

Original articles

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EPIDERMAL GROWTH FACTOR (EGF) EXPRESSION IN HUMAN SALIVARY GLANDS. AN IMMUNOHISTOCHEMICAL STUDY

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Epidermal growth factor (EGF) is a biologically active peptide involved in differentiation, growth, regeneration and repair of human and animal tissues. Quantitative biochemical studies showed in man the highest concentration of EGF in the parotid gland. The aim of the present study was to define EGF immunolocalization in the individual segments of the human major salivary glands (salivon). The material consisted of sections obtained from the surgically removed salivary glands: parotid, submaxillary and sublingual. Immunohistochemical studies were performed by PAP method using monoclonal antibody against human epidermal growth factor. EGF expression was found almost exclusively in the efferent pathways of the salivary glands, mostly in the intercalated ducts and Pflüger salivary tubules. These segments of the salivon are most developed in the parotid gland in which the staining was stronger than in other salivary glands.

Key words: *epidermal growth factor (EGF), salivary gland, salivon, immunolocalization.*

In the last 30 years a number of biologically active peptide growth factors have been isolated. They play a major role in differentiation and growth of tissues and organs (1, 2).

An epidermal growth factor (EGF) was first isolated by Cohen from the submaxillary salivary glands in male mice. In 1975 Gregory isolated from human urine a polypeptide strongly inhibiting gastric secretion and called it beta urogastron. It was found that the epidermal growth factor in mice and human urogastron were homologous substances and that urogastron may be considered as human EGF. EGF is synthesized as a precursor protein composed of 1207 aminoacids with low biological activity. An active form of EGF contains 53 aminoacids (1).

The EGF has been found in many organs. It is present in the alimentary tract: in the salivary glands, Brunner's glands, Paneth's cells, pancreas as well as in the renal tubules, brain, lungs, skin, breast, prostate and thyroid (1). A high concentration of EGF in man has been also found in the prostatic and seminal secretion, in sweat and tears (1—6).

Since the salivary glands are a significant source of EGF, its expression and cellular localization in these glands is obviously interesting, especially that there are only few data available regarding localization of EGF in the individual segments of the salivon.

The purpose of this study was to define the immunolocalization of EGF in the individual segments of the salivon of human salivary glands: parotid, submaxillary and sublingual.

MATERIAL AND METHODS

The material consisted of 33 sections obtained from normal human salivary glands: parotid, submaxillary and sublingual, removed surgically during operations for neoplasms of the oral cavity, larynx and parotid glands. Only histologically normal salivary glands were chosen and those distant from neoplastic infiltration. Immunohistochemical studies of the paraffin sections were performed by using PAP method. A monoclonal antibody against human epidermal growth factor (EGF r-UG recombinant DNA process-Hitachi Chemical Co.) produced by fusion of spleen cells from Babl/c mouse immunized with rEGF with myeloma SP 2/0 was used (7). The primary antibody diluted 1:10 was applied to the sections at room temperature for 30 min. Then, after washing the link antibody the PAP complex (DAKO) was applied for 20 min each. Carbazol (3-amino-9-ethylcarbazol: AEC) was used as a chromogen. The tissue was counterstained with Mayer's hematoxylin and mounted in glycerogelatin. Distribution of EGF in respective morphologic segments of the salivon was evaluated under light microscope by 3 investigators.

RESULTS

The Parotid Gland (*Fig. 1*)

EGF was strongly expressed in the distal parts of the salivon i. e. cytoplasm of the intercalated duct epithelial cells, striated ducts, intra- and interlobular and main ducts, the expression of EGF was strong and of similar intensity in all segments (*Fig. 2, 3*). In addition, EGF was found sporadically in the

secretion present in the lumen of the intralobular ducts (*Fig. 4*). EGF was absent in the serous cells forming serous vesicles and within the mucous

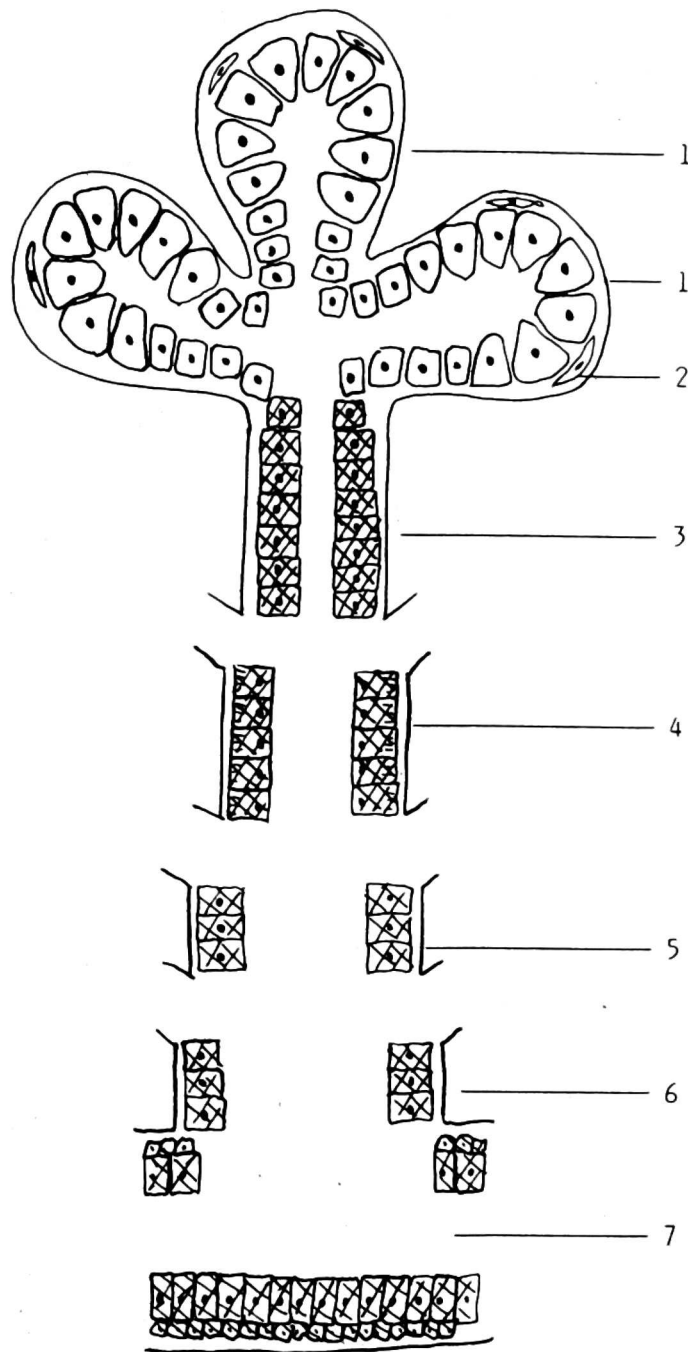


Fig. 1. The presence of the epidermal growth factor (EGF) in the parotid gland — a diagram. Squared fields corresponds to EGF expression. 1 — serous vesicle, 2 — myoepithelial cell, 3 — intercalated duct, 4 — striated duct, 5 — intralobular duct, 6 — interlobular duct, 7 — main duct.

cells forming mucous tubules which are very rare in this gland. The myoepithelial cells forming a basket on the serous vesicles did not contain the EGF, either.

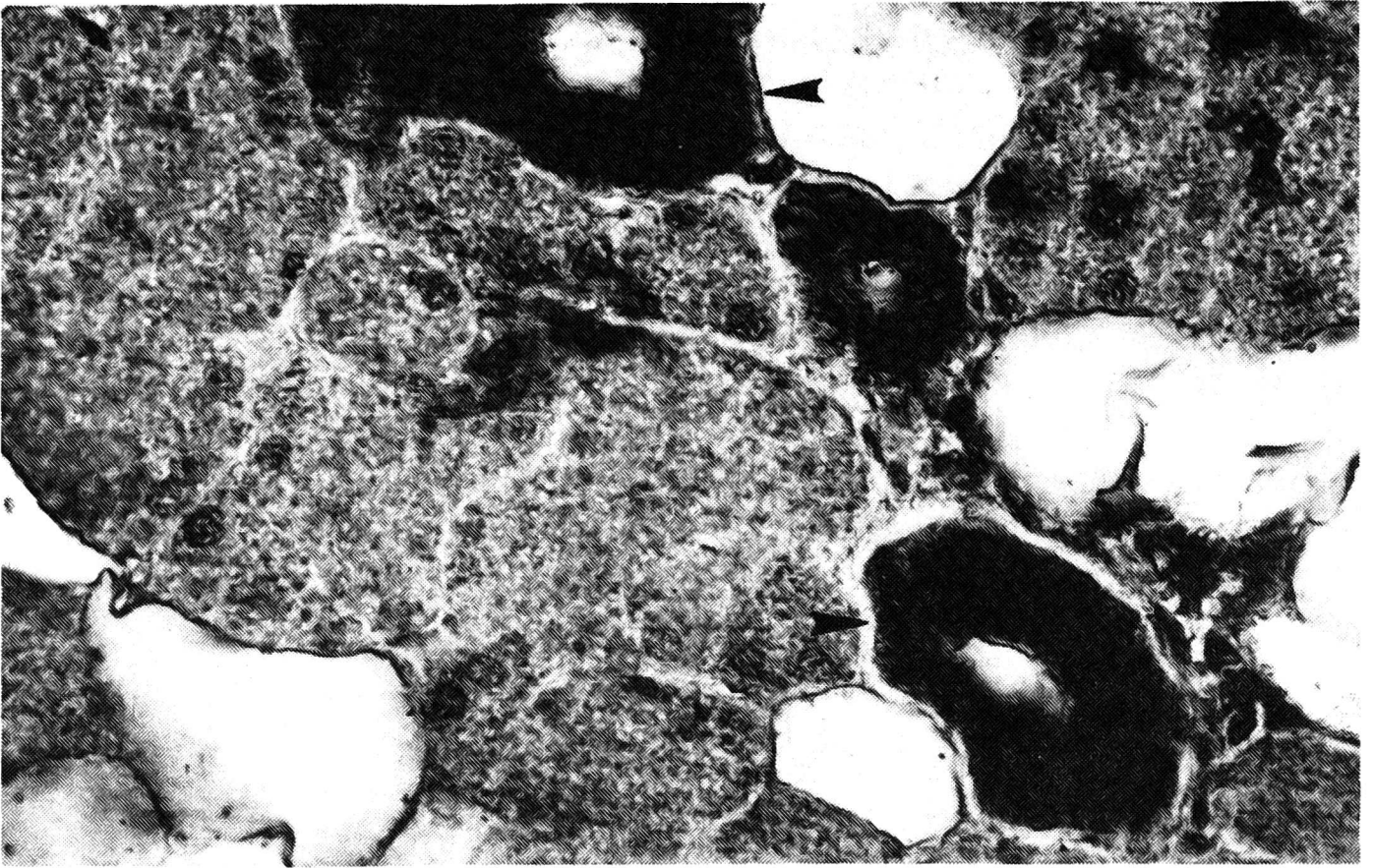


Fig. 2. EGF in the parotid gland. A positive reaction in the epithelium of the intercalated duct (arrow). PAP, hematoxylin counterstain, Magn. 450x.

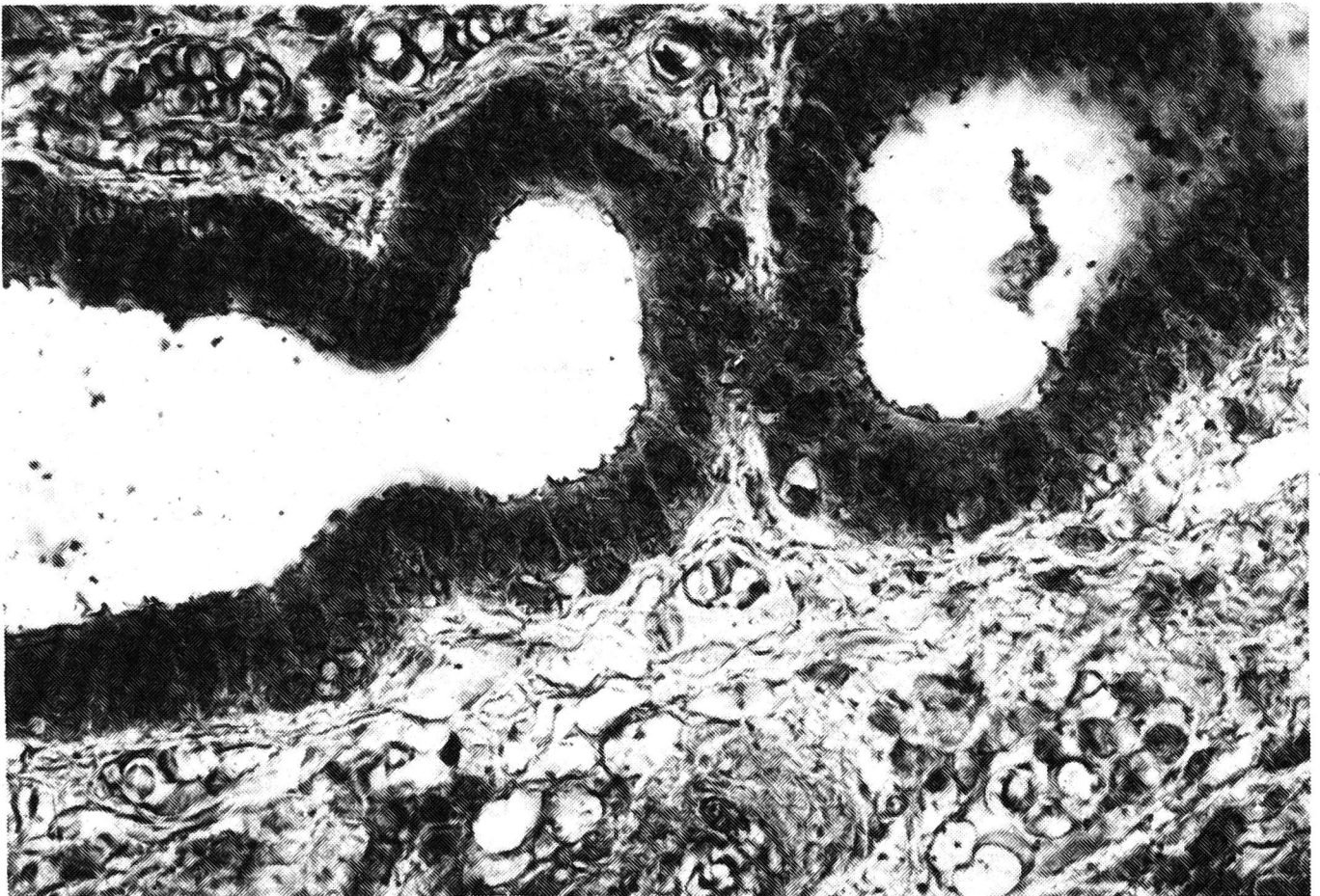


Fig. 3. EGF in the parotid gland. A positive reaction in the epithelium of the interlobular ducts. PAP, hematoxylin counterstain, Magn. 450x.

The Submaxillary Gland (*Fig. 5*)

The expression of the EGF in the submaxillary glands was slightly weaker than that in the parotid glands. The immunostaining for EGF was present in the main ducts, intra- and interlobular ducts, striated ducts and intercalated ducts, whereas neither the serous vesicles nor mucous tubules showed the presence of EGF. Only in sporadic mucous tubules containing serous caps, EGF was found in the serous cell cytoplasm forming the demilunes (*Fig. 6*).

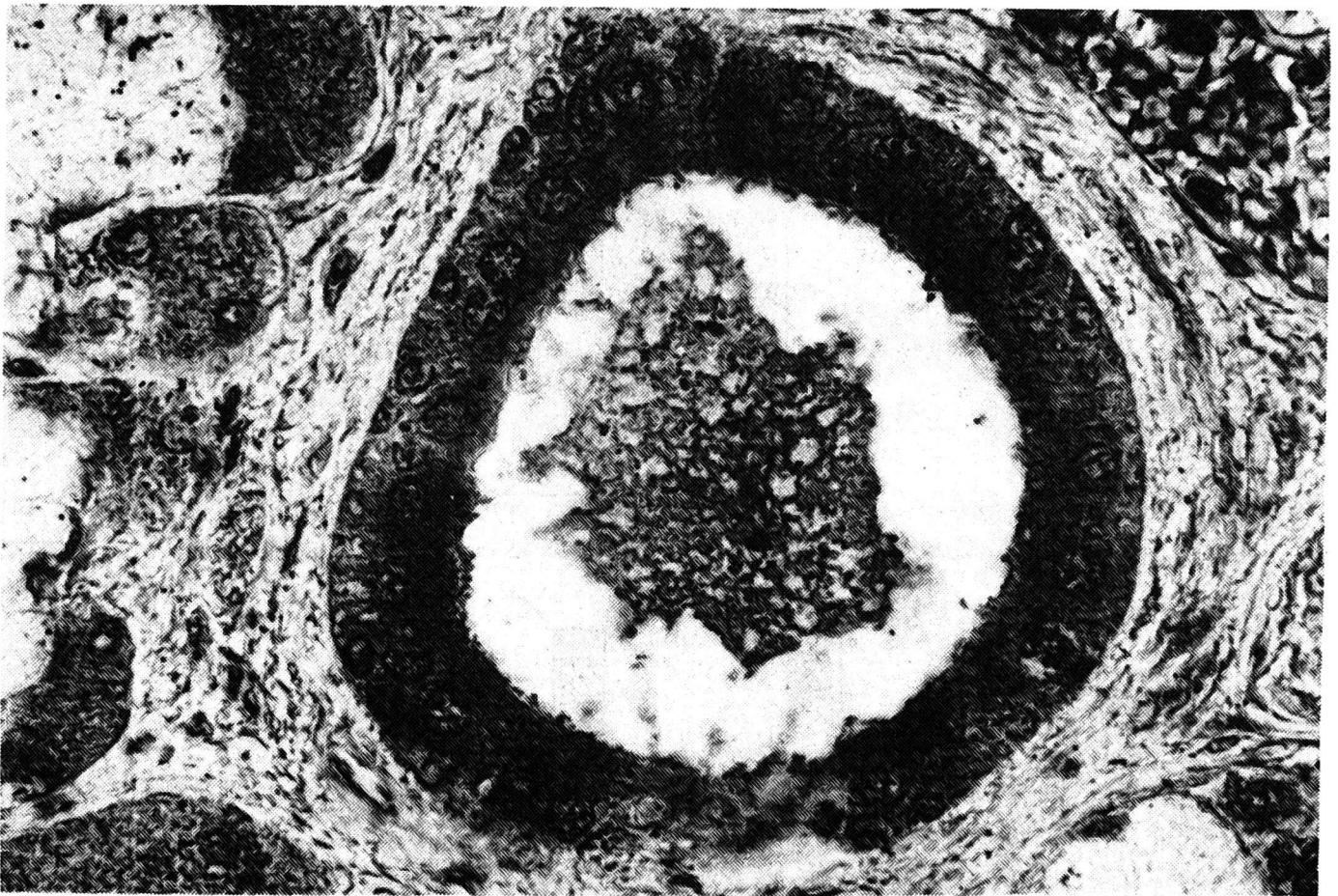


Fig. 4. EGF in the epithelium of the intralobular duct and within its lumen. PAP, hematoxylin counterstain, Magn. 450x.

EGF staining was negative in the basket cells (myoepithelial) and stroma connective tissue.

The Sublingual Gland (*Fig. 7*)

The histological structure of the sublingual gland is different from the parotid and submaxillary glands and the mucous component is predominant. The intercalated ducts in the sublingual gland are fewer and they are very short

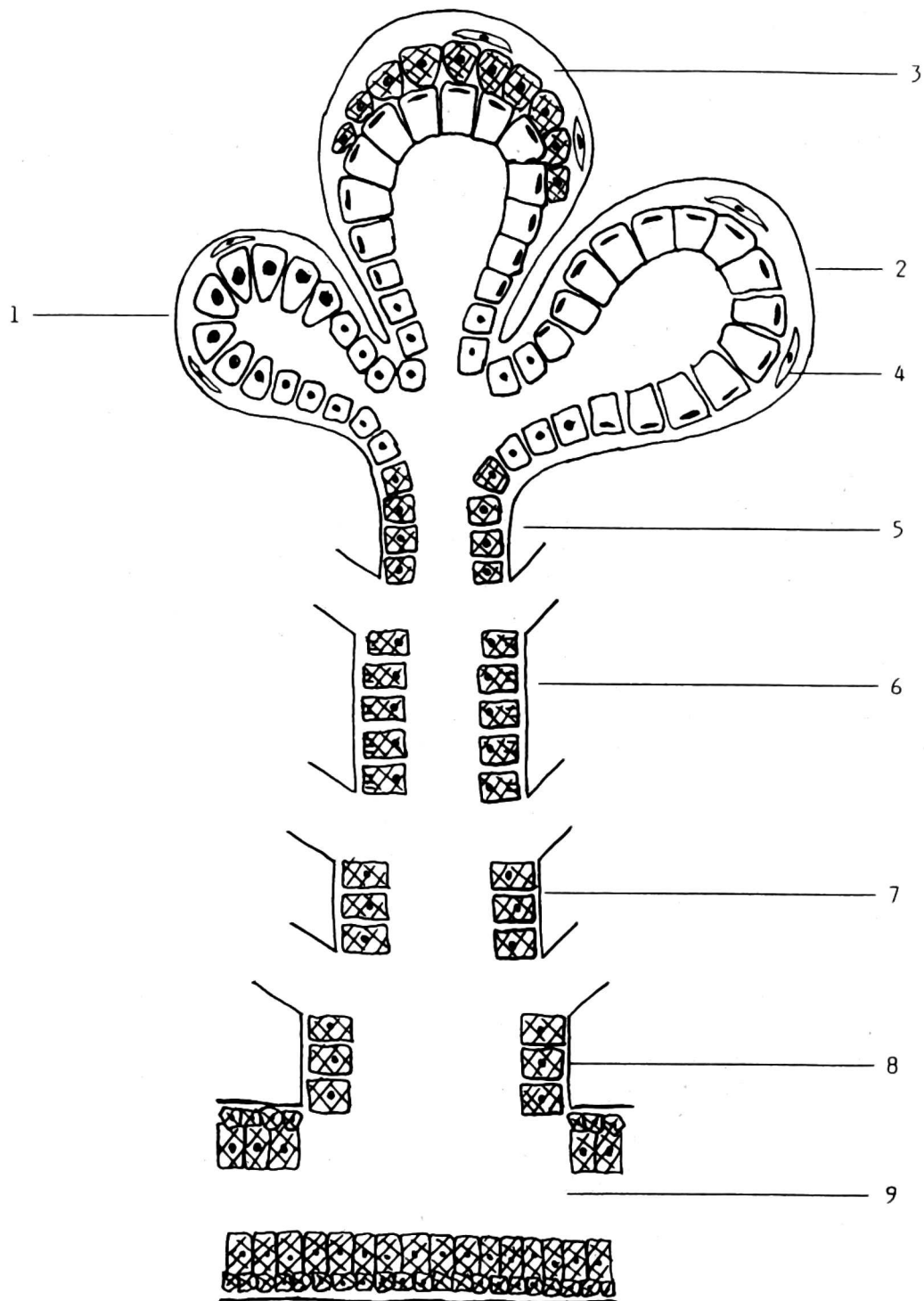


Fig. 5. The presence of EGF in the submaxillary gland — a diagram. Squared field corresponds to EGF expression. 1-serous vesicle, 2-mucous tubule, 3-demilune of Gianuzzi, 4-myoepithelial cell, 5-intercalated duct, 6-striated duct, 7-intralobular duct, 8-interlobular duct, 9-main duct.

and narrow. The striated ducts are also definitely shorter. The sublingual glands were diversified in terms of the presence and distribution of the EGF. A strong expression was found in the main ducts, intra- and interlobular ducts. The infrequent intercalated ducts and striated ducts also showed EGF expression. In addition, the presence of EGF was found in the majority of the cytoplasm of the serous cells forming the demilunes on the mucous tubules (*Fig. 8, 9*). The myoepithelial cells and stroma were immunohistochemically negative for EGF.

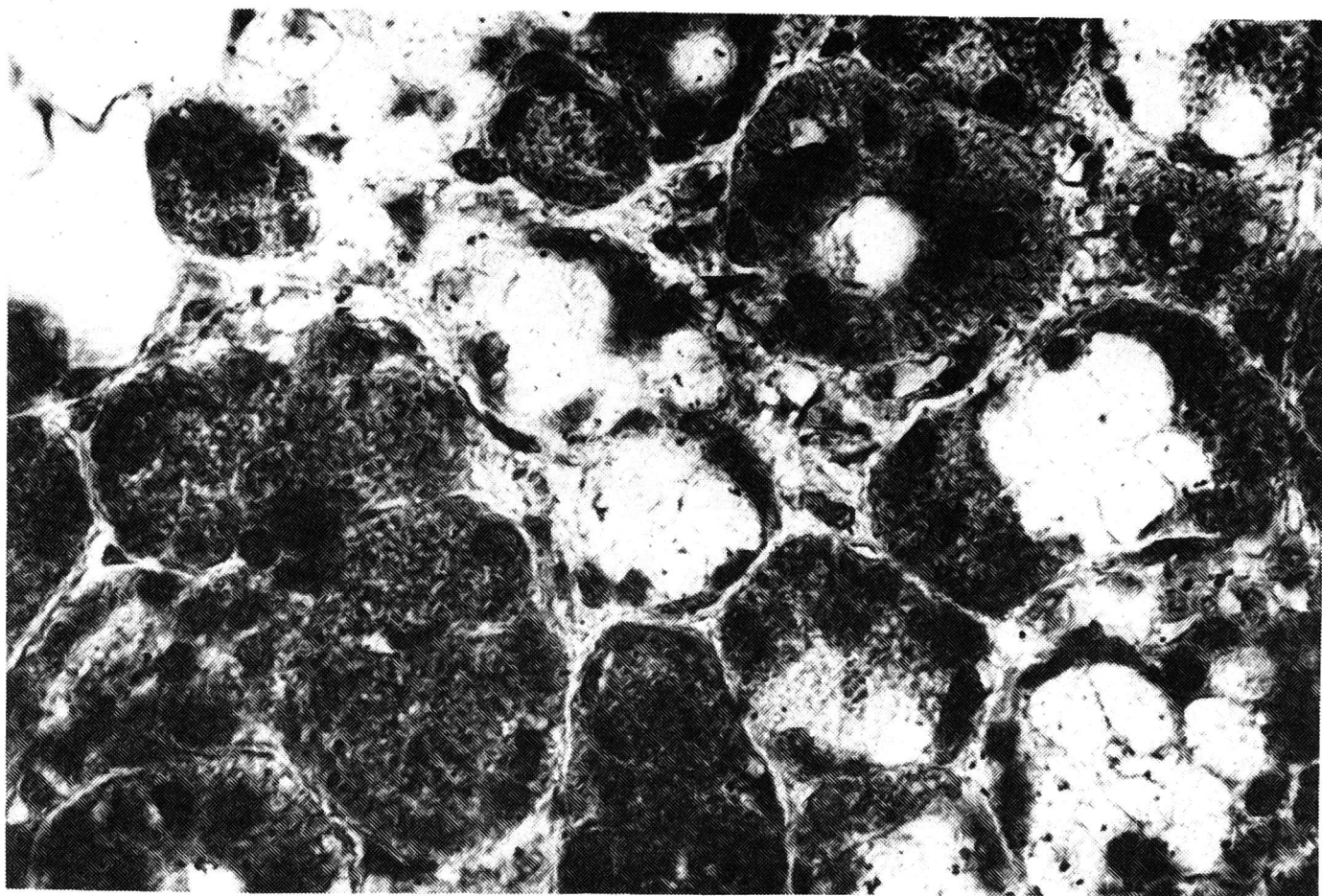


Fig. 6. EGF in the submaxillary gland. EGF expression in the epithelium of the intercalated duct (arrow) and in the demilune of Gianuzzi (asterisk). PAP, hematoxylin counterstain, Magn. 450x.

DISCUSSION

An active EGF circulates in the blood and body fluids, thus affecting the distal organs (1). Its autocrine, paracrine and transcrine action has also been postulated. The EGF effect on the target cells is mediated by a cell surface receptor. The binding of EGF with the receptor stimulates the process of protein phosphorylation inducing eventually the response of the target cell manifested as an increased DNA synthesis, which in turn is closely related to cell replication and transformation. Thus, EGF may be considered an effective mitogenic factor (1, 4).

It is likely that EGF plays an important physiological function in the lumen of the alimentary tract where its concentration is high (2). The activities of EGF in relation to the mucous membrane of the alimentary tract include:

- inhibition of gastric secretion
- protection against ulcerogenic factors
- improved healing of ulcerations in the alimentary tract
- trophic action (2, 4, 8—10).

The present study shows that EGF is located mainly in the efferent pathways of the salivary glands. Human salivary glands contain secretory segments and well developed efferent ducts. The secretory segments are

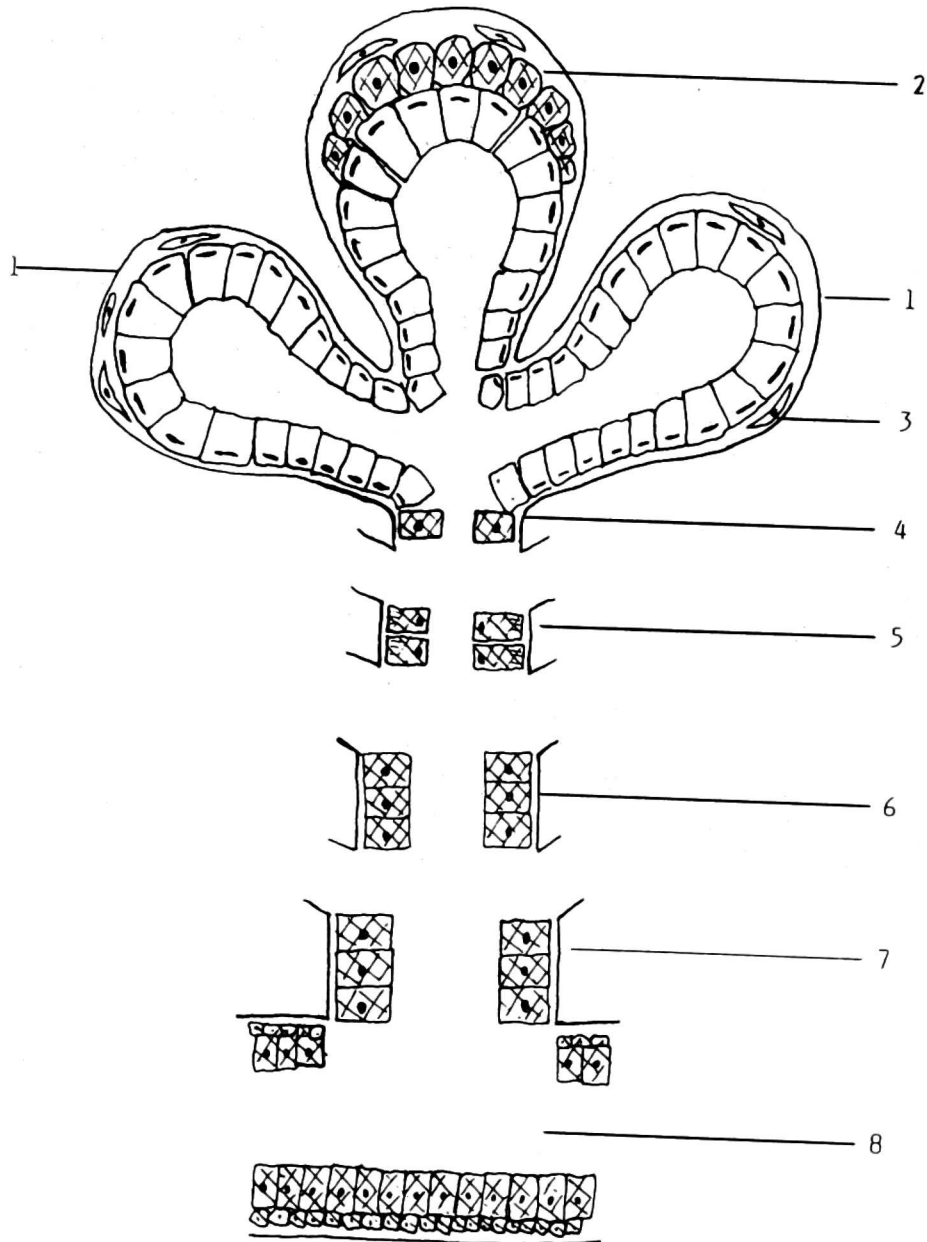


Fig. 7. The presence of EGF in the sublingual gland -a diagram. Squared field corresponds to EGF expression. 1-mucous tubule, 2-demilune of Gianuzzi, 3-myoepithelial cell, 4-intercalated duct, 5-striated duct, 6-intralobular duct, 7-interlobular duct, 8-main duct.

composed of serous cells forming serous vesicles or of mucous cells forming mucous tubules. The lumen of the mucous tubules is slightly wider than that of the serous vesicles. Certain mucous tubules contain clusters of serous cells forming a cap on the mucous tubule — a demilune of Gianuzzi. The serous vesicles and mucous tubules are surrounded by myoepithelial cells forming a basket (basket cells of Boll). The parotid gland is a serous gland, the submaxillary gland is of mixed type with a predominant serous component, whereas the sublingual gland contains mainly the mucous component. The serous vesicles and mucous tubules empty to the intercalated ducts — fairly long (except sublingual salivary gland) ducts with simple cubic epithelial lining also surrounded by myoepithelial cells. Several intercalated ducts join to form the so-called Pflüger salivary tubule or striated duct, with simple

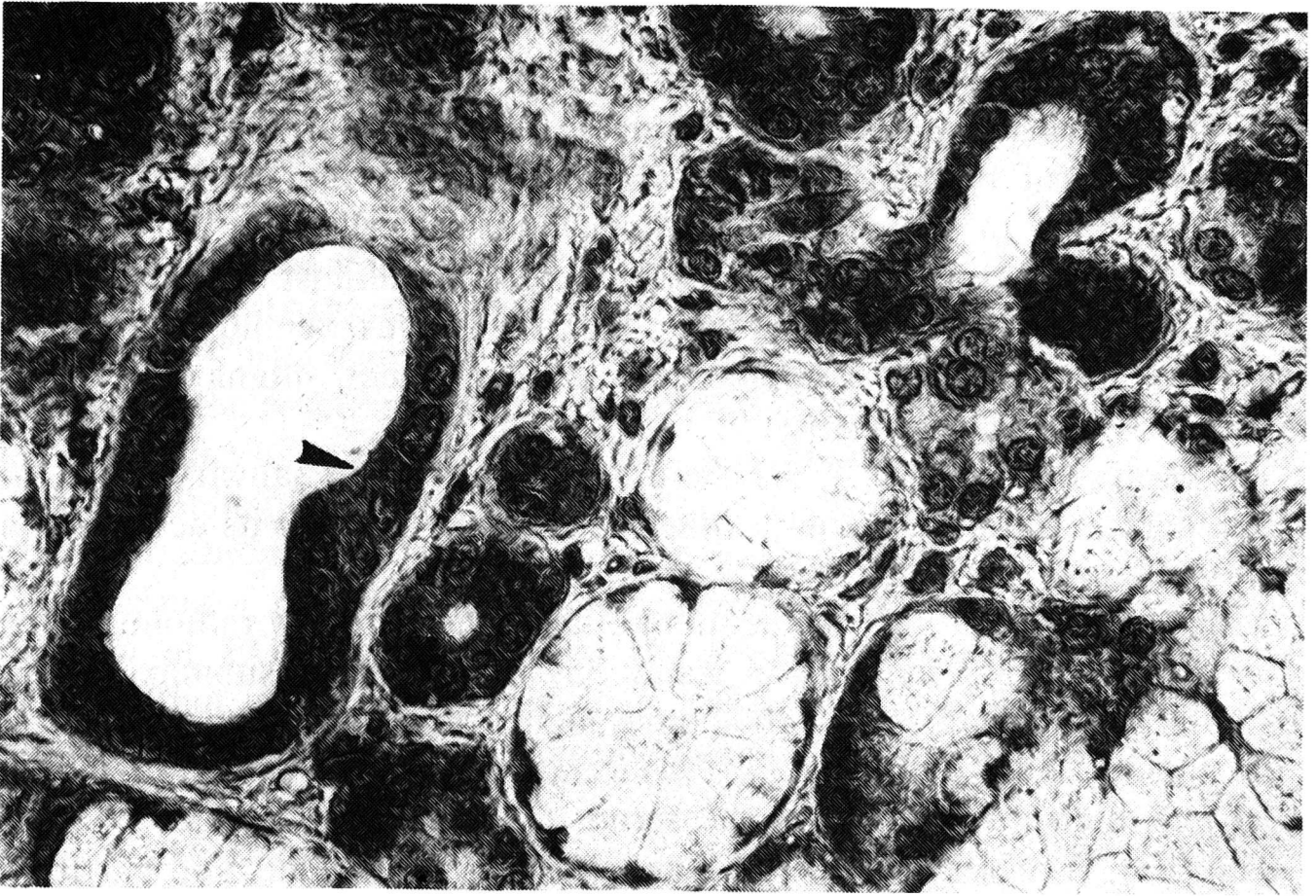


Fig. 8. EGF in the sublingual gland. A positive reaction in the intralobular duct (arrow) and in the small intercalated duct (asterisk). PAP, hematoxylin counterstain, Magn. 450x.

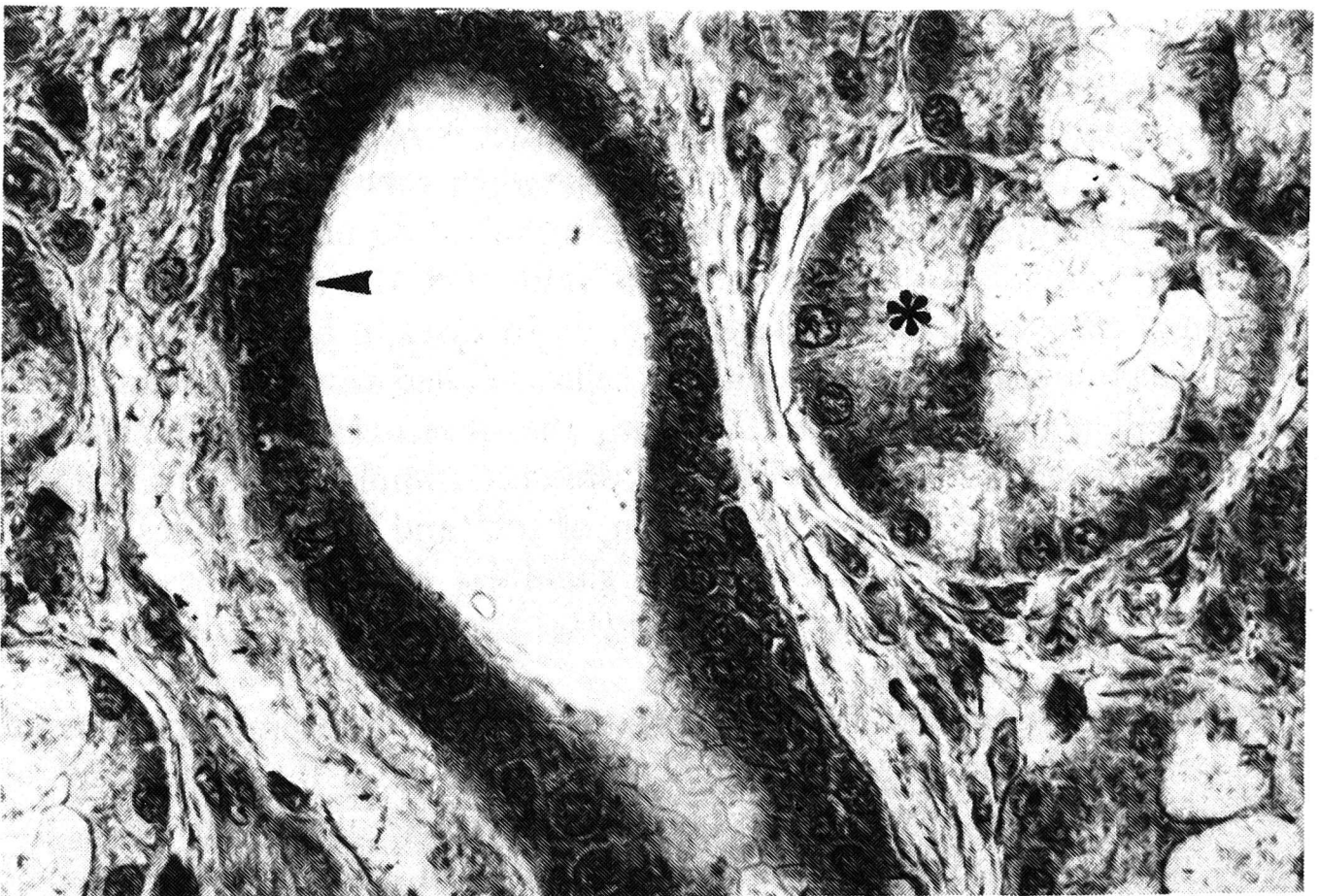


Fig. 9. EGF in the sublingual gland. A positive reaction in the large efferent duct (arrow) and in demilune of Gianuzzi (asterisk). PAP, hematoxylin counterstain, Magn. 450x.

cylindrical epithelial lining. The cytoplasm shows a characteristic striation due to the presence of mitochondria located in the cellular membrane invaginations. The salivary tubules join to form intra- and interlobular ducts with simple cylindrical epithelial lining, which empty to the main ducts with twolayered lining (a layer of cubic and cylindrical cells) (11, 12).

Our present study demonstrated EGF expression almost exclusively in the ductal secretory part of the salivon. Only the serous demilunes of certain mucous tubules of the sublingual and, to a lesser extent, submaxillary glands showed EGF immunoreactivity.

The expression of EGF in the efferent pathways of the salivary glands and its high concentration in the alimentary tract lumen indicate its action mainly beyond the salivary glands.

A concentration of EGF in the human saliva measured by radioimmunoassay is 2704 pg/ml for the parotid gland, 357 pg/ml for the submaxillary and sublingual glands, that is 100—1000 times higher than that in the serum (2, 13). In the pancreatic juice whereas EGF concentration is 2,3—8,5 ng/ml, which is 3—10 fold higher than that in the serum (2).

The EGF is present in the blood as a free plasma peptide, and partially is also platelet-bound (1, 9). The origin of the circulating EGF is not clear. Probably it is mainly produced by the salivary glands which release EGF into the circulation under adrenergic stimulation (1, 9). On the other hand fluctuations in the salivary level of EGF only to a small degree correlate with the concentration of EGF circulating in the serum (1).

EGF present in the lumen of alimentary tract is resistant to hydrochloric acid action and proteolytic enzymes (2, 9). The main source of the gastric EGF is the saliva. Sialadenectomy in rodents leads to a loss of about 90% of the gastric EGF content, it does not affect its content in the duodenum and has only limited effect on its serum level (4, 5, 9). In cases of healing of intestinal ulcers it was found that the regenerating cells so-called new cell lineage which formed small glands communicating with the alimentary tract lumen (14). These newly formed cells secrete EGF. Its presence stimulates the expression of two genes responsible for the production of pS2 and hPS peptides (human spasmodic polypeptide) in the adjacent glandular cells. Both these proteins belong to a group of regulatory peptides (14). These findings indicate a local function of EGF in healing processes.

Although the animals after sialadenectomy do not develop spontaneous ulcerations of the alimentary tract mucosa, a removal of the salivary glands precipitates the formation of experimentally induced ulcerations e. g. cysteamine induced duodenal ulcers (5). Ohmura et al. showed that the EGF content in the saliva of patients with chronic ulcers is markedly lower than that in the healthy population (2, 9). A deficiency of endogenous EGF probably attenuates mucosal resistance to ulcerogenic factors.

Quantitative studies of salivary glands showed the highest EGF concentration in the parotid gland (2, 13). Although the immunohistochemical method of EGF expression used in the present study cannot be considered a quantitative method it has clear advantage by demonstrating cellular localization of EGF.

EGF expression is present mainly in the efferent pathways of the salivary glands and it is strongest in the cells lining intercalated ducts and Pflüger salivary tubules. These segments of the salivon are most developed in the parotid gland where the reaction is strongest as compared with the remaining salivary glands. The fact of markedly lower EGF concentration in the submaxillary and especially sublingual glands found in a biochemical study (13) also correlates with our findings of its weaker expression in these salivary glands.

In summary present immunohistochemical study demonstrated that the main site of EGF production are the proximal segments of the efferent pathways (that is the intercalated ducts and salivary tubules), which are most abundantly represented in the parotid gland.

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Received: November 11, 1991

Accepted: November 29, 1991

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