Some Practical Simplifications of Perspiration Electrolyte Analysis ("Sweat Test")

Kurt M. Dubowski*

A simple, practical, rapid, and effective routine system for recovery and analysis of abdominal perspiration samples is described. The results of its application to control subjects and patients with cystic fibrosis of the pancreas are reported and compared with those of other investigators.

Cystic fibrosis of the pancreas is now acknowledged to be one of the most serious chronic diseases of childhood, with an estimated incidence in its fully manifested form of 1–1.7 per 1000 live births (1). Chances for early diagnosis and treatment—essential before the pulmonary disease that dominates the clinical picture has caused irreversible changes—were greatly enhanced with the introduction of perspiration electrolyte analysis (the "sweat test") by Shwachman (2) and others, following the reports by Darling et al. and di Sant'Agnese et al. (3, 4) of uniquely elevated sodium and chloride concentrations in the sweat of 99 per cent of patients with cystic fibrosis of the pancreas.

The quantitative "sweat test" because of its inherent reliability, relative simplicity, and generally clear-cut results has become the cornerstone for diagnosis of cystic fibrosis (1), and remains preferable to the several recently proposed simplifications, screening tests, or substitute procedures (5-10). The originally proposed and widely adopted "sweat test" technic (2) consisted essentially of absorption of 1 gm. or more of perspiration by a pre-weighed gauze pad from midback or midabdomen of the plastic-bag-enclosed patient who was under thermal stress stimulation. This was followed by elution of

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the perspiration sample from the gauze with water and analysis of the eluate for sodium and/or chloride content.

Two unfortunate difficulties have been noted in connection with the test. First, at least 6 fatalities have been recorded to date as a sequel to the thermal stimulation (11), chiefly in infants with severe pulmonary involvement and only a few weeks of age, and usually with marked hyperpyrexia following external heat application or prolonged sweating in confining garments used to produce a perspiration sample. Obviously, many more untoward experiences not terminating fatally, and therefore not reported, have been encountered during attempts to procure the several grams of perspiration required by the original elution methods of analysis. On several occasions in our series of "sweat tests" by the original Shwachman technic (2, 12), the pediatricians supervising the collection phase of the test found it necessary to discontinue the sweating because of adverse responses of the patient, including hyperpyrexia. Secondly, the original technic often produced samples quantitatively inadequate for an analytical procedure requiring elution of the perspiration specimen, thus requiring repeated testing of the patient. There were also several annoying practical difficulties, such as frequent breakage or contamination of the preweighed flask containing the gauze sponge for the sweat collection, or confusion of flasks and sponges when several sweat tests were performed simultaneously.

A simpler, more effective method of recovering, without weighing, in unaltered and undiluted form essentially all of the perspiration collected would, therefore, have marked advantages for the routine application of the "sweat test." In addition to reducing materially the length and intensity of the sweating required to collect an adequate perspiration specimen and, therefore, the potential hazard to the patient, the following procedure based in part upon a suggestion of Gibbs et al. (13) greatly simplifies the recovery and subsequent analysis of the perspiration sample.

Procedure

Sweat Collection

Because of the potential hazards of the patient phase of the sweat test and the consequent necessity for constant medical supervision of the patient, actual collection of the perspiration sample is not carried out by laboratory personnel. The laboratory supplies to the nursing units a chemically clean 100-ml. wide-mouth screw-capped
polyethylene bottle containing a 3x3-in. 12-ply U.S.P. Type VII all-gauze pad, which has been previously rinsed 4 times in demineralized water and dried in an oven at 100°. (We have found 20X12-mesh 12-ply Red Cross Steri-Pads* consistently free of demonstrable Na or Cl and, therefore, now omit this washing or other sponge preparation.) Nursing and medical staff then carry out the modified sweat collection.

No special preparation of the patient is required. The abdomen is thoroughly washed with distilled water and dried with electrolyte-free gauze or filter paper. The 3-in. square of gauze is then placed on the midabdomen with forceps and covered with a 3.5-in. square of polyethylene sheeting which is adhesive-taped to the skin. The patient is then suitably covered and wrapped in or covered with blankets, but no external heat is applied. After 20-90 min., depending upon the rate of sweating, in a room at ordinary temperature, during which the patient is constantly observed for signs of hyperpyrexia or other adverse reaction, the gauze pad is removed with forceps and immediately returned in the closed polyethylene bottle to the laboratory. Usually, 0.3-1.5 ml. of perspiration is readily obtained, although the gauze pad often does not appear grossly wet.

**Perspiration Electrolyte Analysis**

With forceps, the perspiration-containing gauze pad is loosely packed into a 12-ml. heavy-wall borosilicate centrifuge tube containing a conventional 43-mm. plastic golf tee and the tube tightly closed with a polyethylene cap† or a 50 mm.-square of parafilm M (Fig. 1). The tube is then centrifuged for approximately 10 min. at an RCF of 800-1000, using a balance tube similarly loaded. The dry sponge and the golf tee are then removed from the tube, and sodium and chloride determinations performed on the clear supernatant perspiration.

**Sodium Determination**

This is usually performed by the modification of the Dryer method (14), used routinely in our laboratories for all body fluid sodium determinations during the past 4 years. A 0.1-ml. sample is washed into a 10-ml. glass-stoppered volumetric flask with flame photometry diluent, containing 4.04 mEq. lithium/L. and 0.0202% v/v Sterox

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†Capall, size C, cat. no. 68128, Scientific Products Division, American Hospital Supply Corp., Evanston, Ill.
Fig. 1. Centrifuge tube assembly for sweat extraction.

Fig. 2. Perspiration sodium and chloride concentrations in patients with cystic fibrosis of the pancreas and control subjects.
SE, and is made to volume with the same diluent. This 1:100 dilution of perspiration is then analyzed directly for sodium with a Baird Model DB-4 flame photometer, using a single calibration for original specimen concentrations from 0 to 200 mEq./L.† If desired, a portion of the same diluted specimen is also analyzed directly for potassium content with the flame photometer, using a single calibration for original specimen concentrations from 0 to 10 mEq./L. When other flame photometric procedures for Na determination are to be used that require dilutions different from that routinely employed for serum analysis, it is convenient first to perform the chloride determination and to base appropriate sample dilution for flame photometry upon that result.

Chloride Determination

If an ample perspiration specimen is available, a 0.05 or 0.1-ml. sample is coulometrically analyzed for chloride using the Cotlove Automatic Chloride Titrator (15) and titrating at high rate or medium rate settings. Alternatively, a 0.1 or 0.2-ml. sample can be directly titrated for chloride by the mercurimetric-diphenylcarbazone procedure of Schales (16). Samples of limited volume are analyzed for chloride by an ultramicro modification of the Schales titration procedure (17-19) requiring 10 or 20 μL of specimen.

Results

Perspiration electrolyte analyses by the above methods were performed on 146 control subjects of 8 weeks to 20 years of age and either free of demonstrable disease or with various abnormal conditions other than cystic fibrosis of the pancreas, and on 19 pediatric patients who were 14 months to 9 years of age, with cystic fibrosis independently established by at least two criteria other than abnormal sweat test results.

The results are illustrated in Fig. 2 and a summary included in Table 1, which also contains the results of other investigators. The range of perspiration sodium concentration for patients with cystic fibrosis was 60–138 mEq./L, with a mean of 103.7 and a standard deviation of 18.2, as contrasted to a range of 5–65 mEq./L, with a mean of 26.6 and a standard deviation of 13.8 for the control group.

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†On our Baird DB-4 flame photometers, it has been necessary to mask the Na-Il sodium filter with a tinfoil square with a central 1.0-cm. aperture.
<table>
<thead>
<tr>
<th>Investigator di Date</th>
<th>No. of cases</th>
<th>Sodium (mEq/L)</th>
<th>Chloride (mEq/L)</th>
<th>Na/Cl Ratio</th>
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<tr>
<td></td>
<td>No. of cases</td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
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<tr>
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<td>25.6</td>
<td>5-65</td>
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<td>23.1</td>
<td>1-120</td>
<td>24.8</td>
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<td>...</td>
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<tr>
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<td>55</td>
<td>32</td>
<td>8-72</td>
<td>23</td>
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<td>50</td>
<td>59</td>
<td>10-120</td>
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The concentration of perspiration chloride in cystic fibrosis patients ranged from 55–134 mEq./L. with a mean of 100.7 and a standard deviation of 21.7, compared to a range of 4–56 mEq./L. with a mean of 25.4 and a standard deviation of 11.7 for the control group. The mean perspiration sodium/chloride concentration ratio was 1.05 for the control subjects and 1.03 for the patients with cystic fibrosis (Fig. 3), with an overall mean ratio of 1.04.

Several of the control subjects with perspiration sodium and chloride concentrations above 60 mEq./L. displayed marked respiratory disease such as pulmonary fibrosis, but in the absence of gastrointestinal tract disorders or pancreatic enzyme deficiency they were not considered to be cases of cystic fibrosis of the pancreas by the pediatric staff. Volumes of perspiration recovered with the present technic averaged about 0.5 ml., with a range of 0.2 to 1.8 ml.; no significant differences were noted between volumes of sweat obtained from patients with cystic fibrosis and from the control subjects.

**Discussion and Conclusions**

The results here reported confirm previous findings (1-4, 9, 13, 20) that perspiration sodium and chloride levels are markedly increased in patients with cystic fibrosis of the pancreas, and generally agree
well with recently reported findings of other investigators using the
original Swachman "sweat test" procedure or modifications thereof
(Table 1). This demonstrates a significant difference between the
perspiration sodium and chloride concentrations of normal subjects
and those of cystic fibrosis patients. In our series, there was little
overlap between perspiration sodium and chloride levels of the con-
trol group and those of cystic fibrosis patients (Fig. 2). Further-
more, most of the higher sodium and chloride concentrations (in the
"control" group) accounting for this overlap were found in hetero-
zygous relatives of cystic fibrosis patients, who have been previously
reported to show intermediate elevations of sweat electrolytes (20).
The several variables in the collection of perspiration, such as site of
sweat collection, intensity and duration of thermal stimulus, individual
sweat excretion rates, etc., should have negligible effects upon in-
terpretation of "sweat test" results if the collection phase of the
test is performed similarly in all patients in a given laboratory. We
attempted to minimize these effects by similar collection technics for
our control subjects and cystic fibrosis patients.

Unreported data from our series show that there is no consistent
relationship between levels of sodium and chloride in perspiration
and those in blood serum in control subjects or patients.

The results reported here support the previously stated view that
the normal perspiration sodium concentration rarely exceeds 60
mEq./L. (21), and that the concentration of perspiration sodium is
usually slightly higher than that of chloride. This difference is quite
consistent and lower in our series than previously reported by some
investigators (Table 1), thus seemingly indicating approximately
equal usefulness for diagnostic purposes, of either sodium or chloride
determination in the sweat. The analytical methods outlined here,
however, permit ready determination of both ion concentrations and
consequently there seems little need to make a choice of analyses.

References
5. Baseman, S. P., et al., Determination of saliva chloride for diagnosis of cystic fibrosis
    of the pancreas. Exhibit at meeting of American Academy of Pediatrics, Chicago,
    October 1955.
ELECTROLYTE ANALYSIS OF SWEAT