MICRO-DISC ELECTROPHORESIS OF PROTEINS IN PILOCARPINE-INDUCED SWEAT

M.JIRKA

Research Institute of Child Development, Faculty of Pediatrics, Charles University, Prague, Czechoslovakia

1. Introduction

Raunio has used a micromodification of the conventional disc electrophoretic method for analysis of proteins present in low concentration in aqueous humor [1]. The same method has been used in this study to analyse sweat which has a protein concentration of not more than 40–60 mg per 100 ml [2–7]; in previous studies these proteins were analysed by immunoelectrophoresis [8–10].

2. Material and method

Sweat was collected in glass capillaries [11] from both forearms after application of pilocarpine hydrochloride by iontophoresis in normal individuals (babies, children and adults).

Samples were centrifuged and those not analysed immediately were stored at $-20°$. Samples (0.2 ml) were mixed with 10 volumes of ethanol in a centrifuge tube and stored overnight at 4°. The precipitate was collected by centrifugation in the cold and dissolved directly in one twentieth of the original volume of a mixture of polyacrylamide and sacharose solution [12]. A micromodification of the disc electrophoretic method of Ornstein and Davis [12] was performed using glass capillaries 65 mm by 2 mm and a constant voltage of 100 V per 12 polyacrylamide micro-gel columns for 2 hr. Proteins were stained with amido black.

The components of the micro-disc electrophoresis pattern were characterized with specific rabbit anti-sweat protein immune sera [8–10] as follows: The unstained micro-gel column was placed on an agar plate containing one per cent agar dissolved in a veronal buffer of pH 8.6 for immunodiffusion; after standing overnight two parallel troughs for antiserum were cut out at a distance of 0.5 cm from the column. The precipitin lines developed were compared with the localization of bands on the microgel column stained with amido black.

3. Results

This electrophoretic technique separated six major protein components in sweat localized to prealbumin (A,B), albumin (C), "fast" $alpha_2$- (D), "slow" $alpha_2$- (E) and gamma-globulin (F) zones (fig. 1). In addition, one or two prealbumin components were usually observed (fig. 1 marked with arrows). By immunochemical techniques the chief components of the micro-disc electrophoresis pattern of sweat appeared to correspond to an $alpha_2$-glycoprotein present also in serum (D) and $alpha_1$- (A,B,C) and $alpha_2$-globulins (D) of glycoprotein character having no counterpart in serum (fig. 2).

No striking differences in the micro-disc electrophoresis patterns were observed in normal individuals of different age, but protein concentrations seem to be lower in sweat of babies and children in comparison with adults.

4. Discussion

The character of proteins in human sweat is not

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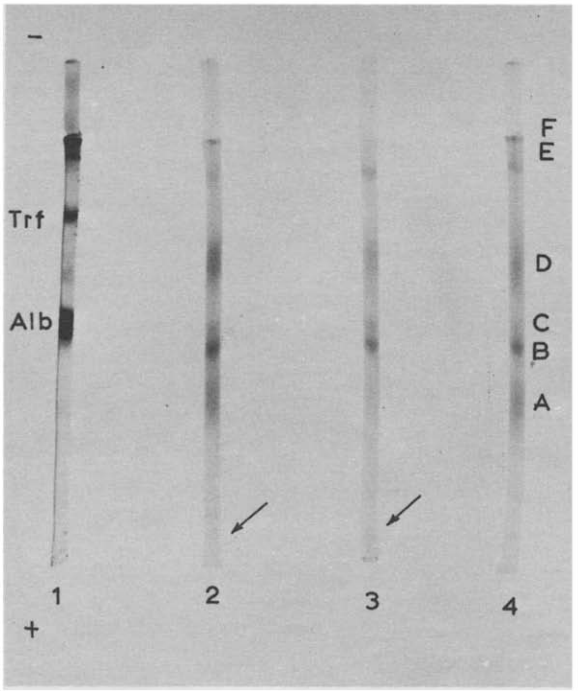

Fig. 1. Micro-disc electrophoresis of sweat proteins. 1, normal serum (0.01 ml of a twenty fold diluted sample); 2-4, pilocarpine-induced sweat of normal children aged 3–8 years (0.01 ml of twenty fold concentrated samples). Alb = albumin, Trf = transferrin (see text).

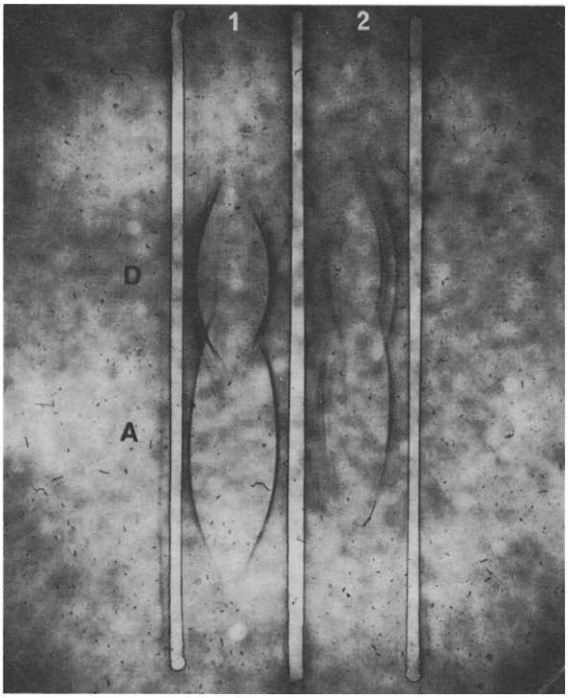

Fig. 2. Immunoprecipitation of the sweat protein electrophoretic components. 1, thermal sweat; 2, pilocarpine-induced sweat. Rabbit immune serum against sweat proteins absorbed with normal serum 5:1 (in the center trough) and diluted with saline 5:1 (in the side-troughs). A–D: Approximative localization of protein components in agar gel corresponding to that in polyacrylamide micro-gel columns (see fig. 1).

yet known in detail; they are mostly muco- or glycoproteins, some of which are probably of non-serum origin [2–7]. According to our findings on immunochemical behaviour of sweat proteins using specific rabbit anti-sweat protein immune sera [8–10], the major immunoelectrophoretic components of thermal as well as pilocarpine-induced sweat are serum Zn-alpha$_2$-glycoprotein and albumin, and the "specific" alpha$_2$- and alpha$_1$-globulin of glycoprotein character.

The major fractions of the micro-disc electrophoresis pattern of proteins in thermal as well as in pilocarpine-induced sweat appeared to correspond to these same components. Some may represent polymorphic forms of sweat glycoproteins. Artefacts occur with some native as well as denaturated proteins when the conventional disc technique is used [13–15]; however pre-running the gel without samples (to remove catalyst residues) did not alter the character of micro-disc electrophoresis pattern of sweat proteins.

The reproducibility of the ethanol precipitation method for concentration of protein samples was found very good without evident signs of protein denaturation; no differences were observed in samples concentrated by dialysis and evaporation.

Micro-disc electrophoresis is a sensitive method for the analysis of protein components in small amounts of sweat obtained by local application of pilocarpine. It might be useful for the clinical study of mucoviscidosis and other disorders associated with anomalies in sweat secretion.

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