MORPHO–PHYSIOLOGICAL FEATURES DISTINGUISHING GLANDS PRODUCING A HYPOTONIC SECRETION

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The large salivary glands of mammals are capable of producing a secretion with a lower osmolar concentration than the blood plasma [1]. However, the mechanism of the process of secretion of a hypotonic saliva has not been adequately studied. Earlier attempts to analyze the evolution of the osmoregulatory systems have shown that hypotonic fluids are formed in osmoregulatory organs by the active absorption of sodium salts through the wall of the renal tubules, impermeable for osmotically bound water [3]. The results of histochemical investigations have shown that the cells of the tubules of the nephron where the reabsorption of sodium takes place are characterized by high activity of certain respiratory enzymes and, in particular, of succinate dehydrogenase [7, 8], and their impermeability for the movement of water along an osmotic gradient is due to the presence of solid mucopolysaccharides of the hyaluronic acid or chondroitin sulfate type in the wall of the tubules [2, 5, 6]. Information about the presence and distribution of acid mucopolysaccharides and oxidative enzymes in the salivary glands is extremely limited [14-16].

Because of these circumstances, in the present investigation a more detailed histochemical analysis was made of the salivary glands of mammals secreting hypotonic saliva. For comparison the lacrimal and mammary glands, whose secretion is isotonic with the plasma, were also investigated.

EXPERIMENTAL METHOD

The investigation was conducted on the submandibular and sublingual salivary glands, lacrimal glands, and mammary glands of adult albino rats and the parotid salivary glands of puppies aged 6, 14, and 20 days. Acid mucopolysaccharides were detected with alcian blue by Steedman's method, by Hess and Hollaender's reaction of metachromasia, and with colloidal iron by Hale's method [12]. As an enzyme control, parallel sections were treated with testicular hyaluronidase (Reanal). The activity of succinate, α-ketoglutarate, isocitrate, and malate dehydrogenases was detected histochemically by the reaction with neotetrazolium using a modification of Farber's method [9], and by the reaction with nitro-blue tetrazolium by Nachlas's method [12].

EXPERIMENTAL RESULTS

Acid mucopolysaccharides were located in the investigated salivary glands in the cytoplasm of the cells of the secretory portions and in the connective-tissue stroma of the glands. They were found in the ground substances of the connective-tissue membrane of the interlobular ducts, and were seen particularly clearly in the basement membranes of the salivary ducts (Fig. 1). The basement membranes of the alveoli and of the intervening portions did not contain solid mucopolysaccharides. Judging by the fact that the mucopolysaccharides in the stroma of the salivary glands stained clearly with alcian blue, colloidal iron, and the metachromasia reaction and were sensitive to the action of testicular hyaluronidase, they were compounds of the hyaluronic acid or chondroitin sulfate type.

The results of the investigation of the activity of the dehydrogenases in the salivary glands showed that dehydrogenation of succinate and α-ketoglutarate took place most intensively in the cells of the interlobular ducts, while the reaction for succinate dehydrogenase was particularly clear in the cells of the salivary ducts (Fig. 2). Meanwhile, in the cells of the alveoli and intervening portions, these enzymes were almost completely absent. The intensity of dehydrogenation of malate and isocitrate was equal and relatively low in the cells of the alveoli and interlobular ducts (see table).

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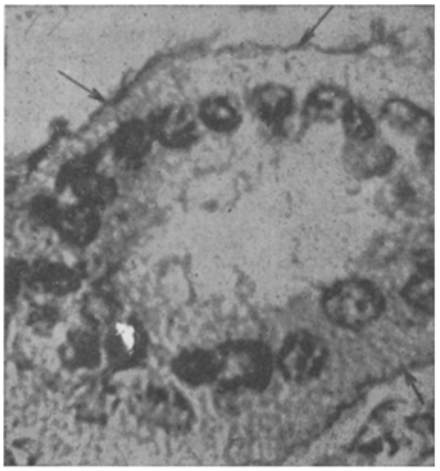

Fig. 1. Acid mucopolysaccharides in the basement membrane of a salivary duct of the sublingual gland of a rat. Hale's histochemical reaction. Photomicrograph, 40 × 12.5.

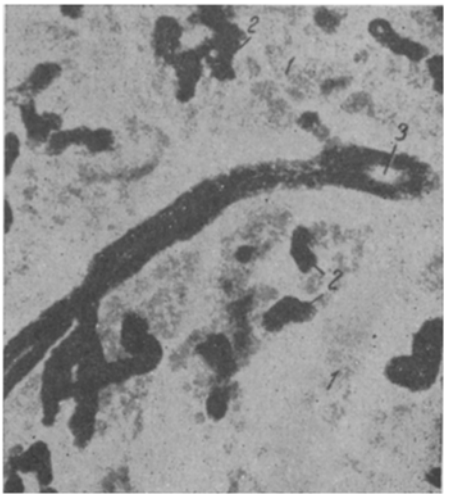

Fig. 2. Reaction for succinate dehydrogenase in the cells of the sublingual salivary gland of a rat. 1) Secretory portions; 2) salivary ducts; 3) interlobular ducts. Photomicrograph, 3.5×12.5.

Activity of Dehydrogenases in the Cells of Different Parts of the Submandibular and Sublingual Salivary Glands

Substrate of action of dehydrogenases	Portions of salivary glands			
	alveoli	intervening portions	salivary ducts	interlobular ducts
Succinate	±	±	+++	++
α-Ketoglutarate	±	±	++	+++
Isocitrate	+	+	+	+
Malate	+	+	+	+

Note. The "+" sign denote the degrees of intensity of dehydrogenation of the investigated substrates.

The differences in the activity of the various dehydrogenases in the cells of different parts of the salivary glands may probably be explained on the grounds of variations in the oxidative metabolism of cells specialized for transport of particular cations [10, 11]. The results of the investigation of the osmoregulatory organs of the vertebrates have shown that maximal succinate dehydrogenase activity is always found in the cells of the tubules, where active aerobic transport of sodium takes place against an osmotic or chemical gradient [7, 8]. Possibly the high succinate dehydrogenase activity in the salivary ducts also indicates that in this part of the gland intensive absorption of sodium salts takes place. This is in agreement with modern views of the mechanism of saliva formation [18].

The saliva formed in the acini is initially isotonic with the plasma; in the interlobular ducts the sodium is partly absorbed and the saliva becomes hypotonic. Since the maximal hypotonicity of the saliva is attained in glands with maximal development of the salivary ducts, it has been postulated [17], that these portions of the ducts are the site of reabsorption of sodium.

The importance of the salivary ducts in the production of hypotonic saliva was revealed by the results of an investigation of succinate dehydrogenase activity in the parotid glands of puppies in early ontogenesis. The glands of puppies at the age of 6 days do not yet contain differentiated salivary ducts and the succinate dehydrogenase activity in them was very low. At this age, they secrete a saliva isotonic with the blood plasma. The ability to produce hypotonic saliva does not appear until the second week of life in the puppy. By this time, the salivary ducts were clearly differentiated in the glands and the activity of the enzyme in the ducts has increased considerably.

The results of a histochemical investigation of the lacrimal and mammary glands carried out for comparison showed that acid mucopolysaccharides were present in the ground substance of the connective-tissue stroma of these

glands only in very small amounts. The basement membranes both of the secretory portions and of the ducts of these glands did not contain acid mucopolysaccharides. The cells of the alveoli and ducts of the lacrimal and mammary glands, unlike those of the salivary glands, were identical as regards dehydrogenase activity. In the lacrimal gland the intensity of dehydrogenation of all the investigated substrates, including succinate, was low. Conversely, in the mammary gland the succinate dehydrogenase activity was fairly high in all parts.

The secretion products by the lacrimal gland contains practically as much sodium as the plasma [13]. The sodium concentration in the milk is much less than in the plasma, for in the process of milk formation some of the sodium is actively absorbed [4]. This may account to some extent for the high succinate dehydrogenase activity in the mammary gland. The absence of acid mucopolysaccharide in the walls of the alveoli and ducts of the lacrimal and mammary glands accords well with the iso-osmotic character of the secretion of these glands. The histochemical analysis thus showed that the distinguishing features of glands producing a hypotonic secretion are high activity of certain oxidative enzymes in the cells of those parts where active absorption of sodium takes place, and the presence of acid mucopolysaccharides around these portions, evidently making them impermeable for osmotically bound water. These same histochemical properties are shared by the renal tubules, which excrete hypotonic urine. It is possible that this histochemical similarity between different organs producing hypotonic secretions or excretions is determined by the close similarity between the physiological mechanisms of formation of the hypotonic fluids in these organs.

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