Intercellular contacts at the epithelial–mesenchymal interface of the developing rat submandibular gland in vitro

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SUMMARY

An ultrastructural study of the development of the rat submandibular gland (SMG) anlage in vitro was undertaken to determine if epithelial–mesenchymal and epithelial-nerve contacts were integral events in the differentiation of the gland in vitro as they are in vivo. SMG rudiments were removed at the stalk-bulb stage (15 days in utero) and cultured for 6 days on a millipore filter in supplemented McCoy's 5A media. Rudiments were taken at daily intervals, fixed and processed for electron microscopy. The overall development of the explanted rudiments closely paralleled their maturation in vivo although cultured glands lagged 24–36 h behind their normal counterparts. Direct epithelial–mesenchymal contacts were seen after the morphogenetic patterning of the gland had been established but prior to functional differentiation of the rudiment. Epithelial-nerve contacts were not seen although healthy axons were seen in the stroma throughout the culture period. The study indicates that epithelial-nerve contacts are probably not required for morphogenesis of cytodifferentiation of the rat SMG. However, direct epithelial–mesenchymal contacts appear to be an integral part of the developmental sequence of the rat SMG.

INTRODUCTION

In an earlier report (Cutler & Chaudhry, 1973a) direct epithelial–mesenchymal and epithelial-nerve contacts were observed at the epithelial–mesenchymal interface in the developing rat and submandibular gland (SMG). These contacts were seen during the period of rapid branching and budding of the early glandular anlage and were only observed along the epithelial–mesenchymal interface of the end-buds from the initial 4–12 branches. It was noted that these contacts appeared after the morphogenetic branching pattern of the rudiment had been established but prior to the onset of secretion and structural maturation in the gland. Thus, it was hypothesized that these contacts might play a role in the functional differentiation and continued morphodifferentiation of the gland subsequent to the establishment of the morphogenetic branching pattern.

A recent report by Coughlin (1975a) indicated that during the phase of rapid

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morphogenesis of the mouse submandibular gland epithelial–mesenchymal contacts but not epithelial-nerve occur in vivo. On the other hand, Coughlin (1975a) showed that epithelial-nerve contacts occur during morphogenesis of the mouse SMG in vitro. The function of these epithelial-nerve contacts in morphogenesis was questioned since normal morphogenesis occurred in the total absence of the neural tissue (Coughlin, 1975b).

An ultrastructural study of the development of the rat submandibular gland anlage in vitro was undertaken to determine if epithelial–mesenchymal and epithelial-nerve contacts could be observed and if so were such contacts important in morphogenesis and/or functional differentiation of the rudiment cells.

**Materials and Methods**

Holtzman strain, Sprague-Dawley rats were bred under the controlled conditions reported previously (Cutler & Chaudhry, 1973a). On the 15th day of gestation fetuses were aseptically delivered by sterile laparotomy. The submandibular glands were quickly excised, washed twice in Hanks' balanced salt solution and then transferred to a Falcon organ culture assembly and cultured for 6 days on Millipore filter in supplemented McCoy's 5A media as described previously (Cutler & Chaudhry, 1973b). Rudiments were taken at daily intervals and fixed in 3% phosphate buffered glutaraldehyde, then post-fixed in 1% osmium tetroxide, stained *en bloc* with 0.25% aqueous uranyl acetate and routinely processed and embedded for electron microscopy (Cutler & Chaudhry, 1973a, b, c). For orientation, 1 μm thick sections were cut and then stained with 1% methylene blue and examined by light microscopy. Thin sections (60–90 μm) were double stained with methanolic uranyl acetate (Stempak & Ward, 1964) and lead citrate (Reynolds, 1963). Thin sections were examined on a Siemens Elmiskop 1 or a Zeiss EM 10 electron microscope.

**Results**

At the time of explantation the SMG rudiments were composed of a stalk and solid terminal bulb (Fig. 1) which occasionally showed cleft formation indicative of the initiation of branching. One day after explantation the rudiments were actively branching and budding and by 2 days in vitro they consisted of multiple branches and end-buds (Fig. 2). The branching and budding activity of the explants decreased on the third day in culture but lumen formation was seen in the main stalk and many of the end-buds. Occasionally, secretory granules were seen in the end-bud cells bordering on the newly formed lumina (Fig. 3). By 6 days in culture the rudiments were richly arborized and there was a marked accumulation of secretory product and ductal differentiation was well under way (Fig. 4). Thus, while the overall development of the explanted rudiments closely paralleled their in vivo maturation the cultures lagged 24–36 h behind normal.
Direct epithelial–mesenchymal contacts were observed between the epithelium of the end-buds of the initial 6–15 branches and the surrounding mesenchyme. These contacts were seen predominantly on the second day and occasionally very early on the third day of the culture period. Contacts of this nature were not seen prior or subsequent to this time point.

Most of the contact zones were formed by the junction of a mesenchymal cell process with the plasma membrane of an end-bud cell (Fig. 5). Infrequently an epithelial cell gave off an extension which contacted a neighboring mesenchymal cell (Fig. 5). The predominant configuration of these junctions was that of an intermediate contact zone. The opposing cell membranes were separated by a gap of about 15 nm which frequently contained an amorphous material. The opposed membranes were occasionally thickened but often showed no observable changes. A band of electron-dense material was seen in the cytoplasm beneath the contact zone.

Contact zones in which a mesenchymal cell projection penetrated through the basal lamina and extended into the end-bud in the intercellular space between two end-bud cells (Fig. 6) were also seen. Areas of apparent fusion of opposed epithelial and mesenchymal cell membranes were only rarely seen.

No epithelial-nerve contacts were observed although axons were seen in the stroma throughout the culture period.

**DISCUSSION**

The epithelial–mesenchymal contacts observed in the current study appeared at about the same stage of development as those observed *in vivo* (Cutler & Chaudhry, 1973a). In both systems the contacts were seen during the period of rapid branching and budding of the rudiment which followed the establishment of the morphogenetic pattern of the gland. Similarly, the contacts preceded the onset of glandular secretory activity, morphodifferentiation of myoepithelium and the maturation of specialized ductal components (Cutler & Chaudhry, 1974, 1973a, b). In both cases, the contacts were seen only along the epithelial–mesenchymal interface of the developing end-buds and the morphology of the contact zone was remarkably similar.

Despite the continued vitality of nerves in the stroma throughout the culture period, no epithelial-nerve contacts were seen. This is in contrast to observations made *in vivo* (Cutler & Chaudhry, 1973a). These observations are consistent with and extend those of Coughlin (1975a, b). Since glandular morphogenesis and functional differentiation proceeds *in vitro* in the absence of direct epithelial-nerve interactions the role, if any, of such contacts in the cytodifferentiation of the SMG rudiment is probably minimal. However, direct epithelial–mesenchymal interactions are always seen and their role in cytodifferentiation may be significant.

The classic transfilter experiments of Grobstein (1956) and his associates
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(Grobstein & Dalton, 1957; Koch & Grobstein, 1963; Kallman & Grobstein, 1965, 1966) suggested the presence of diffusible molecules which lead to 'embryonic induction' in the absence of direct epithelial–mesenchymal contacts. However, reports by Nordling, Mirttinen, Wartiovaara & Saxén (1971) and Saxén (1972) have indicated that the length of time required for induction to occur between interacting cell populations in a transfilter situation tends to rule out diffusion of molecules as a mechanism for information transfer. They postulated that inductive information transmission might be mediated by direct cellular contact or extracellular materials which were bound to the cell periphery. Both of these hypotheses require that the plasma membranes of cells from the interacting tissues be brought into relatively close apposition. Reports by Wartiovaara, Nordling, Lehtonen & Saxén (1974), Lehtonen (1975) and Saxén et al. (1976) indicate that direct epithelial–mesenchymal contacts are essential for the induction of kidney tubule morphogenesis. Recent studies have added embryonic lung (Bluemink, van Maurik & Lawson, 1976) and tooth germs (Slavkin & Bringas, 1976) to the growing list of tissues in which transient epithelial–mesenchymal contacts are seen. In both cases, these contacts seem more closely associated with functional differentiation of cells rather than morphogenesis of the structure, a situation analogous to that in the developing salivary gland.

The present study indicates that epithelial–mesenchymal contacts are an integral part of the developmental sequence of the rat submandibular gland both in vivo and in vitro while epithelial-nerve contacts are only seen in vivo and are probably not of major importance in the cytodifferentiation of the SMG. Moreover, the appearance of the epithelial–mesenchymal contacts after

**Figures 1–6**

*Fig. 1.* 1 μm thick Epon section of a typical SMG rudiment at the time of explantation. Note that the rudiment is composed of a stalk (S) and a terminal bulb (TB). × 350.

*Fig. 2.* 1 μm thick Epon section of a 1-day explant. Note the numerous end-buds (EB). × 175.

*Fig. 3.* 1 μm thick Epon section of an end-bud from a 3-day explant. Note the dense secretory granules (arrows) around the small lumen. × 225.

*Fig. 4.* 1 μm thick section of SMG rudiment after 6 days in culture. Note that the ducts and end-buds show lumina. × 80.

*Fig. 5.* Electron micrograph showing two epithelial-mesenchymal junctions (arrows). Junction A shows thickening of the opposed membranes and is made by a mesenchymal cell extension (ME) contacting the basal plasmalemma of an end-bud cell. Junction B is made by an epithelial extension (EE) from an end-bud cell which contacts the mesenchymal cell extension (ME) in junction A. Section from an end-bud of a 2-day explant. × 75000.

*Fig. 6.* Electron micrograph showing a mesenchymal cell extension (ME) penetrating through the basal lamina (BL) and extending into the bud-end (SMG) in the intercellular space between end-bud cells. Section from 2-day explant. × 32000.
the establishment of the morphogenetic branching pattern and prior to the functional differentiation (production of cell specific secretory protein) of the rudiments suggests a potential role for these contacts in the 'induction' of cytodifferentiation rather than morphodifferentiation in the rat SMG. Bernfield, Banerjee & Cohn (1972) have previously shown that deposition of proteoglycan is a central phenomenon in the development of the classic arborized salivary gland structure.

It is possible that the contact zones observed in epithelial-mesenchymal interactions in the salivary gland and other tissues might act as sites of transfer for 'informational molecules'. Such molecules could be normal or specialized constituents of the cell surface. Contacts such as these might also lead to focal depolarization of the membranes allowing divalent cations such as Ca$^{2+}$ to serve as informational molecules. In any case, the efficiency of such interactions would be greatly facilitated by such anatomical arrangements. The molecules would not have to travel (diffuse) over large distances, thus reducing their dilution or loss in the extracellular space. The rate of information transfer would be enhanced since the interactions would occur over a short distance and finally, such an arrangement could bring 'molecular inducers' into close proximity with specific receptor sites on the opposing cell membrane.

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REFERENCES


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