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Electrophoresis of human salivary secretions at acid pH

Electrophoretic methods have been extensively used in investigations concerning the protein composition of human salivary secretions. Thus, paper[1,2], polyacrylamide[3,4], cellulose acetate[5], paper curtain[6,7], immunoelectrophoresis[8], and more recently iso-electric focusing[9] have been shown to be valuable tools in the separation and identification of proteins from parotid and submandibular, as well as mixed saliva. An excellent review on the subject has been published[10]. Polyacrylamide electrophoresis has also been utilized in an attempt to determine abnormal protein patterns in certain pathological conditions such as cystic fibrosis[11] and osteoporosis[12]. All of the above-mentioned studies have been carried out at alkaline pH, although in one case[3], an attempt to separate the cathodal components of submaxillary saliva was made by reversing the electrodes, a theoretically objectionable procedure. Thus, although the behavior of human salivary proteins in electrophoresis above pH 7 has been studied extensively, their behavior under acid conditions has not yet been investigated and is therefore poorly understood. This report describes the use of acidic, 6 *M* urea polyacrylamide gels for the separation of parotid and mixed human saliva.

Materials and methods

Human salivary secretions. Human parotid fluid was collected from healthy volunteers using a modified stainless-steel Carlson–Crittenden vacuum cup[13] at a flow rate of 1.09 ml/min with a mean protein concentration of 2.90 mg/ml. Mixed human saliva was obtained from healthy laboratory personnel in a cold room (2°) by expectoration into a vessel containing thymol crystals in order to inhibit bacterial growth. All samples were stored at 2° and processed no later than 2 h after collection. The saliva was then cleared by centrifugation at 48,200 × *g* for 30 min and the supernatant fluid lyophilized and stored at −40° until used.

Sample preparation. Samples were prepared by dissolving a given amount of lyophilized saliva (50–100 mg) in 1.0 ml of 0.1 *M* Tris–citric acid buffer, pH 3.8 containing urea in 4 *M* concentration; insoluble proteins were then removed by centrifugation (27,000 × *g* 30 min, 2°) and the supernatant fluid utilized for the electrophoretic analysis. It was found necessary to use only freshly dissolved samples as further precipitation occurred, especially with parotid fluid, during prolonged storage (20 h or more).

Electrophoresis. Electrophoresis in acidic, 6 *M* urea polyacrylamide gels was carried out as previously described[14], but modified in order to obtain the following experimental conditions: voltage, 250 V (constant); current, 100 mA; pre-run, 4.0 h; electrolyte, 0.37 *M* glycine–citric acid buffer, pH 2.9; load, 25–75 μl (approximately 0.90 mg protein); separations were carried out for 5–6 h at room temperature but coolant (4°) was circulated through the cooling plates at all times. In all instances a 14.0 % Cyanogum-41 gel was used.

The gels were stained for 24 h with Amido Schwarz and destained electrophoretically (40 min) with a mixture of methanol–acetic acid–water (5:1:5).

Results and discussion

Excellent resolution of human mixed (20 zones)and parotid (17 zones) saliva can be achieved under the present conditions of electrophoresis (Fig. 1); furthermore, there is no problem in identifying the origin of each particular secretion, mainly because parotid and whole saliva differ drastically not only in the number but also in the concentrations of the basic components migrating faster than lysozyme.

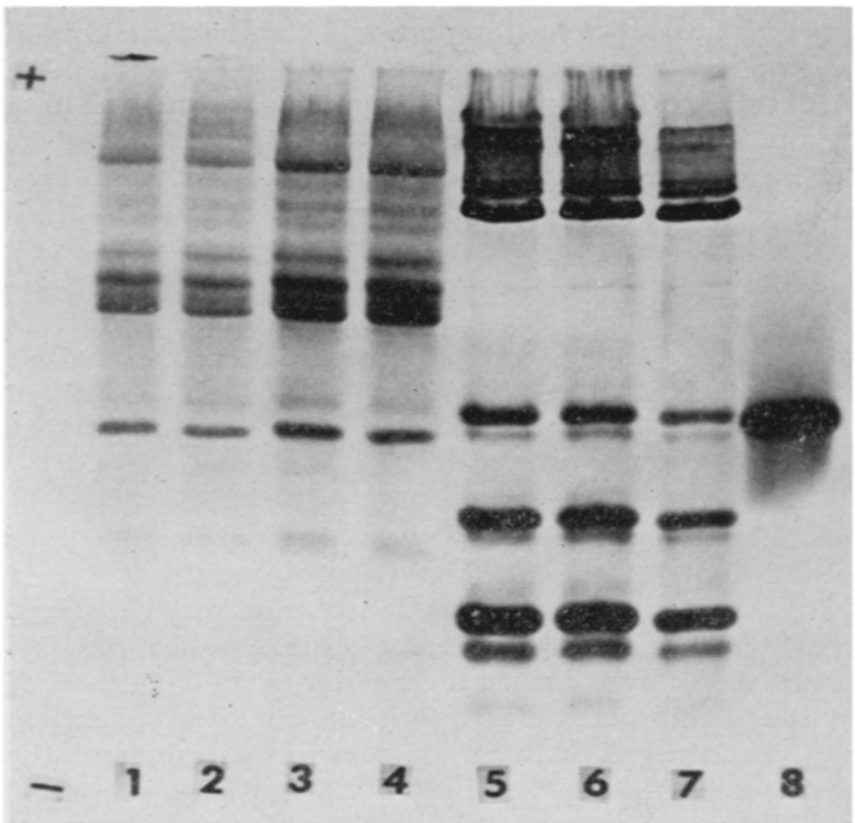

Fig. 1. Polyacrylamide electrophoresis of human salivary secretions at acid pH in the presence of 6 *M* urea. Channels 1 and 3 duplicate samples (25 and 50 μl) of whole human saliva from a 28-year-old normal male; channels 2 and 4 (25 and 50 μl) of whole saliva from a 26-year-old normal female. Channels 5, 6 and 7 parotid fluid from three different normal individuals. Channel 8, lysozyme marker.

It is of great interest to note that the lysozyme zone (see arrow, Fig. 2) can also be used to differentiate mixed from parotid saliva. Two zones, differing in concentration, are seen to migrate at the level of hen's egg white lysozyme. In mixed saliva, the band appearing in greater concentration has a mobility identical to lysozyme, while in parotid fluid the same zone has a slightly lower mobility. The reverse is true for the less concentrated protein band. At this time no explanation can be given for this phenomenon, but it is likely that these bands represent two distinct salivary lysozymes. MILLIN AND SMITH[15] reported the existence of three chromatographically

distinct amylases in human saliva. We have obtained identical results using parotid lysozyme isolated by the method of BALEKJIAN *et al.*[16] as a standard in lieu of hen's egg white lysozyme. With the exception of lysozyme[16] little is known about the basic proteins in human salivary secretion. It is of interest to note, in this regard, the presence of several very basic proteins in both parotid and mixed saliva which are seen to migrate at faster rates than lysozyme (see Fig. 1). These basic proteins constitute less than 1 % (0.86) of the total protein in parotid fluid and are characterized[17] by having isoelectric points above pH 11.0 (range, 11.0–12.6) and very small molecular weights (range, 3256–8500) and have been partially purified by adsorption chromatography on highly cross-linked polyacrylamide gel[18], dialysis and ion-exchange chromatography on carboxymethyl cellulose. The human parotid basic proteins are significant from an evolutionary standpoint since similar proteins have been isolated from the venom gland secretion of several species of North American Crotalid snakes[19].

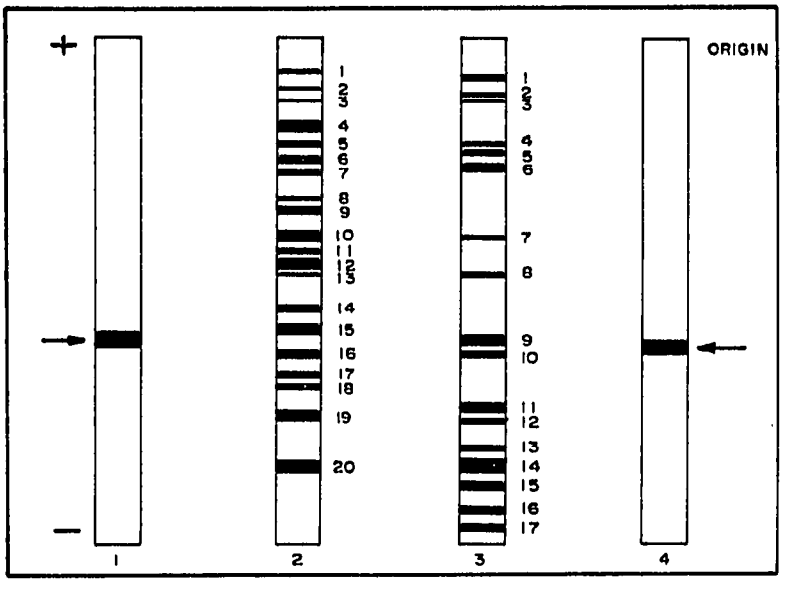

Fig. 2. Schematic representation for the electrophoretic separation of human salivary secretions under the conditions described in the text. Channels 1 and 4 represent the lysozyme marker (arrows). Channel 2, location of 20 protein zones in whole saliva, five of which (15–20) lose most of their intensity upon destaining. Channel 3, location of 17 protein zones in parotid fluid, two of which (16 and 17) are lost during extensive destaining. The bands with lysozyme-like mobility referred to in the text correspond to 14 and 15 in whole saliva and 9 and 10 in parotid fluid. Bands 13–17 in parotid fluid (channel 3) constitute the small-molecular-weight basic proteins described in the text.

Since some of the weaker components are lost when transcription of the gel pattern to the photograph is made, a schematic representation of the electrophoretic separation is shown in Fig. 2. In summary, as shown in Figs. 1 and 2, polyacrylamide electrophoresis in acidic 6 *M* urea can become a very useful tool for the study of the basic proteins and polypeptides in human salivary gland secretions.

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