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THE ROLE OF EPITHELIO-MESENCHYMAL INTERACTION IN THE ORGANOGENESIS OF THE PAROTID SALIVARY GLAND OF THE CAMEL FETUSES

(With 6 Figs.)

By

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دور تفاعل النسيج الطلائى والميزنشيمى
فى تخليق الغدة النكفية لأجنة الجمـــــــــال

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أوضحت الدراسة أن بدائية الغدة النكفية فى أجنة الجمال تظهر عند طول ٢ر٣ سم مـــن الخلايا الطلائية المبطن للفم ويبدأ تفرع بدائية الغدة مصحوبا بتحلل الغشاء الطلائى القاعدى عند قمتها ، مع زيادة تركيز الألياف والأوعية الدموية رقيقة الجدار حولها ، وكذا الزيادة المطـــردة فى محتوى الخلايا الطلائية من الحبيبات المخاطية متعادلة التفاعل · وكذا عند طول ١٤ سم تظهر كبيسات الغدة على هيئة كريات خلوية معتمة ، وبتقدم العمر تجوف هذه الكريات وتتمايـــز خلاياها لطبقة واحدة من الخلايا الهرمية · وفى الأطوار الجنينية المتقدمة تصبح الغدة النكفيـــة مكونة من تراكيب عنقودية معقدة وتصبح خلاياها قادرة على إفراز المخاط المتعادل مع قليـــل من المخاط الحامضـــى ·

SUMMARY

The primordia of the fetal parotid salivary gland of the camel was firstly observed at the 32 mm CVRL stage as an outgrowth from the stomadeal epithelium. Hence, it is suggested that it has an ectodermal origin.

The initiation of the epithelial branching was associated with a high mitotic activity at the growing tip of the epithelial anlage, degradation of the basal lamina surrounding this tip, condensation of fine collagenic fibers and thin-walled blood vessels around the same area and with a prominent accumulation of neutral mucins and glycogen granules into the cytoplasm of the epithelial cells.

The differentiated ductal epithelium showed some goblet cells containing acid carboxymucins.

The undifferentiated acini were firstly detected at the 140 mm CVRL stage as solid cellular balls. Cytodifferentiation and luminization of such acini were advanced with progressed age.

In the full-term fetus the gland becomes of the compound acinar variety and its acinar cells were predominatly serous in addition to few mucous cells.

INTRODUCTION

The study of the prenatal development of the major salivary glands of the domestic animals has taken very little attention, as observed from the available data, except for the detailed description of the prenatal development of the major salivary glands of the buffalo given by YADM (1985). In the expermintal animals, histogenesis of the major salivary glands were studied by BORGHESE (1950), POSPISILOVA (1970), REDMAN and SREEBNY (1970), POSPISILOVA, et al. (1972) and CUTLER (1977).

The role of epithelio-mesenchymal contacts in the growth and differentiation of the fetal epithelial rudements of the salivary glands has been discussed by WESSELLS and COHN (1968), COUGHLIN (1975), WESSELLS (1977), DAVID and BERNFIELD (1981) and BERNIFIELD and BANERJEE (1982).

The present investigation is a trial to determine the mode of formation of the parotid salivary gland of the camel and to explain some of the mystery about its prenatal growth and differentiation.

MATERIAL and METHODS

The material comprised 30 apparently normal camel fetuses collected from Cairo Slaughter house. Their curved crown rump length (CVRL) was ranging from 28 to 1090 mm. The smallest fetuses and the resected glands from the larger fetuses (where the gland could be defined) were fixed in Bouin's fluid and 10% formol saline for 24 and 48 hours, respectively. The specimens were prepared by ordinary routine methods and 4-6 Um thick paraffin sections were stained with H & E, Van Gieson, PAS, Alcian blue, Alcian blue-PAS, Alcian blue-Aldehyde fuchsin and Best's carmine (BANCROFT and COOK, 1984).

RESULTS

The organogensis of the fetal parotid salivary gland of the camel passed into four constructive stages; primitive, branching, glandular and cytodifferentiation stages.

In the primitive stage; the anlage of the parotid gland was firstly observed at 32 mm CVRL as a solid cellular bud proliferating from the buccal epithelium at the angle of the mouth sulcus (Fig. 1). The bud was made up of densely packed undifferentiated cells whose comparatively large, ovoid, nuclei were surrounded with pale eosinophilic, vacuolated cytoplasm having a high affinity for PAS and Best's carmine. This epithelial proliferation was forced into exclusively cellular mesenchyme which was made up of polymorphic cells with branched and anastomosed cytoplasmic processes which were positive to both PAS and Alcian blue, but the latter was predominated. The epitheliomesenchymal interface was represented by a thin PAS-positive basal lamina and scanty, fine argyrophilic fibers. It was absent at the growing tip of the epithelial bud, where a direct communication between some of its cells and the adjacent mesenchyme was visible. Some thin-walled blood vessels were firstly observed

in the vicinity of the epitheliomesencymal interface at 40 mm CVRL-stage.

The branching stage was firstly observed at 80 mm CVRL, where the parotid anlage became in the form of a canalized epithelial structure with a bifurcated growing tip (Fig. 2). The latter was made up of a sprout of densely packed vacuolated cells with pale eosinophilic, PAS-positive cytoplasm and angular nuclei. The cells showed many mitotic figures. The canalized portion was lined with stratified columnar epithelium with an average thickness 31 Um. The basal cells were ovoid or polyhedral with moderately basophilic granular ovoid nuclei and vacuolated, pale eosinophilic and PAS-positive cytoplasm; meanwhile, the upper columnar cells were closely packed, their oval nuclei were lodged in their proximal halves, and their cytoplasm was more vacuolated and paler eosinophilic in their infranuclear portion than in their smaller supranuclear portions. The cytoplasm was strongly PAS-positive and showed numerous, fine Best's carmine-positive granules.

The epitheliomesenchymal interface was represented by a prominent PAS-positive basal lamina associated with fine acid fuchsin-positive fibers. The fine fibers were found elsewhere among the surrounding mesenchyme especially in the vicinity of the epithelio-mesenchymal interface. The thin-walled blood vessels were also increased in number especially near the interface and between the bifurcated epithelial growing arms. Fibroblast and few histiocytes were observed in addition to the numerous mesenchymal intercellular alcianophilia was markedly reduced.

Glandular stage (140 mm CVRL) it was observed that the previously described epithelial duct has given off repeated dichotomous divisions resulting in the formation of a large number of similar ducts with a gradual decline in their diameters. The terminal ducts were associated, along their course,with solid cellular balls (future acini), each of which had a short rod-like connecting piece (Fig. 3). The resultant was the formation of compound acinar complex. The largest orders of the branched duct system, whose diameters were gradually decreased with the advancement of age, had a diameter ranging between 270 to 120 Um. They were lined with stratified columnar epithelium with an average height of about 35 Um. The cells were similar to those described in the previous stage, but sporadic goblet cells were observed among the columnar cells especially in the large ducts. These cells were increased in number with the advancement of age, and they were positive to the stains of both neutral and acid mucins with a greater affinity to the latter. The smaller orders of the duct system were characterized by narrow lumina and their epithelial stratification were reduced to two or a single layer of cuboidal cells showing high mitotic activity. The number of goblet cells decreased in the smaller ducts. The terminal ducts, which were associated with the solid future acini, showed no or very narrow lumina and their simple cuboidal epithelial lining showed weaker reaction to PAS and Best's carmine than the larger ducts did.

The future acini and their short connecting pieces were made up of undifferentiated polyhedral cells, having comparatively large, nuclei and little amount of highly eosinophilic cytoplasm. The latter, contained little amount of PAS-positive material.

The interstitial tissue was characterized by a prominent increase in the amount of the acid fuchsin-positive fibers especially around the larger ducts, and their amount was less around the developing acini. The fibers became more concentrated in the form of strands around groups of the branched duct system to form incomplete lobulation (Fig. 4). It was observed that these strands were surrounding differentiated arterioles, venules, and nerve trunks. The thin-walled blood vessels were often associated with the branched glandular elements and followed their pattern of branching (Fig. 4). Moreover, the basal lamina of both the developing gland and their associated vessels were closely adjacent. The number of mesenchymal cells was sharply decreased with a substantial increase in the number of fibroblasts and to a lesser extent histocytes, mast cells, plasma cells, lymphocytes and erythroblastic hemopoietic cells.

Cytodifferentiation stage; this stage was characterized by a high increase in the formation of the glandular elements and their differentiation with a substential reduction in the amount of the interstitial tissue.

From stage of 250 mm CVRL through term, the main developmental events were the differentiation of spherical cellular balls (future acini) and their associated highly branced duct system. The acini became lined with a single layer of cubiodal to truncated pyramidal cells resting on a thin basal lamina. The cytoplasm of these cells was firstly light basophilic, strongly PAS-positive and showed numerous supranuclear Best's carmine-positive granules. With the increase of age, the cytoplasm showed a grandual eosinophilic affinity with a substantial decrease in the amount of PAS-and Best's carmine-positive materials. The spherical nuclei were almost moderately basophilic being insinuated near the basal lamina (Fig. 5).

Among the majority of the differentiated acini, few cells with a strongly alcinophilic cytoplasm resembling goblet cells were observed between the usual aciner cells. The differentiated acini were characterized by the presence of pervious narrow lumina which were entirely empty. Associating with the acinar basal lamina, scarce attenuated cells with oval or flattened nuclei were observed. These cells may represent myoepithelial cells. The average diameter of the acini was ranging between 18-35 Um. It was observed that the lobular architecture of the gland became gradually occupied with increasing number of acini and their associated ducts as the age was advancing. At the same time, there was a gradual diminution in the intralobular fibrous stroma (Fig.6).

The small-sized intralobular ducts were lined with a single layer of squamous to cuboidal cells with few number of goblet cells, meanwhile, the larger interlobular ducts were lined with pseudo-stratified to stratified columnar epithelium with larger number of goblet cells. The latter ducts were always surrounded with dense acid fuchsin-positive fibers which represent the interlobular septa and they were associated with prominent bundles of nerve fibers and blood vessels of different orders.

DISCUSSION

The present invesigation has revealed that the primordium of the parotid salivary gland of the camel fetus was firstly observed at the 32 mm CVRL stage as an un-

differentiated cellular bud originating from the epithelium of the primitive mouth sulcus. This observation confermed that this gland originates from the stomadeal ectoderm. Arey (1954) mentioned that there is no general agreement between embryologists about the exact origin of the salivary glands and whether it is endodermal or ectodermal. The ectodermal origin of the parotid salivary gland has been confermed in rat (POSPISILOVA, 1970; REDMAN and SREEBNY, 1970) and human fetus (VALDES-DAPENA, 1979).

It is observed that a basal membrane, made up of neutral mucins and fine collagenic fibers surrounded the outer margin of the epithelial bud except at its free tip. This membrane represented the epithelio-mesenchymal interface. The absence of the latter at the free tip (Growing tip) created a sort of contact between the epithelial cells, at this area, and the surrounding mesenchymal elements (cells and matrix). The mesenchyme at this area was often vascularized. The aforementioned observations suggest the presence of epithelio-mesenchymal interaction at the area where the limiting interface was missing. Three types of contact between the epithelium and the surrounding mesenchyme were described by GROBSTEIN (1955) and SAXEN, et al. (1976); cell-cell contact, cell-matrix contact and diffusion. COUGHLING (1975) has concluded that contact between epithelial and mesenchymal cells can often be seen at the areas where the basal lamina is interrupted. The degradation of the basal lamina surrounding a salivary gland rudiment was attributed to the presence of mesenchyme at the areas of epithelio-mesenchyme contact, due to its ability to secrete hyaluronidase (BANERJEE and BERNFIELD, 1979).

The begining of epithelial branching was firstly detected in the 80 mm CVRL stage. The branching was always detectable at the growing tips of the epithelial bud in the form of dichotomous splitting. This process was continuous during most of the prenatal morphogenesis of this gland. It is observed that the begining of branching was accompanied by a prominent increase in the amount of collagenic fibers and thin-walled blood vessels among the surrounding mesenchyme. This increase was more frequent around the epithelial growing tips than around their stalks. It is also observed that the process of branching was associated with a distinct accumulation of neutral mucins and glycogen within the cytoplasm of the branching epithelial rudiment. Thus, it is concluded that the initiation of the salivary epithelial branching is achieved by; a- degradation of the basal lamina at the growing tips. b- accumulation of neutral mucins and glycogen in the cytoplasm of the epithelial cells c- condensation of fine collagenic fibers and thin-walled blood vessels around the growing tip. WESSELLS (1977) clarified that the removal of mesenchyme from the surface of embryonic mouse soliviary epithelium by enzymatic embryonic digestion resulted in the failure of epithelial branching. The role of mesenchyme and its collagen in the initiation of the epithelial branching is explained by BERNFIELD and BANERJEE (1982).

The process of branching was followed by luminization of the duct system. The present investigation suggested that the luminization of the epithelial ducts begins in the older (larger) ducts and gradually proceeds to the younger (smaller) segments.

A result which conferm the observation of YADM (1985) in buffalo fetuses. Following the luminization, is the differentiation of the ductal epithelium into stratified columnar with many goblet cells. The latter showed a high content of acid carboxylated mucins, meanwhile the columnar variety contained neutral mucins. The number of goblet cells was greater among the epithelium of larger than that of the smaller ducts.

The undifferentiated acini were firstly observed at the 140 mm CVRL stage as solid cellular balls. Soon, during the succeeding fetal stages, luminization and cyto-differentiation of such acini were gradually advanced. The differentiated acinar cells were often truncated pyramidal and their cytoplasmic secretory precursors were predominatly neutral mucins and glycogen granules. In addition to the latter cells, one or two mucous cells were observed among the majority of acini containing acid carboxylated mucins. Accompanying acinar differentiation, is the great diminution of the interstitial stroma with substantial increase in the glandular elements. From the aforementioned data, it is concluded that the parotid salivary gland of the camel fetus at term becomes compound acinar in form and its secretory products include both the neutral mucins, which predominate, and the mucous mucins produced from the few mucous acinar cells and the ductal goblet cells and the gland is ready to perform its function just after birth.

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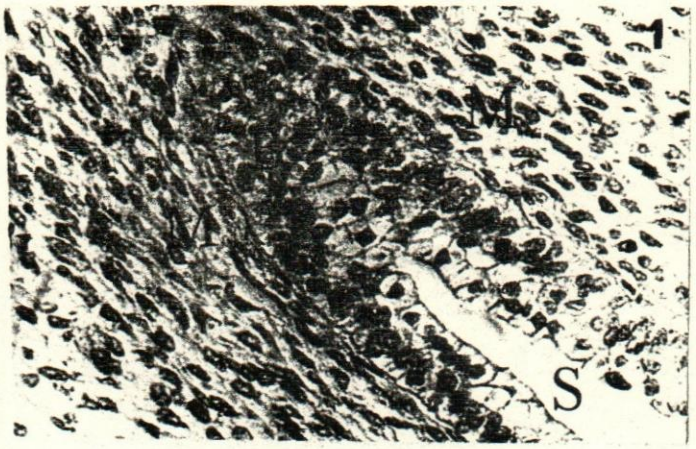
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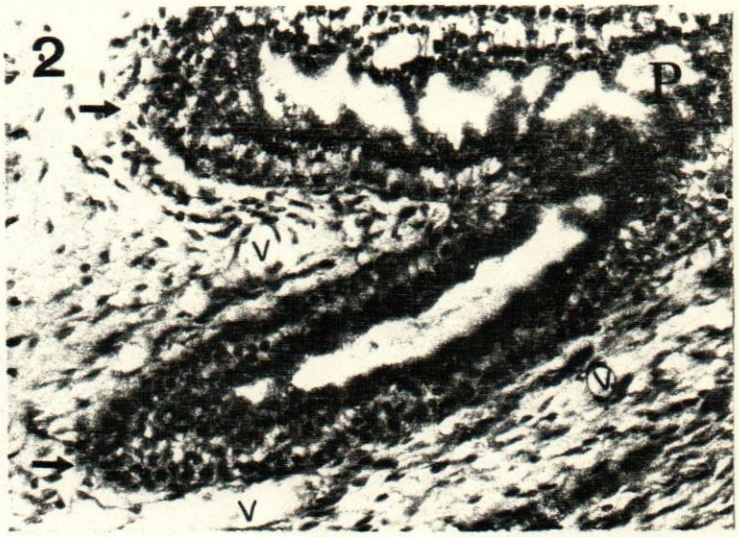
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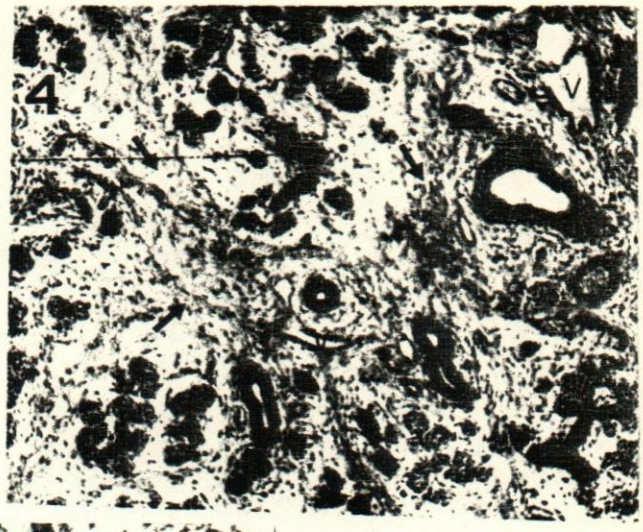
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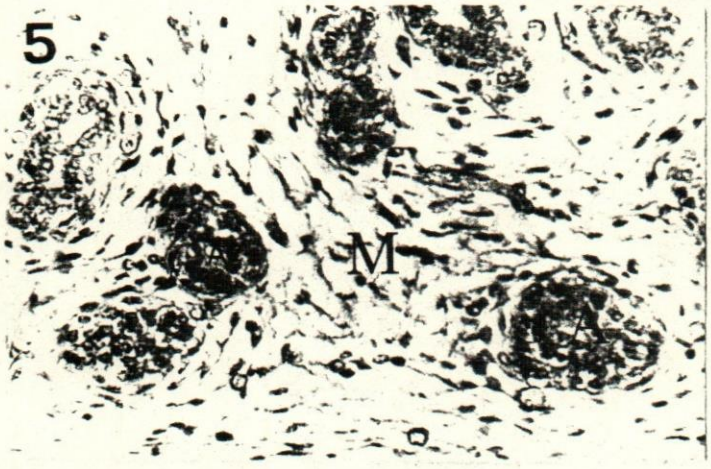
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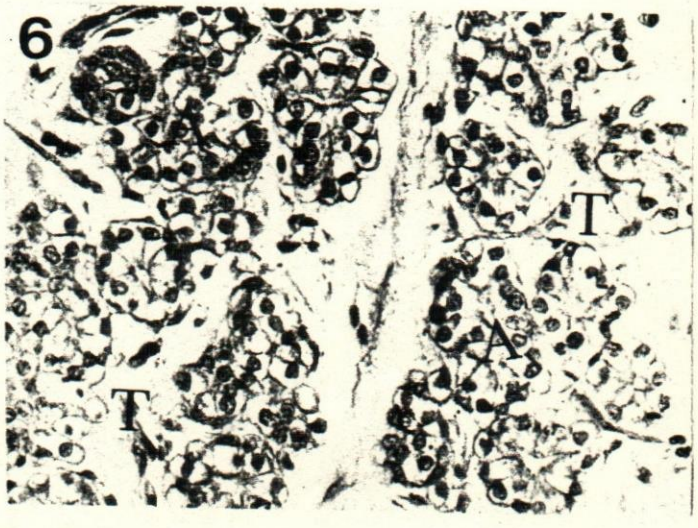
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LIST OF FIGURES

Fig. (1): Photomicrograph of a section of the parotid salivary gland primordium in a fetus of 32 mm CVRL showing; mouth sulcus (S) surrounded with cellular avascular mesenchyme (M). The cellular bud at the mouth sulcus (B) represented the primitive parotid salivary gland. (H & E stain x 500).

Fig. (2): Photomicrograph of a section of the parotid gland primordium in a fetus of 80 mm CVRL showing:
The parotid anlage (P) became canalized epithelial structure with bifurcated tip. The surrounded mesenchyme showed many thin-walled blood vessels (V). Areas of epithelio-mesenchymal contact (arrow). (Van Gison stain x125).

Fig. (3): Photomicrograph of a section of the parotid gland primordium in a fetus of 140 mm CVRL showing, an epithelial duct (D) associated with solid cellular balls (B) which represent the future acini. (PAS technique, x 312.5).

Fig. (4): Photomicrograph of a section of the parotid salivary gland primordium in a fetus of 200 mm CVRL showing, the initiation of lobular formation through the condensation of collagenic fibers (arrow) around groups of the glandular elements which were associated with many thin-walled blood vessels (V). (Van Gieson stain, x 125).

Fig. (5): Photomicrograph of a section of parotid gland primordium in a fetus 290mm CVRL showing, differentiated acini (A) surrounded with a large amount of mesenchyme (M). (H & E. stain, x 500).

Fig. (6): Photomicrograph of a section of parotid gland primordium in a fetus 950 mm CVRL showing,marked increase in the number of the differentiated acini (A) in a given lobule with a substantial decrease in the interstitial tissue (T). (H & E. stain, x 500).