Sodium Transport: Inhibitory Factor in Sweat of Patients with Cystic Fibrosis

Abstract. *A factor inhibitory to sodium transport exists in the sweat of patients with cystic fibrosis of the pancreas. When the duct system of the rat parotid was perfused with sweat from patients, marked inhibition of sodium reabsorption was observed. Perfusion with sweat from normal subjects caused no change in sodium reabsorption. The factor thus demonstrated may be responsible for the increased sodium concentrations in the sweat of patients with cystic fibrosis.*

The sweat of patients with cystic fibrosis of the pancreas has higher concentrations of sodium and chloride than the sweat of normal children of the same age and sex (*1*). This abnormality is the most characteristic and constant symptom of the disease and most valuable in diagnosing it. The nature of this defect has remained unknown mainly because little direct information about the function of the human sweat gland has been available. In recent years, however, significant progress has been made in this field.

By using micropuncture and microanalytical techniques, Schulz and her co-workers (*2*) demonstrated that in the secretory coil of the human sweat gland a primary secretion with osmolality and concentrations of sodium, potassium, and chloride similar to those of plasma is produced. As this fluid passes through the duct of the gland it is made hypotonic by reabsorption of sodium chloride in excess of water. Recently, Schulz and Peter (*3*) showed that, like the primary secretion of the sweat gland in normal subjects, that of patients with cystic fibrosis has a composition similar to that of plasma. However, the final sweat of single sweat glands of these patients has higher osmolality and sodium and chloride concentrations than the sweat of normal subjects. Apparently, this abnormality is the result of a defect in the reabsorption of sodium chloride by the duct of the sweat gland. However, the sweat gland in cystic fibrosis is remarkably free of gross morphologic changes (*4*) which might account for the dysfunction of the gland. In addition, numerous enzymes in sweat gland tissue isolated from patients with cystic fibrosis and normal subjects do not differ in activity (*5*). Furthermore, with respect to the activity of the major enzyme involved in sodium transport, a Na^+- and K^+-activated adenosinetriphosphatase that is sensitive to ouabain, the sweat glands of patients with cystic fibrosis and those of normal subjects do not differ (*6*).

In view of the absence of demonstrable anatomical or enzymatic defects in the sweat glands of patients with cystic fibrosis, we propose that the sweat abnormality in this disease may be due to a factor (or factors) that inhibits sodium reabsorption in the sweat glands. If such a factor were secreted in the primary fluid produced in the secretory coil of the sweat glands, it would decrease the reabsorption of sodium in the duct and thus cause the increased sodium concentrations of the final sweat of patients with cystic fibrosis. To test this hypothesis, we perfused the duct system of the rat parotid gland with sweat from patients and controls and studied the effects of this retrograde perfusion on the sodium excretion in the parotid saliva of the rat. In a previous micropuncture study (*7*) we demonstrated that the duct system of the rat parotid is composed of three functionally different segments: the acini-intercalated ducts, striated ducts, and excretory ducts. The acini-intercalated ducts secrete a primary fluid with plasma-like osmolality and sodium and potassium concentrations; the striated ducts modify the primary fluid by reabsorbing sodium in excess of water, thus producing hypotonicity; and the excretory ducts convey the saliva to the mouth. We also demonstrated (*8*) that retrograde perfusion of the duct system with Ringer's solution or 0.154*M* NaCl solution does not disrupt the function of the gland. Using this technique, we were able to introduce different substances into the various duct segments of the gland and study their effects. Ouabain in concentrations of 10^{-3} mole/liter partially inhibited sodium reabsorption in the striated ducts. Sodium cyanide, an inhibitor of cell respiration, abolished the sodium reabsorption in these ducts.

The subjects with cystic fibrosis were among those followed in the Cystic Fibrosis Clinic of the University of Wisconsin at Madison. They all had the symptoms and signs of the disease, and the diagnosis was confirmed by the finding that there were increased sodium and chloride concentrations in their sweat. The control subjects were healthy children unrelated to those with cystic fibrosis but of the same age and sex. They had normal sodium and chloride concentrations in their sweat. Sweat

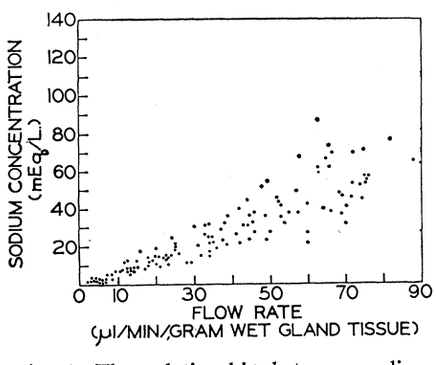

Fig. 1. The relationship between sodium concentration and flow rate in the parotid saliva of ten rats. The parotids of these animals did not undergo retrograde perfusion before stimulation with pilocarpine.

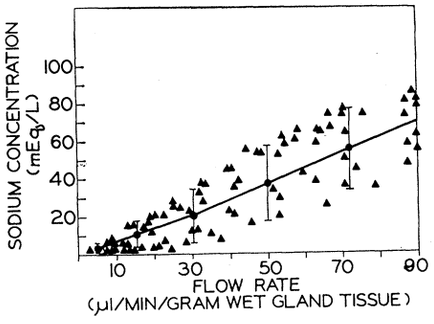

Fig. 2. The relationship between sodium concentration and flow rate in the parotid saliva of ten rats. The parotids of these rats underwent retrograde perfusion with sweat from control subjects. The dark circles represent the mean values of the points in Fig. 1; the vertical lines represent plus or minus two standard deviations.

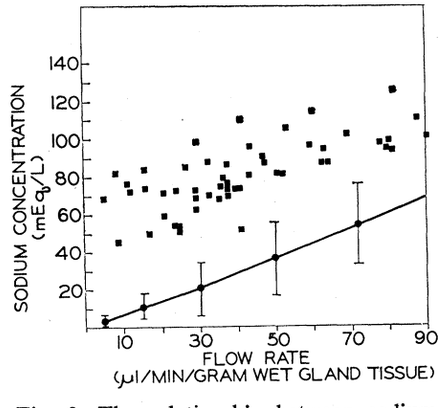

Fig. 3. The relationship between sodium concentration and flow rate in the parotid saliva of eight rats. The parotids of these rats underwent retrograde perfusion with sweat from patients with cystic fibrosis. Dark circles and vertical lines as in Fig. 2.

was collected from the skin of the scapular area of patients and control subjects during thermal stimulation of the sweat glands. Because the sodium concentrations of the sweat from patients and controls were different, the sodium concentrations of all solutions used for retrograde perfusion of the rat parotid were corrected to 140 to 145 mEq/liter by addition of proper amounts of NaCl. The methods of preparation of the rats and retrograde perfusion of the parotids have been described previously (*7, 8*). Male albino rats of the Sprague-Dawley strain were used. Sodium concentrations in samples of rat parotid saliva (1 to 5 μl) were measured with a flame photometer (Instrumentation Laboratories) with an internal lithium standard.

Following stimulation of the rat parotid with pilocarpine, the sodium concentration of the saliva was related to the salivary flow rate (Fig. 1). At low flow rates, the gland excretes saliva with very low sodium concentrations. At progressively higher flow rates, the salivary sodium concentration gradually increases. This relationship is accounted for by the limited sodium reabsorptive capacity of the striated ducts of the rat parotid gland (*7*). As the production of a plasma-like primary fluid increases, the sodium reabsorptive mechanism becomes saturated and the salivary sodium concentration increases. When the duct system of the gland was perfused with sweat from normal children, there was no effect on the reabsorption of sodium (Fig. 2). When sweat from patients with cystic fibrosis was used for the retrograde perfusion, there was a marked increase in the salivary sodium concentration at all flow rates (Fig. 3). Since the rat parotid gland reabsorbs sodium mainly in the striated ducts, it may be concluded that the sweat of patients with cystic fibrosis contains a factor (or factors) that inhibits sodium transport in the striated ducts of the rat parotid.

Although the exact nature of this factor is not yet clear, we have determined some of its physicochemical properties. The factor is heat labile; heating of the sweat at 100°C for 5 minutes resulted in the disappearance of the sodium transport inhibitory activity. This activity also disappeared when the sweat samples were frozen and thawed. Storage of the sweat at 4°C for 24 hours caused a reduction in the activity by 30 to 40 percent. The activity remained in the dialysis chamber when the sweat was dialyzed against 0.154*M* NaCl solution at 4°C for a period of 3 hours.

We propose the following mechanism that may be responsible for the sodium transport abnormality in cystic fibrosis: The secretory process of the sweat gland is normal, and a plasma-like primary fluid is produced in the secretory coil of the gland; this fluid, however, contains the sodium transport inhibitory factor. When the primary fluid reaches the duct of the gland, the site of production of hypotonicity, this factor inhibits sodium reabsorption; thus, the final sweat emerges from the sweat pore with a sodium concentration higher than normal. It is hoped that final identification of this factor may lead us closer to the detection of the molecular defect of cystic fibrosis.

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Antennae and Sexual Receptivity in Drosophila melanogaster Females

Abstract. *For the female to be normally responsive to the display of wing vibrations by males, the arista and funiculus of the female's antenna must be intact and able to move freely. The arista probably acts as a sail, twisting the funiculus and thus stimulating units of Johnston's organ at its base.*

Female *Drosophila* whose antennae have been removed mate less readily than normal females do. The wing vibrations, which are a conspicuous element in the courtship of many *Drosophila* species, may serve to waft a current of scented air over the female's antennae; that there are air-current receptors at the antenna's base has been suggested (*1*). Petit (*2*) made a more detailed analysis of the sense organs involved by amputating different antennal segments in *D. melanogaster*.

The *Drosophila* antenna has three main segments (*3*): the basal scaphe; the pedicel, containing Johnston's organ; and the distal flagellum or funiculus, attached to the pedicel by a stalk whose movements can stimulate the elements of Johnston's organ directly. Projecting from the outer margin of the funiculus near its proximal end is the branched arista, which together with small sclerites at its base probably represents the three reduced terminal segments of the antenna.

Petit (*2*) measured the sexual receptivity of females after four different operations: (i) removal of both antennae, (ii) removal of one antenna, (iii) removal of the funiculus and arista on both sides, and (iv) removal of the arista on both sides. The control value for receptivity was 88 percent; that of the operated groups was (i) 8 percent, (ii) 62 percent, (iii) 30 percent, and (iv) 43 percent, respectively. She concluded that the most important receptors were the arista, "receptor for tactile and chemical stimuli," and Johnston's organ, "the vibration receptor."

Petit did not watch courtship but scored the number of inseminated females after a 48-hour confinement with males. Since one can tell whether a female is receptive or not within 15 minutes of her being courted, this technique has drawbacks if one wishes to record different degrees of reduced receptivity. Further, after long periods of confinement with males, forced matings are common. My experiments avoid these difficulties and provide further evidence on the way in which the antennal sense organs operate in the perception of courtship stimuli.

Receptivity was measured by direct observation of single pairs of flies in small observation cells. More than 95 percent of females either accept males within 15 minutes of courtship (that is, are receptive) or are unreceptive and refuse to accept for long periods. In practice 30 minutes of more or less continuous courtship is a suitable criterion (*4*).

The same operations as in Petit's experiments were performed on females anesthetized with ether on days 2 or 3