Quantification of Chloride in Sweat with the Cystic Fibrosis Indicator System

Warren J. Warwick, Leland G. Hansen, and Mark E. Werness

We examined the relation between chloride concentration and the area of complexed chloride of Medtronic's Cystic Fibrosis Indicator System, using a high-resolution x-y coordinated digitizer to measure the circumference of the chloride precipitation ring. These digitized points were entered directly into an IBM PC computer, where the area of the chloride precipitation was calculated with use of a repetitive rectangular estimation program. Using these data, we determined the relationship between the area of chloride precipitation and the chloride concentration of the standard NaCl solutions. When the area of the ring of chloride precipitation in the system's patch is measured immediately after the sweat test is completed, the concentration of chloride in the sweat can be calculated with a reproducibility equal to that of the Gibson-Cooke sweat test.

Additional Keyphrases: screening · Gibson-Cooke test compared

Results of sweat tests done by laboratory technicians who do the test only occasionally are not considered to be as reliable as those for sweat tests done by a cystic fibrosis (CF) sweat-test laboratory (1). The Cystic Fibrosis Indicator System (CFIS) (2, 3) was developed to be so standardized that any CFIS sweat test done in a non-CF Center laboratory would provide a "yes," "no," or "maybe" (intermediate) diagnostic result as reliably as the Gibson-Cooke sweat test (GCST) (4) done in a sweat-test laboratory at a CF center (5-8). We were interested to see how well the CFIS could quantify sweat chloride over a wide range of chloride concentrations. In this study, designed to supplement and extend the observations of Kollberg and Hellsing (9), we used saline solutions of known chloride concentration to estimate the relation between chloride concentration and the area of complexed chloride on the patch. We then used the measured areas of complexed chloride from CFIS tests of subjects with known or suspected CF to demonstrate the quantitative power of the CFIS indicator patch as compared with that of the GCST.

Materials and Methods

Cystic Fibrosis Indicator System

The CFIS (cat. no. 4930; Medtronic, Inc., Minneapolis, MN 55440) was used as described (7) for comparison with the GCST. CFIS indicator patches were used alone to develop the reference standard curve.

The "indicator" part of the CFIS, a small circular patch placed over the site of pilocarpine iontophoresis stimulation, provides a quantitative sweat test and is designed to function as a screening test (3, 7). Sweat absorbed through the pores of the patch diffuses radially through a medium containing a chloride-complexing chemical. The patch is designed so that any sweat sample containing <45 mmol of chloride per liter will not diffuse past a defined area (designated as indicating "normal" sweat chloride content) but all samples containing greater concentrations of chloride will uniformly cross the entire boundary between the two areas that denote "normal" and "CF" (Figure 1).

Statistical analysis of a large quality-control study done by Medtronic, Inc., in 1982 showed that the most likely sweat chloride concentration for a partial crossing of the boundary is 40 mmol/L and for a complete crossing is 55 mmol/L. These statistical cutoff points are comfortably similar to the consensus values for the upper limit for normal and the lower limit for CF subjects as measured with the GCST.

Gibson-Cooke Equipment

We performed the Gibson-Cooke sweat test with the equipment, supplies, and technique described by Warwick et al. (10).

Experimental Design

In this study we tested 11 concentrations of sodium chloride ranging between 5 and 100 mmol/L. For each concentration we placed a 75-μL aliquot of test solution on a nonporous chloride-free surface and covered and sealed this area with one CFIS patch. This sample volume had been previously determined to take 20 to 25 min to fill the patch, the usual in vivo filling time for the CFIS patch (7). The process was performed at 32 °C, the average skin temperature of the skin after pilocarpine iontophoresis, and was repeated 10 times for each chloride concentration.

As soon as the end-of-test tab was half filled, indicating the collection of 30 mg of the test solution, we removed the patch from contact with the damp surface and blotted the contact surface with dry, chloride-free gauze. The clearly visible boundary of the chloride precipitation area was

---

Fig. 1. Three CFIS patches, showing results for (A) normal, (B) intermediate (at risk for CF), and (C) classic CF

The area of color change caused by the complexing of chloride in the sweat is linearly related to the concentration of chloride in the sweat. (Used with permission of Medtronic, Inc., Minneapolis, MN 55440)
marked with a fine-tip pen.

Using a high-resolution x-y coordinate digitizer (Model 7024 XY Plotter and Model DT12-241 series 7000 Digitizer; Bausch & Lomb, Austin, TX 78753), we traced the circumference of the chloride precipitation ring and digitized about 60 points around the circumference. Via an RS-232 interface, the coordinates for these digitized points were entered directly into an IBM PC computer, where the area of the chloride precipitation was calculated by using a repetitive rectangular estimation program. We studied the relationship between the area of chloride precipitation and the chloride concentration of the standard NaCl solutions.

We used a second set of patches to repeat the immediate area measurement just described, then let the patches sit for two days, to allow the boundary of the precipitation area to expand and to become stable. The circumference of this larger area was measured, the area was calculated as above, and the relationship of these areas to the chloride concentration of the standard NaCl solutions was studied.

Polaroid photographs of 76 CFIS patches from six CF and 30 normal subjects were obtained from a previous study (7) of the CFIS and the GCST. In that study, each volunteer had one CFIS and one GCST sample taken on each arm. In all, 36 paired samples and one unpaired sample were available for comparison. We determined the CFIS quantitative sweat chloride estimate by measuring the areas on the photographs, using the 11-point standard curve described above.

Results

Ten CFIS patch area measurements were made at each of the 11 chloride concentrations. Table 1 shows the mean, standard deviation, and coefficient of variation of each set. Standard regression analysis of all 110 data points showed a very strong linear relationship between the area of chloride precipitation (y, mm²) and the chloride concentration (x, mmol/L): y = 1.61x + 7.1 (r = 0.991; P < 0.001). The equation for predicting chloride concentration from the area of precipitation was x = 0.62y - 4.4.

Analysis involving the patches for which the area of precipitation had been allowed to stabilize for 48 h showed a similar strong linear relationship between the area of precipitation and chloride concentration (Figure 2). The initial and the stabilized areas were virtually identical in

![Fig. 2. Stabilized area vs chloride concentration](image)

The CFIS patch is designed to be read promptly after the sweat sample is collected because of expansion of the area complexed in the "developing region" (see Fig. 1) of the patch. The area complexed immediately after collection of chloride (---) is compared here with the area 48 h later (--). A linear relationship is shown for both the blanking region (inside the inner circle) but were increasingly different from each other as the area of chemical complexing of chloride in the testing area (outside the inner circle) increased. We limited the analysis to the testing areas, because we saw no obvious change in the precipitation area in the blanking area. The graph in Figure 2 is therefore truncated and limited to the chloride concentration range 40 to 100 mmol/L. Over this range the correlation coefficient was 0.999 and the t-test for significance has a P value of <0.001. The regression line for the saline solutions with concentrations of 70 to 100 mmol/L is y = 2.646x - 14 (x and y variables as above). The equation to calculate chloride concentration from the stabilized area of precipitation for areas >160 mm² is x = 0.378y + 5.3.

To compare sweat chloride concentrations in CFIS measurements and GCST measurements done simultaneously, we calculated the CFIS chloride concentration from the formula for nonstabilized area (see above) and the GCST concentration by the technique of Warwick et al. (10). For the CFIS calculations, we used the area of chloride precipitation measured from photographs taken immediately after collection of sweat was completed. The area of the patch in the photographs was checked and proven to be the same as the area of the original measurement of the patches, which, like the photographs, had been retained for documentation of the results of a comparison of the CFIS and the GCST (6). The duplicate results are graphed in Figure 3.

The regression equation for the two estimates of sweat chloride on these subjects is CFIS = 0.825GCST + 0.273 mmol/L (r = 0.972; P < 0.001).

Discussion

The data presented show a highly significant relation between the area of chloride complexed by the 30-mg CFIS
The CFIS patch and the concentration of chloride in both saline solution and pilocarpine-stimulated sweat, in harmony with the suggestion of Kollberg and Hellsing (9) that the area of chloride complexed might be used as a quantitative sweat test.

Results for area measurement to estimate the chloride content compared very well with the results of the GCST, the correlation coefficient being 0.972. The difference between the mean difference for two repeated Gibson–Cooke sweat tests (2.2 mmol/L) and for two repeated CFIS sweat tests (3.3 mmol/L) is not significant.

Although the estimation equation for the calculation of sweat chloride from the area complexed in the CFIS patch gave values that were consistently lower than those from the traditional GCST, subjects with cystic fibrosis were clearly separated from non-CF subjects (Figure 3); i.e., there is no practical difference in interpretation of sweat chloride values derived from either test.

This preliminary study suggests an opportunity for enhancing the CFIS sweat test, because quantitative estimates of area are easy to make with a moderately priced tablet digitizer, and the CFIS patch provides a permanent record of the sweat test. Quantitative analysis of chloride concentration from a CFIS patch can be done any time, even long after the CFIS was used as a screening test. Our observations showed, after 48 h, minimal if any increase in the area of complexed chloride in the blanking region, but a large, quantitative increase in the area of complexed chloride in the testing area of the CFIS patch.

This difference in performance of the two areas is not important with regard to immediate or delayed use of the CFIS patch as a screening test for cystic fibrosis. The delayed reading would be "fail safe" as far as the diagnosis of CF is concerned, because a normal result might become indeterminate, a questionable result become more questionable or perhaps positive, a positive result become more positive. Sweat tests with areas of complexed chloride in the indeterminate area for the delayed measurement of 80 to 160 mm² will be seen in <5% of sweat tests in both CF and non-CF subjects. Present equations are not suitable for use in this quantification of sweat chloride in the indeterminate area for delayed measurements, so such tests would have to be repeated. In all cases of positive quantitative tests and of indeterminable area measurements the patient should be referred to a CF center for confirmation.

Research reported here was supported in part by a Cystic Fibrosis Foundation Grant, Research, and Teaching Center Grant and by Specialized Center of Research Grant no. HL27355-04, National Heart, Lung, and Blood Institute, NIH.

References