Liquid-Chromatographic Determination of Chloride in Sweat from Cystic Fibrosis Patients and Normal Persons

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Chloride concentrations in sweat were measured by "vacancy liquid chromatography," a technique in which anions eluting from an ion-exchange column in the presence of an elution buffer which absorbs at 280 nm cause a decrease in absorbance proportional to the concentration of the anion. Less than 1 μL of sweat suffices for this analysis, which takes 5 min per sample. Results were comparable to those obtained by conventional titrimetric determination of chloride. Sweat from cystic fibrosis patients had higher (104 ± 26 mmol/L) chloride concentrations than sweat from normal persons (16 ± 7 mmol/L). This technique can be used in the diagnosis of cystic fibrosis.

Additional Keyphrases: electrolytes · reference interval · titrimetry compared · lactate measurement · measurement of anions by "vacancy" chromatography · pediatric chemistry

Sweat chloride measurements are widely used in the diagnosis of cystic fibrosis (CF). The most generally used test, the pilocarpine iontophoresis sweat test, was introduced by Gibson and Cooke (1) in 1969. Chloride concentrations in sweat that exceed 60 to 70 mmol/L are considered diagnostic for CF (2, 3). The current investigation was undertaken in an effort to apply to the analysis of sweat the newly described technique of "vacancy" chromatography on a liquid chromatograph equipped with an anion-exchange column. Vacant chromatography involves equilibrating the column with a buffer, such as potassium phthalate, that absorbs light in the ultraviolet range. When potassium phthalate buffer is used as the eluent buffer with an anion exchange column, A280 decreases when anions are eluted from the column.

Here, we define the retention times for various anions on this column and demonstrate that the chloride concentration in sweat can be accurately measured by this technique.

Methods

With informed consent from subject or parent, we obtained sweat (>50 μg) from human volunteers by pilocarpine iontophoresis (Lancor Cystic Fibrosis Analyzer; Sherwood Medical Industries, Inc., St. Louis, MO 63103). Sweat was measured as the difference in weight of a piece of gauze before and after squeezing to express all of the absorbed sweat. Subjects included 39 males and 17 females, ranging in age from three days to 30 years, with a mean age of 2.6 years. Conventional chloride measurement was performed for diagnostic purposes by mercuric nitrate titration in acidic medium in the presence of 2,3-diphenylcarbazone (4). Sweat samples were then frozen, stored at -20 °C for one to three weeks, and subsequently thawed and diluted with deionized water.

For vacant chromatography we used a liquid chromatograph equipped with a variable-wavelength ultraviolet light detector set at 280 nm (Spectromonitor III), and a 20-μL sample loop (all from L.D.C. Corp., Riviera Beach, FL 33404). An anion-exchange column (PRP X-100, 150 × 4.4 mm; Hamilton Co., Reno, NV 89520) was used that contained 10-μm spherical particles of a macroporous copolymer of styrene and divinyl benzene chemically bound with trimethyl ammonium groups, specified capacity 0.17 mmol/g. The running buffer was potassium acid phthalate (1.5 mmol/L, pH 5.15), and the flow rate was 1 mL/min. Standard solutions and buffers were prepared with deionized water and filtered (0.2-μm pore-size filter) before use.

Sweat samples were not identified as "normal" or "CF" before analysis by vacant chromatography, and results obtained in the titrimetric reaction were not revealed until after chromatographic chloride determinations were completed. Values of sweat chloride greater than 60 mmol/L were considered diagnostic for CF, while chloride concentrations between 40 and 60 mmol/L were considered intermediate in value and were not included in the comparison between CF and normal samples presented below in Table 1.

Results

Figure 1 illustrates the vacant chromatography profile obtained with standard solutions of NaCl. Retention time for the chloride ion was 3.0 min, and increasing concentrations of chloride in the range of 0.1 to 1 mmol/L gave progressively and proportionately larger deflections in the absorbance at 280 nm. Other ions did not interfere with chloride. Retention times for several common anions were: fluoride (2.4 min), phosphate (2.6 min), and sulfate (10.2 min). These findings are similar to the results of others using this same technique to measure anion concentrations in various types of samples (5–7). The reproducibility of this analysis was evaluated by repetitive measurements of NaCl standards and human sweat samples. Day-to-day variability (CV) of the retention time for chloride was <4% (3.0 ± 0.1 min, n = 7), as was same-day variability (3.0 ± 0.1 min, n = 19). Day-to-day variability of the peak height for a 1.0 mmol/L NaCl standard was <3% (10.3 ± 0.3 mm, n = 5); same-day variability was <2% (10.2 ± 0.2 mm, n = 8).

For analysis of human sweat we routinely used 0.25 μL of sweat (20-μL samples of sweat diluted 80-fold with water). Sweat from normal persons and CF patients both give two prominent peaks (Figure 2), the first of which has a retention time of 2.6 min. This corresponds to the retention time

| Table 1. Chloride Concentrations in Normal and Cystic Fibrosis Sweat |
|-----------------------|-----------------------|
| Mean (and SD) chloride concentration, mmol/L |
| Titrmetric assay | Liquid chromatography |
| Normal sweat (n = 42) | CF sweat (n = 10) |
| 15 ± 8 | 16 ± 7 |
| 104 ± 17 | 104 ± 26 |
Normal negative quantities represents the first negative peak, which appears for all samples regardless of anion content, has a retention time of 1.2 min and a height of 280 nm. The second negative peak, with a retention time of 3.0 min, represents chloride. Sweat samples diluted with known quantities of lactate or chloride, respectively, showed the appropriate increase in the size of the corresponding negative peak. The CF sample (Figure 2B) shows a much larger negative peak for chloride than does the normal sample. From the standard curve constructed previously, we calculated the mean concentration of chloride to be 14 mmol/L in normal sweat (Figure 2A), 85 mmol/L in CF sweat (Figure 2B).

The reliability of this technique was tested in a blind study of human sweat samples, which were first analyzed for diagnostic purposes by the standard titrimetric chloride determination (5, 6) and then by vacinity chromatography. There was a strong correlation between the results obtained by the standard titrimetric analysis and the results obtained by vacinity chromatography (Figure 3). Linear regression analysis of this data gave a line with a y-intercept of 3.6 mmol/L, a slope of 0.89, and correlation coefficient of 0.99 (n = 56). There were no false positives or false negatives among the samples tested, which included 42 normal per-
sons and 10 CF patients. Four patients in this group had intermediate concentrations of sweat chloride (40–60 mmol/L), and this finding was the same by both types of chloride analysis. Table 1 contains the tabulated results of chloride analysis for normal and CF sweat, excluding these four values in the intermediate range. These results show no statistically significant difference between the data obtained by the standard titrimetric analysis of chloride and the vacancy chromatography method.

Discussion

Cystic fibrosis is generally diagnosed by analysis for chloride in sweat (2, 3). We have shown here that sweat chloride concentrations can be accurately measured by vacancy chromatography on an anion-exchange column. An advantage of this new technique over standard titrimetric chloride analysis is that one can simultaneously evaluate the concentration of chloride and lactate in the sample, and that it may be possible to analyze for other trace anions by increasing the sensitivity of the detector. Analysis for anions in relatively small samples of other biological fluids should also be feasible. The volume of sweat required for analysis of chloride concentrations by vacancy liquid chromatography is unusually small, 0.25 µL.

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References