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SCANNING ELECTRON MICROSCOPY OF RAT EMBRYONIC SUBMANDIBULAR GLAND

MICROSCOPIA ELETRÔNICA DE VARREDURA DA GLÂNDULA SUBMANDIBULAR EMBRIONÁRIA DE RATO

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Embryonic organ morphogenesis results from epithelial-mesenchymal tissue interaction (SPOONER, 1974; CUTLER & CHAUDHRY, 1974). Interactions between the salivary gland epithelium and the mesenchymal capsule have been shown to control the development of the gland's characteristic branching pattern (CUTLER & GREMSKI, 1991). The mesenchyme exercises control over the morphogenetic behavior of the salivary epithelium through the selective production and destruction of specific

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extracellular matrix molecules (SPOONER **et al.**, 1985; FUKUDA **et al.**, 1988). There is strong indication that collagen, produced by the capsular mesenchyme, is a critically important molecule in the regulation and maintenance the branching pattern of developing salivary glands. The synthesis and deposition of interstitial collagen appears to be required for salivary glands branching morphogenesis to be initiated and maintained (SPOONER & FAUBION, 1980; FUKUDA **et al.**, 1988). In addition, basement membrane has also been shown to play an important role in the regulation of salivary gland morphogenesis (SPOONER **et al.**, 1986).

The current report describes an overall topographic view of 20-day embrionic submandibular gland (SMG), using rudiments which were frozen in liquid nitrogen and cracked into pieces and then submitted to a long term maceration in OsO4 (TANAKA & MITSUSHIMA, 1984).

MATERIALS & METHODS

Wistar rats were bred under rigidly controlled conditions as previously reported (CUTLER & CHAUDHRY, 1974). On the 20th days of gestation, fetuses were aseptically delivered by sterile laparotomy. The SMG rudiments were excised and fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 1.0 hr at room temperature. The specimens were then cracked after the immersion in liquid nitrogen and maceration in 0.1% OsO4 in cacodylate buffer (pH 7.4) and dried in a critical point dryer withour metal coating.

RESULTS & COMMENTS

Scanning electron microscopy is a very useful technique to determine the differentiation and branching of SMG. Fibrilar structure of the lobular epithelia and their complementary mesenchyme

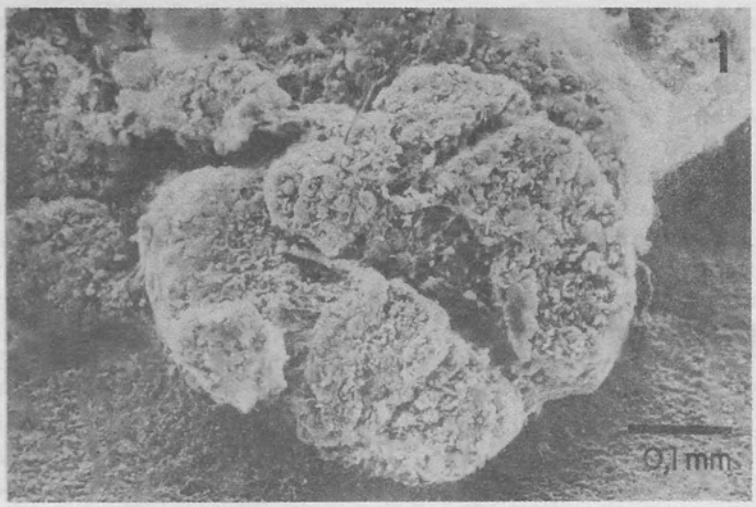
can be followed topographically during cleft formation. Also the relationship between the epithelia and the extracellular matrix in general can be seen.

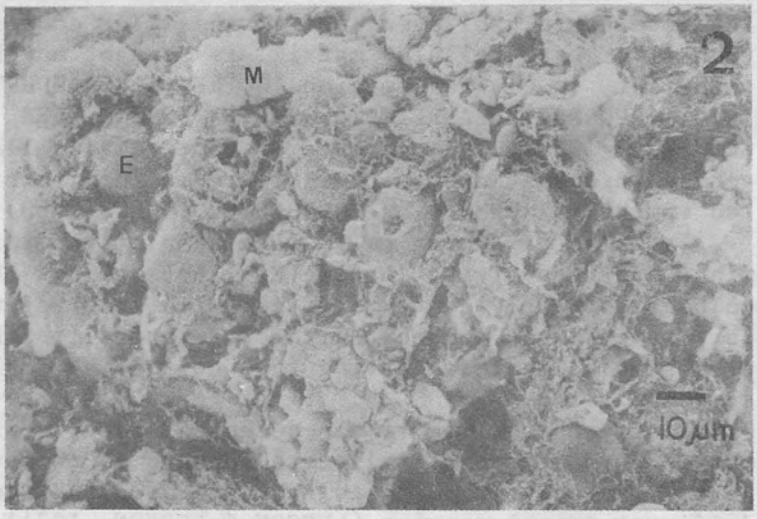
In the case of the 20-day rat SMG rudiment, the epithelial organization and their mesenchyme are clearly seen (Figs. 1 and 2). The lobuli are irregularly shaped and present several indentations, as reported by NAKAMISHI **et al.** (1986) in mouse embryonic SMG. Bundles of fibrillar material were found scattering over the adjacent lobuli (Fig. 3). Recovering the surface of the lobuli also can be seen fibrils showing a random array with a less denser organization (Figs. 2 and 4). Rabbit parotid gland treated in HCl-collagenase (WATANABE, OGAWA & YAMADA, 1989) had the connective tissue components including basal lamina removed. The acinar surfaces were clearly uncovered. According the literature, collagen, one of the major constituents of extracellular matrix, is thought to be important in cleft formation (CUTLER & CHAUDHRY, 1974; SPOONER, BASSET & SPOONER, 1989; CUTLER & GREMSKI, 1991). The presence of commercial collagenase preparations in the culture medium inhibited epithelial branching (WESSELS & COHEN, 1968).

Profiles of acinar cells were seen on the terminal portion of the lobuli (Fig. 5) showing the colunar, slightly convex surface, with intercellular spaces well demarcated. Short microvilli-like processes appeared leaving the epithelial cell surface.

No myoepitheliocyte was detected. The distribution of the myoepitheliocyte appears to be markedly varied according to the type of gland and different animal species (LEESON & LEESON, 1971).

The results obtained by the method adopted revealed the lobular epithelia and their complementary mesenchyme. It was possible to determine also differences in the fibrillar structures existing on the surface of the lobular epithelia and those in the clefts, making connection between the lobuli. The basement membrane was not defined as well





Figs. 1 e 2. Scanning electron micrograph of 20-days rat embryonic submandibular gland. 1, overal view of the SMG rudiment; the lobules with several indentations and fibres are seen. 2, e=epitelium, m=mesenchyme; tope view of the lobule; lobular epithelia and their mesenchyme in addition with fibrillar material can be seen.

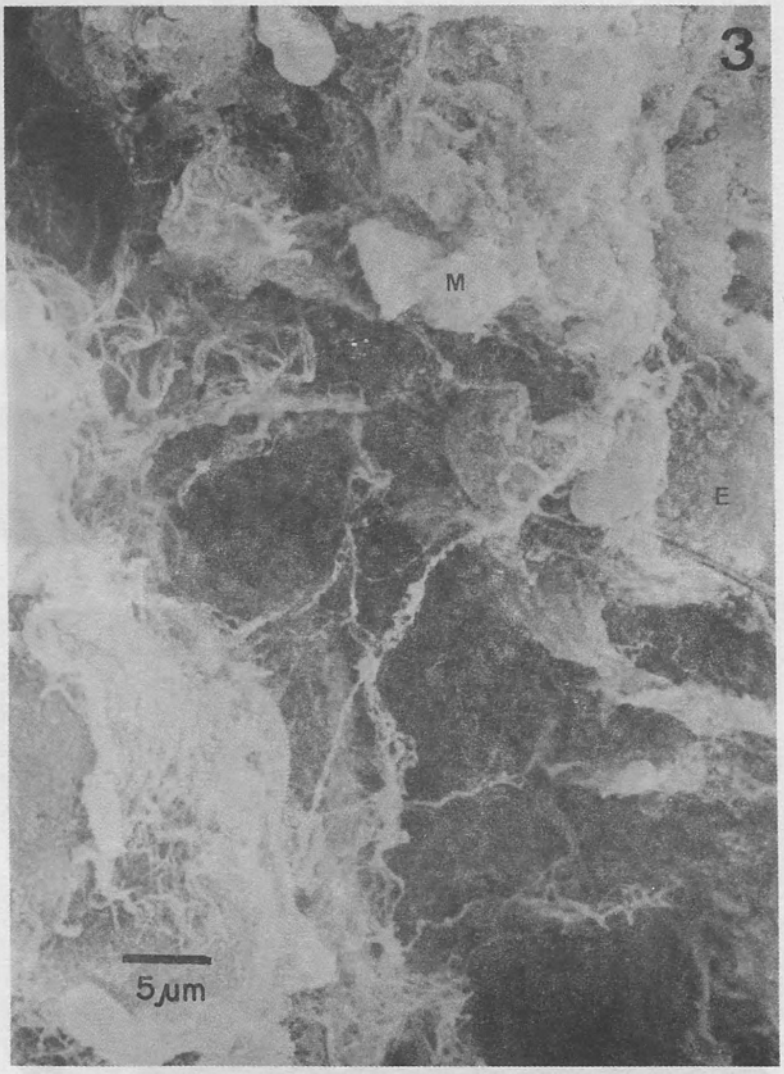
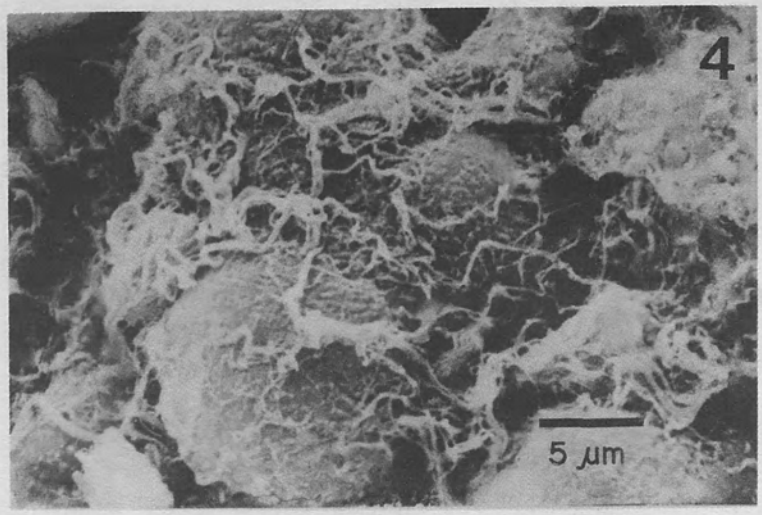
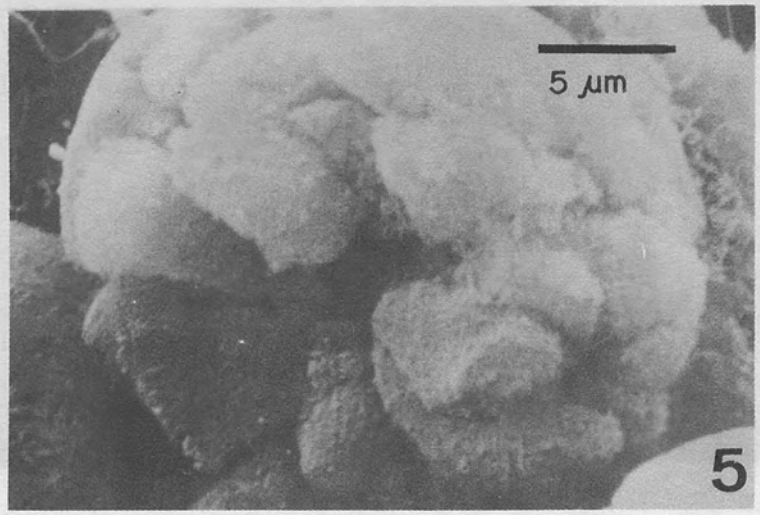


Fig. 3. Scanning electron micrograph of 20-day rat embryonic submandibular gland. e=epithelium, m=mesenchyme. High magnification view showing bundles of fibrils between two adjacent lobules. Fibrils are associated with the epithelia and the mesenchyme.





Figs. 4 e 5. Scanning electron micrograph of 20-days rat embryonic submandibular gland. 4, high magnification view of an area of the lobule showed in the figure 2; fibrils with different diameter are recovering the acini; 5, e=epithelium; m=mesenchyme; profiles of acinar cells are seen.

as internal structure of the cells.

RESUMO

Foi utilizada a microscopia eletrônica de varredura para determinar aspectos topográficos da glândula submandibular de rato em fase embrionária bem como relacioná-la com a matriz extracelular. Os rudimentos examinados mostram os lóbulos com várias fendas, regiões de ductos e o mesênquima. Material fibrilar encontra-se disposto sobre a superfície dos lóbulos e estabelecendo ligação entre as fendas e entre os ácinos. Nessa fase de desenvolvimento embrionário as fibras são também observadas entre os lóbulos adjacentes. O mesênquima, que confronta com o epitélio, estabelece pontos de contato com as fibras.

PALAVRAS CHAVE: glândula-submandibular, morfogênese, matriz-extracelular, microscopia-eletrônica-de-varredura.

SUMMARY

Scanning electron microscopy was employed to determine the topographic aspects of 20-day rat embryonic submandibular gland and its relationship with the extracellular matrix. An overal view of the rudiment showed the lobules with several indentations, stalk regions, and the mesenchyme. Fibrilar material was scattered over the lobules and between the clefts. At this stage fibres were observed also between two adjacent lobules. The mesenchyme that confronted with the epithelium formed ridges on or in which the fibres were often found.

KEY WORDS: submandibular-gland, morphogenesis, extracellular-matrix, scanning-electron-microscopy.

RÉSUMÉ

La microscopie electronique de balayage a été utilisé pour déterminer les aspects tòpographiques de la glande sous-mandibulaire d´un rat en periode embryonnaire ainsi que pour la mettre en rapport avec la matrice extracellulaire.

Les rudiments examinés montrent les lobules, ayant des fentes, des regions de ducte et le mesenchime. Sur la superficie des lobules, on trouve du matériel fibreux que établit des liaisons entre les fentes et entre les acines. En cette phase de d´éveloppement embryonnaire les fibres sont également observé entre les lobules assemblés. Le mesenchime que est en contact avec l´epitelium a également de points de contact avec les fibres.

MOTS CLÉS: grande sous-mandibulaire, morphogenese, matrice extracellulaire, microscopie electronique de balayage.

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