E and F, respectively, in Exps. 4 were 6.7, 6.2 and 6.8. The destruction clearly must have been very considerable in these samples and possibly also in those of Exp. 3. Thus the rate of renal loss of ascorbic acid in both experiments could have been appreciably greater than the low rate suggested merely by the assays of the voided urine samples.

Attention may be drawn to the fairly uniform nature of our values for the thiamine contents of the arm-sweat samples for all subjects, whether they had or had not received a supplement of L-ascorbic acid and irrespective of the type of climate (range 0.095–0.140 mg/100 ml.; mean 0.123 mg/100 ml.) These values are of similar order to the mean derived from the far less uniform series of results of Mickelsen & Keys (1943). As with ascorbic acid, it would seem that losses of thiamine even in prolonged profuse sweating are normally extremely small.

SUMMARY

1. Samples of arm sweat obtained from tropically acclimatized European men exercising in oppressively warm environments were found to contain, as means of the more precisely estimated values, 0.00 mg ascorbic acid, 0.02 mg dehydroascorbic acid and 0.123 mg thiamine/100 ml.

2. No significant changes were observed in the values as a consequence of administering a dietary supplement of 500 mg L-ascorbic acid during the 26 h before sweat collection.

3. The mean urinary ascorbic-acid output during and somewhat beyond the period of exercise was increased markedly by the ingestion of an L-ascorbic-acid supplement, but the mean urinary dehydroascorbic-acid 'apparent' output (uncertain because of rather rapid spontaneous decomposition of the substance to diketogulonic acid) was not significantly affected.

4. On the evidence available it would not appear to be necessary to supplement the ascorbic-acid or thiamine intakes of European men working in warm environments in the tropics to offset such losses as occur in the sweat, provided they are already receiving normal amounts of these vitamins in their daily diet.

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