

Original articles

Improved sweat test method for the diagnosis of cystic fibrosis

E P CARTER, A D BARRETT, A F HEELEY, AND J A KUZEMKO

Departments of Paediatrics and Clinical Chemistry, District Hospital, Peterborough

SUMMARY We describe a new technique of collecting sweat for measurement of osmolality and sodium concentrations. Eighty two subjects were studied—39 controls and 43 patients with cystic fibrosis. Adequate amounts of sweat were obtained in 81 subjects and sweat was analysed for both osmolality and sodium concentrations in 73 subjects. The 34 controls gave sweat osmolality and sodium values ranging from 62 to 196 mmol/kg and 9 to 72 mmol/l respectively. The 39 cystic fibrosis patients gave osmolality values ranging from 220 to 416 mmol/kg and sodium concentrations ranging from 60 to 150 mmol/l. Sweat osmolality alone was determined in eight infants under 50 days of age—four later developed the clinical features of cystic fibrosis and four, in whom cystic fibrosis was suspected, were later excluded. Sweat osmolality values in these two groups ranged from 255 to 345 mmol/kg and 87 to 123 mmol/kg respectively. The simplicity of collecting sweat and the measurement of osmolality offer distinct advantages over techniques previously described.

The sweat test is the single most important and conclusive biochemical investigation in the diagnosis of cystic fibrosis.¹ Over 99% of subjects homozygous for the cystic fibrosis gene have sweat chloride and sodium concentrations above 70 and 60 mmol/l respectively.² Although various techniques have been introduced in the past,³ the most reliable test is based on the quantitative pilocarpine iontophoresis technique described by Gibson and Cooke in 1959.⁴ A minimum of 100 mg of sweat must be obtained for an accurate result, and because of the difficulty in producing this amount of sweat in neonates it is generally considered inadvisable to attempt the test on infants under the age of 6 to 8 weeks. Unless performed meticulously by experienced staff, the Gibson–Cooke procedure is open to many sources of error leading to over diagnosis of cystic fibrosis.^{5,6}

The introduction of a sweat collection technique using an electrically heated metal cup, and the measurement of sweat osmolality had the potential for reducing many of the errors inherent in earlier methods.⁷ Although we found this method cumbersome in routine use, particularly with small children, it showed that sweat osmolality measurements correlated well with sweat sodium and chloride concentrations.⁸

The more recent development of a non-heated capillary sweat collection system (Macroduct) seemed to offer a simple and practical alternative method. We describe our preliminary experience with the Macroduct system of sweat collection and measurements of sweat osmolality and sodium concentrations in normal subjects and in patients with cystic fibrosis.

Subjects and methods

Eighty two subjects were studied; 39 acted as controls and 43 were patients with known cystic fibrosis. Their ages ranged from 2 months to 50 years and 3 days to 27 years respectively. Sweating was induced on the flexor side of the forearm by pilocarpine iontophoresis using pilocarpine impregnated gel discs. A current of 1.5 mA was used for five minutes only. The stimulated area of skin was thoroughly cleaned and the sweat collection device placed in position. (Macroduct, ChemLab Instruments Ltd). The Macroduct consists of a round, 2.8 cm diameter, plastic concave base with a central aperture through which sweat collects into coiled capillary tubing. A spot of dye at the aperture stains the sweat blue so that the rate of production and the volume of sweat may be assessed easily. The

usual duration of this procedure was 15 minutes. The capillary tubing containing the sweat sample was then removed, sealed, and transported to the laboratory without risk of contamination or evaporation. Immediately before analysis the sweat sample was transferred to a container from which the required volumes were taken for osmometry using the Wescor Vapour Pressure Osmometer (ChemLab Instruments Ltd) and the measurement of sodium concentration using the standard method of flame emission photometry. Eight μl of sweat were required for the osmolality analysis which took 90 seconds, and 10 μl for the sodium estimation which required 15 minutes. The reproducibility of the osmolality measurement using the vapour pressure osmometer was determined on electrolyte solutions with osmolalities of the order: 100, 270, and 500 mmol/kg. The coefficients of variation at these levels for 30 determinations within a single assay were 1.82, 0.52, and 0.39% respectively and for 30 determinations of the same solutions over 15 days were 2.80, 0.83, and 0.49% respectively.

Results

In 73 subjects (34 controls and 39 cystic fibrosis patients) sweat was analysed for both the osmolality and sodium concentrations. The values obtained ranged from 62 to 196 mmol/kg for osmolality and 9 to 72 mmol/l for sodium in the 34 control subjects. In the 39 cystic fibrosis patients values for sweat osmolality ranged from 220 to 416 mmol/kg and for sodium 60 to 150 mmol/l. When the sweat sodium concentrations and osmolality values were compared for all these subjects, a highly significant degree of correlation was found (Fig. 1). Estimates of the 98% confidence limits for sweat sodium and osmolality values provided a means of clear discrimination between patients with cystic fibrosis and the control subjects when age was taken into account, except for the 15 year to adult age range (Fig. 2). The mean sweat sodium and osmolality values tended to increase with age in control subjects but this was not found in patients with cystic fibrosis. In one control subject, a child with generalised atopic dermatitis, insufficient sweat was obtained for either analysis. The sweat osmolality was measured in eight other infants within the first few weeks of life—four infants aged 4 to 28 days in whom the diagnosis of cystic fibrosis was later supported on clinical grounds and four aged 7 to 50 days in whom the diagnosis was later excluded. Sweat osmolality in these two groups ranged from 255 to 345 mmol/kg and 87 to 123 mmol/kg respectively.

There seemed to be no significant degree of

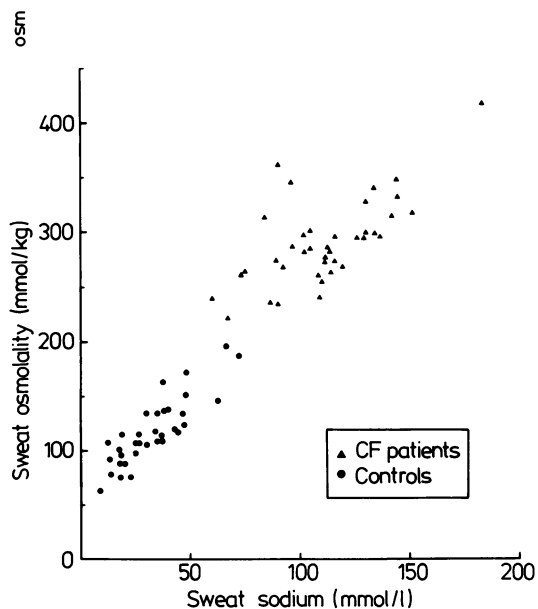


Fig. 1 Comparison of sweat sodium and osmolality values obtained from a single specimen from 39 patients with cystic fibrosis (CF) and 34 controls. Correlation coefficient=0.93.

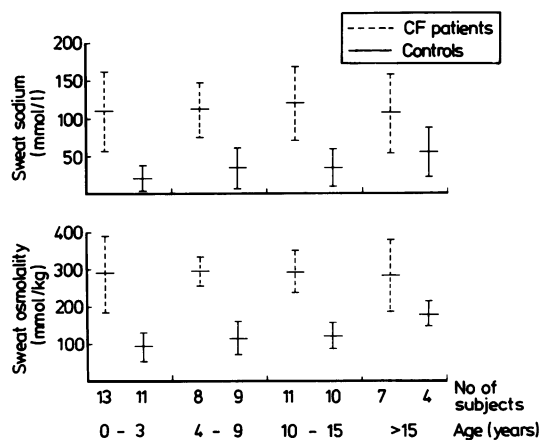


Fig. 2 Range of values for sweat sodium and osmolality (mean (2SD)) found in cystic fibrosis (CF) patients and controls of different ages.

correlation between the sweat osmolality and the rate of sweat production, at least with regard to the cystic fibrosis patients (Fig. 3). Such correlations were not sought in control subjects since age related differences in the sweat osmolality would need to be taken into account.

To determine whether there were variables of

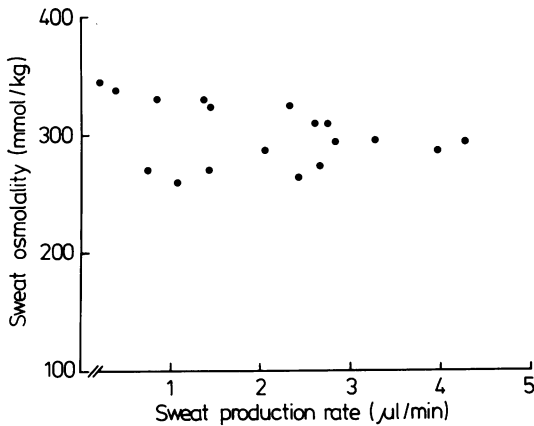


Fig. 3 Relation between sweat production rate and sweat osmolality in 18 patients with cystic fibrosis.

Table Variation in sweat production rate, sodium concentration, and osmolality in two subjects tested on four consecutive days

	Sweat production rate ($\mu\text{l}/\text{min}$)	Osmolality (mmol/kg)			Sodium (mmol/l)		
		Range	Mean	CV (%)	Range	Mean	CV (%)
Normal subject	1.27-2.72	93-102	98	3.9	23-32	28	15.0
Cystic fibrosis patient	1.25-2.11	299-335	310	5.4	112-134	128	8.2

CV=coefficient of variation.

significant magnitude affecting an individual's sweat electrolyte concentration, two subjects (one control and one cystic fibrosis patient) underwent sweat testing on four consecutive days. The results obtained are shown in the Table.

The rate of sweating was also observed in 35 subjects. It took between nine and 25 minutes to collect enough sweat for both the sodium and osmolality estimations, with a mean of 13 minutes and an average rate of sweating of 2 $\mu\text{l}/\text{minute}$.

Discussion

The diagnosis of cystic fibrosis is established on a meticulous clinical history, physical examination, evidence of pancreatic insufficiency, and a raised value of chloride or sodium or osmolality in the sweat. The results of the sweat test must be considered together with the whole clinical findings and it is essential, therefore, that an accurate

technique of collecting and measuring either sweat electrolytes or osmolality, or both, is available. The method of collecting sweat directly into coiled plastic capillary tubing followed by the measurement of sweat osmolality and sodium concentration offer distinct advantages over techniques described previously.

The collection of sweat is straightforward and well tolerated by infants and children. Since the volume of sweat being produced is visualised throughout the procedure, a need for repeat testing is rare. The sweat sample can be transported to the laboratory in the Macroduct, sealed by clamping or some other means to reduce evaporation and contamination. In the present study, although the sweat was collected from patients in a busy outpatient department and the samples transferred to the laboratory for analysis, the results were available to the clinician within an hour of the start of the procedure.

By measuring both the sweat osmolality and a component electrolyte an additional assurance of analytical accuracy is obtained, but the major analytical advantage is derived from the use of osmometry through its minimal requirement for volumetric manipulation. In a number of our subjects duplicate measurements were performed because well over 40 μl of sweat were available for analysis. In one additional normal subject sweat was obtained almost simultaneously from both forearms and analysed for sodium and osmolality. The values were similar for both arms.

Our results indicate that infants and children with cystic fibrosis are clearly identified by the values obtained for their sweat osmolalities and sodium and the extent to which these differ from normal values is the greatest at younger ages. No false positive results were found in children whose medical conditions simulated cystic fibrosis (eg coeliac disease, chronic asthma).

Our results are complementary to those of Kirk *et al*⁶ who collected sweat from their subjects by an electrically heated cup. They mentioned the potential difficulty of maintaining that particular device on the patient's skin for the duration of the sweat collection and implied that it might not be suitable for routine use in infants under the age of 3 months. To keep the time of the sweat collection to a minimum by increasing the sweat production rate, they increased the rate of pilocarpine iontophoresis using a current of 4 mA. Although no additional discomfort seemed to be experienced by their subjects, it is probably advisable, particularly with infants, to keep the applied current to an absolute minimum. In the procedure we describe we have found no evidence that the rate of sweat production affects osmolality appreciably, and using the lower

iontophoresis current we have not experienced difficulties in obtaining sufficient sweat for osmometry in infants during the first few weeks of life. Sweat osmolality values in six infants with cystic fibrosis under four weeks of age were significantly different from the values obtained in four normal infants of comparable ages. Within this particular age range, the test procedure provided a simple and an effective means of confirming the biochemical diagnosis of cystic fibrosis in infants identified initially through a screening programme.⁹

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Correspondence to Dr J A Kuzemko, Department of Paediatrics, Peterborough Hospital, Peterborough.

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