

Whole body sweat collection in humans: an improved method with preliminary data on electrolyte content

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Shirreffs, S. M., and R. J. Maughan. Whole body sweat collection in humans: an improved method with preliminary data on electrolyte content. *J. Appl. Physiol.* 82(1): 336–341, 1997.—Previous methods used to collect human sweat for electrolyte analysis have been criticized because they involve only regional sampling or because of methodological problems associated with whole body-washdown techniques. An improved method for collection of whole body sweat from exercising subjects is described. It involved construction of a plastic frame that supports a large plastic bag within which the subject exercises. The subject and the equipment are washed with distilled, deionized water before exercise begins. After exercise is completed, the subject and equipment are again washed with water containing a marker not present in sweat (ammonium sulfate). Total sweat loss is calculated from the change in body mass, and the volume of sweat not evaporated is calculated from dilution of the added marker. Recovery of added water was $102 \pm 2\%$ (SD) of the added volume, and recovery of added electrolytes was $99 \pm 2\%$ for sodium, $98 \pm 9\%$ for potassium, and $101 \pm 4\%$ for chloride. Repeated trials ($n = 4$) on five subjects to establish the reproducibility of the method gave a coefficient of variation of $17 \pm 5\%$ for sodium, $23 \pm 6\%$ for potassium, and $15 \pm 6\%$ for chloride. These values include the biological variability between trials as well as the error within the method. The biological variability thus appears to be far greater than the methodological error. Normal values for the composition of sweat induced by exercise in a hot, humid environment in healthy young men and women were (in mM) 50.8 ± 16.5 sodium, 4.8 ± 1.6 potassium, 1.3 ± 0.9 calcium, 0.5 ± 0.5 magnesium, and 46.6 ± 13.1 chloride.

sweating; sweat composition; exercise

THE COMPOSITIONAL ANALYSIS of human sweat is of interest in many clinical and exercise situations. A wide range of values for all of the major solute components has been reported (15), reflecting variations between individuals, differences due to the experimental conditions, and differences due to the method of collection. This last factor may be due to errors caused by contamination or to incomplete collection of the sample, or it may reflect a real difference induced by the collection procedure. The composition of sweat has been investigated by using a variety of different methods for collection of the sweat samples. The crudest method is simply to collect sweat as it drips from the skin surface. The problems with this approach are obvious: there will have been a variable amount of evaporation from the skin surface, leading to an unknown degree of concen-

tration of the sample; there is the possibility of contamination with skin cells; and there is also a lack of control over the region from which the sweat is derived. A review of the early studies in this area and of some of the methodological deficiencies was provided by Dill et al. (9).

The two main methods that have been used in attempts to overcome these difficulties involve collection of sweat from a specific region of the body, using some form of enclosing bag or capsule, or a variation on the whole body-washdown technique. The first of these methods can clearly eliminate the problem of evaporation of water from the skin surface, but the method itself may change the sweat composition by preventing water evaporation. This will lead to high levels of skin hydration, which may block sweat gland ducts and lead to a progressive fall in sweating rates (5). Use of a ventilated capsule will allow water evaporation to occur, but this method may alter the skin wetness and alter the local sweat rate. Due to the secretion and reabsorption nature of sweat production within the sweat gland and duct, sweat composition is influenced by sweat rate, at least within single ducts, such that a reduction in rate allows for greater reabsorption of electrolytes from the duct, resulting in a lower concentration in the final sweat produced. There also appear to be regional variations in sweat composition, as evidenced by the different values obtained when the composition of sweat obtained from different parts of the body is compared (7, 17), and the values obtained with this method also differ from those obtained by the whole body-washdown technique (12, 19). Higher electrolyte concentrations are generally observed when local sweat collection procedures are used, probably due to an alteration in composition caused by the restriction on evaporation of the sweat. This method is not, therefore, appropriate when whole body sweating is occurring and where total electrolyte or solute loss must be known. The difficulties caused by the restriction of evaporation can be overcome by using a ventilated capsule or chamber: the water in the effluent air is trapped in a cold trap, and the electrolytes are recovered at the end of the study period by washing out the enclosing apparatus (3). This does not, however, overcome the difficulties caused by regional differences in the composition of sweat.

A number of variations on the whole body sweat-collection method have been developed. In most of

these, total sweat loss is calculated, after appropriate correction, from the change in body mass. In early studies, where subjects exercised in the low humidity of the desert environment, it was assumed that there was no loss of electrolytes from the skin surface resulting from dripping of sweat: subjects were washed before exercise, the body and the clothes worn were washed after exercise with distilled water, and the electrolyte content of that water was measured (8). Other modifications to the whole body-washdown techniques that have been proposed for use in exercising subjects have involved thorough washing and drying of the subject, who then wears an absorbent suit to absorb sweat secreted onto the skin. The problems with this method are that there is the possibility of some loss by dripping of sweat from uncovered skin on the face and hands, and there appear to be difficulties in ensuring complete recovery of sweat electrolytes from the clothing worn. There is also no reliable way to estimate the fraction of the sweat that has evaporated. Also, sweat that drips from the skin is lost, and this has tended to restrict the use of this method to situations where sweat rates are low. In other situations, forced convection has been used to maximize sweat evaporation (12), but again this procedure will alter the thermoregulatory responses to the exercise conditions. In other investigations, subjects have worn minimal clothing and were dried at regular intervals with towels that were then added to the washdown water (1, 11).

Vellar (20) avoided some of these problems by enclosing subjects within a close-fitting plastic bag and exposing them to a hot humid environment to stimulate sweating. This method is not applicable to exercise, and there is again the probability that the high local humidity of the environment close to the skin will influence the sweating characteristics. A similar procedure was used by Malhotra et al. (14). Their report clearly stated that the head was not enclosed, so any sweat from the face and head was not included in the collection, even though this was referred to as "whole body sweat."

With the use of these methods for sweat collection, a wide range of values for sweat electrolyte content has been obtained in normal subjects, and there is a real possibility that some of this apparent variability between individuals is a consequence of deficiencies in the collection method.

The method reported here for collection of sweat from exercising subjects was devised in response to a need for determination of whole body electrolyte losses. We have recently reported that the restoration of water and electrolyte balance after exercise depends on the replacement of electrolyte losses (16), but progress in this direction has been hampered by the lack of a reliable method for determination of sweat electrolyte loss. The present report contains some details of the method used for sweat collection and some information on the reliability of the method.

MATERIALS AND METHODS

Subjects. All subjects participating in this investigation were healthy young men and women, all of whom were more or less physically active on a recreational basis, but none was training systematically at the time of the study. All gave their written informed consent, and the investigation was approved by the local Ethics Committee.

Sweat collection. Sweat collections were made during intermittent cycle ergometer exercise in the heat, as this exercise model is the one most widely used in studies of sweat composition. A standard Monark friction-braked ergometer (model 684, Monark, Stockholm, Sweden) was used. Before each test, the ergometer was washed thoroughly with a power hose and then rinsed with 5 liters of deionized water before being allowed to dry in a warm environment. The shorts and towel to be used by the subject were also washed in deionized water and dried: they were then wrapped in polythene film until required.

Throughout the text, deionized water refers to distilled, deionized water with a specific resistance of $>18 \text{ M}\Omega/\text{cm}$.

On reporting to the laboratory, subjects defecated and micturated if necessary. They showered and then washed themselves with 4 liters of deionized water. Subjects were instructed to wash themselves thoroughly with copious amounts of soap in the shower. On leaving the shower, they stood in a stainless steel tray and washed with the deionized water. Each used 4 liters, contained in four 1-liter sports-drink bottles that subjects used to spray the water evenly over themselves. Subjects then dried themselves carefully with the washed towel and put on a pair of plastic operating theater overshoes to avoid contact with the floor. Nude body mass was measured to the nearest 10 g. Subjects then entered the exercise room.

The cycle ergometer was contained within a large polyethylene bag that was stretched over a plastic frame (Fig. 1). The bags used in this study (Anaplast, Ayrshire, UK) were obtained from an agricultural merchant and were intended to contain silage. These bags, made of 440-gauge polyethylene, have the advantages of being readily available in the correct size, as well as resistance to puncture and low cost. A new bag was used for each trial. Preliminary trials showed that the bags were free from contamination with electrolytes. A frame of dimensions $95 \times 130 \text{ cm}$ and 150 cm high was constructed by using 1-in.- (2.5-cm-) diameter plastic plumbing pipes. Push-fit joints were used for the corners. The purpose of this frame was to ensure that the inner surface of the bag did not come in contact with the subject or the ergometer. Between trials, the frame was dismantled, washed in water and then washed again in deionized water before being dried and reassembled. Disposable plastic gloves were worn when these materials were handled. When the subject entered the laboratory, the frame and ergometer were in place, but the plastic bag was pulled up to a height of only $\sim 600\text{--}800 \text{ mm}$. The subject then removed the plastic overshoes and stepped into the bag, which was then pulled up fully and stretched over the top of the plastic frame. The height of the frame was such that top of the frame was $\sim 20\text{--}40 \text{ cm}$ higher than the top of the subject's head when seated on the ergometer with the hands on the handlebars. The subject then put on the washed shorts and was ready to begin exercise. Subjects were dressed only in shorts; shoes were not worn.

All exercise in these studies was carried out at an ambient temperature of about 34°C and a relative humidity of 60–70%. The exercise was undertaken in 5-min bouts interspersed by 5-min rest periods. Exercise intensity was set at a level that had been established in preliminary tests to

correspond to ~60% of maximum oxygen uptake. A further two preliminary tests, in which subjects exercised for 5 min and then rested for 5 min, during which time body mass was measured, had established the exercise time necessary for each individual to incur a fluid loss equivalent to ~2% of body mass. In all trials reported here, this exercise time was used, varying from ~30 to 70 min. Therefore, the total time required to achieve the necessary loss of body mass ranged from ~55 to 135 min. The total sweat loss in all trials was therefore approximately the same, when expressed relative to each subject's body mass, although sweating rates were very different.

On completion of the prescribed exercise, while still in the bag, the subjects removed their shorts and left them in the bottom of the bag, and washed themselves with 4 liters of deionized water to which had been added ammonium sulfate (20 mM). This water was presented in four 1-liter sports-drink bottles. Subjects were instructed to wash themselves as completely as possible and to remove as much surface water as they could before leaving the bag. One additional liter of deionized water containing ammonium sulfate was then used to wash down the inside of the bag and the bicycle, with care being taken to ensure that as much liquid as possible was flushed toward the bottom of the bag and mixed through the shorts left there.

The liquid in the bag at this stage contained the sweat that had not evaporated, plus the water used to wash the subject down at the end of the trial. The volume of unevaporated sweat was calculated from the dilution of the ammonium sulfate. Pilot studies had established that these ions were not detected if the same procedure was followed without addition of ammonium sulfate to the washdown water. Duplicate samples of the water in the bag were collected, using a syringe and needle, and stored for analysis.

At the end of the investigation, subjects dried themselves completely before nude body mass was again measured. The difference in mass was used to estimate total sweat loss, assuming that the specific gravity of sweat is 1.0 (13). The respiratory water loss (50 ± 9 g) and water loss due to substrate oxidation (45 ± 8 g) were estimated for each subject in the preliminary test (18). These were assumed to be the same in each trial and were taken into account in the estimation of the sweat loss.

Sample analysis. Analysis was carried out by ion chromatography, using a Dionex DX-100 Ion Chromatograph system (Dionex, Sunnyvale, CA). Samples were first diluted by 1:100 in filtered, distilled, deionized water. The average value of the duplicate samples was taken. The coefficient of variation (CV) for the analysis, calculated from eight duplicate values, was 1.5% for sodium, 4.5% for potassium and 1.0% for chloride.

Statistical analysis. CVs were calculated on duplicate values from the SD of the difference and the mean of the duplicates. The intraclass correlation coefficient (R) was calculated to establish the reproducibility of the method. The comparability of the values for sweat electrolyte content obtained by the whole body method and by regional collection was established according to Bland and Altman (4), and difference between the values was obtained by Student's t -test for paired comparisons.

RESULTS

Contamination of the system. The effectiveness of the procedures of washing the bicycle, the plastic frame, and the towel and shorts used by the subject, as well as the presence of possible contamination in the plastic bag itself, was assessed by assembling these items and washing them with 5 liters of deionized water in the absence of a subject. No electrolytes (sodium, potassium, magnesium, calcium or chloride) were detected in the water sampled, indicating that there was no measurable contamination of the system.

Recovery. The completeness of the recovery of electrolytes secreted in the sweat was assessed by assembling the system and, with the subject (who had undergone the prior washing procedures) seated on the cycle ergometer, pouring 1 liter of water containing 50 mmol sodium chloride and 5 mmol potassium chloride over the subject. Immediately after this, the subject completed the washdown procedures as described above. This part of the investigation was carried out at an ambient temperature of 20°C to minimize the possibility of contamination with sweat from the subject and also to minimize evaporative loss. The total time for the

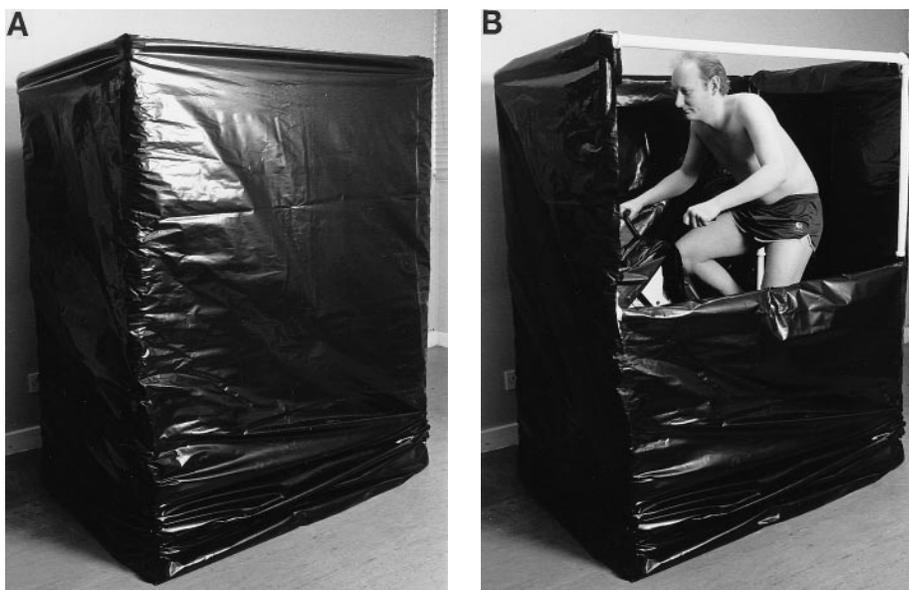


Fig. 1. Sweat-collection apparatus. *A*: intact system; *B*: a section of the bag has been cut away to show subject on cycle ergometer.

Table 1. Recovery of volume and electrolytes added to the system

	Fractional Recovery, %	CV, %
Volume	102 ± 2	3.3
Sodium	99 ± 2	1.8
Potassium	98 ± 9	6.4
Chloride	101 ± 4	2.5

Values are means ± SD of repeated measures on 4 subjects. CV, coefficient of variation, refers to the within-subject variability, calculated from 4 duplicate values.

whole procedure was kept short (10 min) to minimize these effects. This procedure was carried out twice on each of four subjects. The percentage of the 1-liter volume poured over the subject and of the added sodium, potassium, and chloride recovered is shown in Table 1. The recovery ranged from 98% for potassium to 101% for chloride.

Repeatability. The reproducibility of the measurements was assessed from repeated measurements made on five subjects, each of whom followed the same procedures four times. For the 2 days before the first trial, each subject recorded the quantity and types of food consumed and activities undertaken and reproduced these on the 2 days preceding each subsequent trial. On each of these trials, subjects exercised for the same length of time, at the same exercise intensity, and under the same environmental conditions. These trials were carried out at intervals of 7 days and at the same time of day on each occasion. The results are presented in Table 2.

Normal values. The normal values for the major ions measured were obtained from seven subjects (5 men, 2 women) and are presented in Table 3. All these data relate to measurements made in trials where subjects exercised to the point where a sweat loss of ~2% of body mass loss was achieved. Subjects exercised for 5-min periods with 5-min rest periods; total exposure time varied between subjects, with a range from 55 to 135 min.

Comparison with regional collection. Five subjects were investigated to compare the whole body-washdown technique described here with a regional collection method. Subjects exercised as described above, with 5-min exercise periods and 5-min rest periods on four separate occasions. Regional sweat collection was made midway through the exercise period on a small (7.5 cm²) area of the back, between the shoulder blades, using a previously weighed gauze swab enclosed by a polyethylene cover that was taped to the skin. The skin was first washed with distilled water and the swab was

Table 3. Normal values for sweat electrolyte composition obtained from 7 healthy young subjects and values from other sources

	Mean ± SD	Range	Other Sources
Sodium	50.8 ± 16.5	35.2–81.0	20–80
Potassium	4.8 ± 1.6	2.7–6.8	4–8
Calcium	1.3 ± 0.9	0.0–2.9	0–1
Magnesium	0.5 ± 0.5	0.0–1.5	<0.2
Chloride	46.6 ± 13.1	31.6–70.4	20–60

Values are given in mM; other sources, see Ref. 15.

put in place: during the recovery period after the 5 min of exercise, the swab was removed and immediately placed in a weighed container and sealed. The back was chosen as the site of the regional sweat collection, as it allowed easy access to the experimenter and has been used by other investigators.

The swab and container were weighed, and the mass of sweat was derived from the difference. Deionized water (0.05 liter) was added to the swab, and the container was shaken vigorously before samples were taken for analysis, using the same methods as described above. Results are shown in Table 4. For sodium and chloride, the electrolyte concentration from the regional sample was higher than from the whole body method, but the potassium concentration was lower in the regional sample.

DISCUSSION

The results obtained by using this method for sweat collection from exercising subjects demonstrate that it is possible to obtain reliable and reproducible measures of sweat loss and sweat composition from exercising subjects. The system is virtually free from extraneous contamination with electrolytes, and recovery of water and electrolytes added to the system is effectively complete. It does not interfere with the evaporation of sweat from the skin surface, allowing studies of sweat composition and electrolyte loss to be made under physiological conditions. Results obtained in this way compare favorably with previously reported estimates of recovery using alternative whole body-washdown methods. Lemon et al. (12) reported recovery of added urea to be 93.3 ± 18.1 and 103.2 ± 17.1% (SD) for two different operators. The use of a marker that is present in sweat does, of course, complicate the system, and they estimated that an amount equal to ~20% of the added urea was excreted onto the skin during the washdown procedure.

Table 2. Reproducibility of measures made on 5 subjects, with 4 trials on each subject

Measure	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Mean Range	R
Mass loss, g	1,580–1,820	1,250–1,630	950–1,020	970–1,360	1,420–1,900	312	0.920
Unevaporated sweat, g	870–1,110	900–1,160	600–770	420–910	940–1,560	356	0.553
Sweat sodium, mM	68–96	34–52	32–54	31–47	38–62	22	0.800
Sweat potassium, mM	5.7–9.1	5.7–8.0	2.6–6.0	2.1–6.9	3.7–6.5	3.3	0.951
Sweat chloride, mM	62–77	32–51	33–50	34–43	36–58	16	0.640

Values are extremes of range recorded for each subject and mean of the extreme range. R, intraclass correlation coefficient.

Table 4. Comparison of regional and whole body sweat electrolyte composition

	Whole Body	Regional	Difference
Sodium	51.6 ± 18.3	75.3 ± 21.9	23.6 ± 12.8
Potassium	5.6 ± 1.8	3.7 ± 2.2	1.8 ± 3.3
Chloride	48.0 ± 14.2	57.5 ± 15.5	9.5 ± 13.7

Values are means ± SD (in mM) obtained from 20 trials. Regional composition is from sweat collected midway through exercise period from center of the back, between the shoulder blades. Difference is mean of individual differences.

The recovery of electrolytes added to the system is virtually complete, ranging from 98% for potassium to 101% for chloride. This last value is inflated by one sample where there appears to have been some contamination present, and a recovery of 108% was obtained for this individual sample. If this sample is excluded, the recovery for chloride was 100%. It appears as if this contamination was introduced at the analytic stage, as the sodium and potassium recoveries for that trial were 99 and 95% respectively. Calculation of the recovery of the added volume (1 liter) from the dilution of the added markers in the washdown fluid shows a recovery of 102%, from a 98% recovery of ammonium and 99% recovery of sulfate. The variation in the recovery of the electrolytes ranged from $1.8 \pm 2\%$ for sodium to $6.4 \pm 9\%$ for potassium (Table 1). These are somewhat less than that found by Lemon et al. (12) as noted above, but it would also be possible to reduce the variance of the potassium measurements by analyzing the samples at an increased sensitivity on the ion chromatograph. For the values reported in this paper, sodium and potassium were analyzed on the same run.

The reproducibility of the measures made on five subjects undertaking identical procedures on four separate occasions of body mass loss, of unevaporated sweat, and of sodium, potassium, and chloride concentrations in sweat, were all of a similar magnitude (10–23%). These values include the biological variability between trials, and for sweat composition and volume of unevaporated sweat they also include the error within the sweat-analysis method. Barnett and Maughan (2) found no difference in the quantity of sweat lost during 1-h bouts of moderate-intensity exercise, undertaken three times in the heat at weekly intervals. In the present investigation, the data were collected on the final four of six exercise trials (the first two being used to determine the duration of exercise to be undertaken). There was no pattern to the variation in sweat loss between weeks; rather, for four of the five subjects, the amount of body mass loss was similar on three of their trials but on the other one they lost either a greater or lesser amount of mass. This may contribute in part to the variations in sweat composition recorded.

There seems to be general agreement that the use of plastic bags to enclose limbs causes problems due to the skin becoming waterlogged as well as the fact that the composition of the sweat varies between different regions (5, 7, 17). This may be less of a problem if a repeated-measures design is used to compare the ef-

fects of different treatments, but there is effectively no evaporation of sweat secreted on to the skin surface because the air within the enclosing bag is saturated with water vapor, and this may preclude the detection of any treatment effects. In the present study, the sweat collected over a 6- to 7-min period midway through the exercise period from a small area of the back did not correspond to the values obtained from the whole body method (4). The local collection method gave different values for sodium concentration ($P = 0.000$), potassium concentration ($P = 0.030$), and chloride concentration ($P = 0.007$; Table 4). However, although the value obtained from the whole body method was less than the regional sweat for both sodium and chloride concentration, it was greater for potassium concentration. This suggests that the concentration and composition of sweat varies at different regional sites or that as sweating proceeds, its ionic composition alters in different ways with respect to the individual electrolytes. It should be remembered that the regional sweat values are obtained from one collection point midway through the exercise period. Total electrolyte loss cannot therefore be adequately estimated from a single regional sample. The practice of making regional collection of sweat at a number of sites (7, 12) and making an estimate of total electrolyte loss could, however, be tested for its validity against the whole body collection method reported in this paper.

Previous methods used to collect whole body sweat with a subsequent washdown procedure for electrolyte recovery have relied either on complete evaporation of the sweat from the skin surface (12) or on trapping the sweat in clothing worn by the subject together with the application of towels (1, 11). There are some problems with both these methods, as there must remain some uncertainty as to the completeness of the collection. The method described here ensures that all sweat remains within the bag. This method also removes the need for the wearing of special clothing or for forced convection to ensure sweat evaporation, as used by Lemon et al. (12). As with so many other procedures used in the past, there is a very real possibility that this in itself will alter both sweating rate and sweat composition. The wearing of clothing and application of towels will trap sweat and create changes in the local microclimate.

Our pilot studies established that ammonium ions were not detected in the water recovered after washing down subjects who had exercised until 2% of body weight had been lost. Given the sensitivity of the ion chromatography detection method used, and taking account of the dilution during the washdown process, this suggests a concentration of ammonium and sulfate in sweat of <0.3 mM. Consolazio et al. (6), however, reported values of 4–6 mM for ammonia and 1–5 mM for sulfate, with no data reported for the ammonium ion. Lentner (13) reports sweat ammonia concentration to be 3.02 mM and sulfate concentration within the range of 0.07–2.00 mM; again, no data are reported for the ammonium ion.

The sensitivity at which the ion chromatograph was used for the sample analysis was very much less than that possible with the system. The identification and quantification of other compounds present is certainly possible. No other analyses were performed on the samples collected, but the method can obviously be applied in situations where quantitative recovery and analysis of any nonvolatile solute or organic component present in sweat is required.

In summary, this method can be used to study thermal sweating during passive exposure, or it can be used to collect exercise-induced sweat. The method has the advantage of not interfering with the normal sweating process. It is not applicable to treadmill exercise but appears to be reliable and reproducible when used to quantify water and electrolyte loss in subjects exercising on a cycle ergometer.

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