J. Embryol. exp. Morph. Vol. 66, pp. 209–221, 1981
Printed in Great Britain © Company of Biologists Limited 1981

Mesenchymal control over elongating and branching morphogenesis in salivary gland development

By HIROYUKI NOGAWA[1] AND TAKEO MIZUNO[1]

From the Zoological Institute, Faculty of Science, University of Tokyo

SUMMARY

Recombination of the epithelium and mesenchyme between quail anterior submaxillary gland (elongating type) and quail anterior lingual or mouse submaxillary gland (branching type) was effected *in vitro* to clarify whether the elongating morphogenesis was directed by the epithelial or the mesenchymal component. Quail anterior submaxillary epithelium recombined with quail anterior lingual or mouse submaxillary mesenchyme came to branch. Conversely, quail anterior lingual or 12-day mouse submaxillary epithelium recombined with quail anterior submaxillary mesenchyme came to elongate, though the mesenchyme was less effective with 13-day mouse submaxillary epithelium. These results suggest that the elongating or branching morphogenesis of quail salivary glands is controlled by the mesenchyme.

INTRODUCTION

We have previously described the unique morphogenesis of quail anterior submaxillary gland, which simply elongates without branching (Fig. 1), and demonstrated (1) that mitoses are evenly distributed through the rudiment, (2) that directional mitoses parallel to the long axis of the rudiment have a low probability, and (3) that epithelial cells do not elongate along the long axis of the rudiment (Nogawa, 1981). These observations suggest that the morphogenesis of this gland is not directly prescribed by the mitotic pattern and shape of individual cells of the rudiment.

In branching organs, such as lung, salivary gland and mammary gland of the mouse, it is known that epithelial morphogenesis can be altered under the influence of heterotypic mesenchyme. For example, bronchial mesenchyme induces the tracheal epithelium to branch while tracheal mesenchyme inhibits branching of the bronchial epithelium (Alescio & Cassini, 1962; Wessells, 1970); mammary epithelium branches dichotomously (salivary type) instead of monopodially (mammary type) when recombined with salivary mesenchyme (Kratochwil, 1969; Sakakura, Nishizuka & Dawe, 1976).

Quail has not only elongating-type but also branching-type salivary glands

[1] *Authors' address:* Zoological Institute, Faculty of Science, University of Tokyo, Hongo, Tokyo 113, Japan.

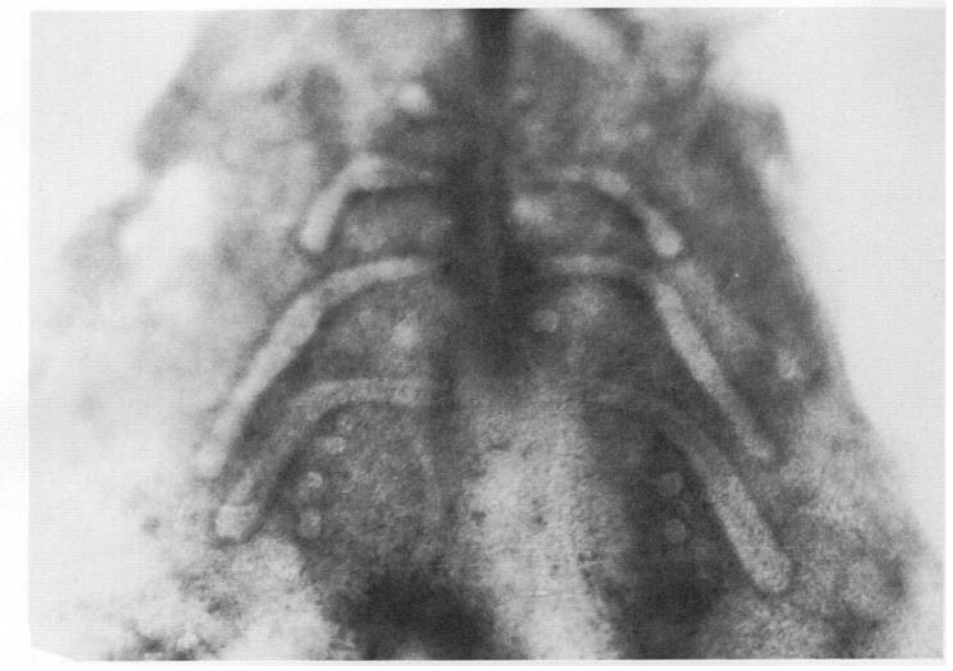


Fig. 1. A dorsal view of 9-day quail anterior submaxillary gland. Three pairs of anterior submaxillary rudiments originate straddling the midline, and elongate obliquely-backwards in the mesenchyme. × 60.

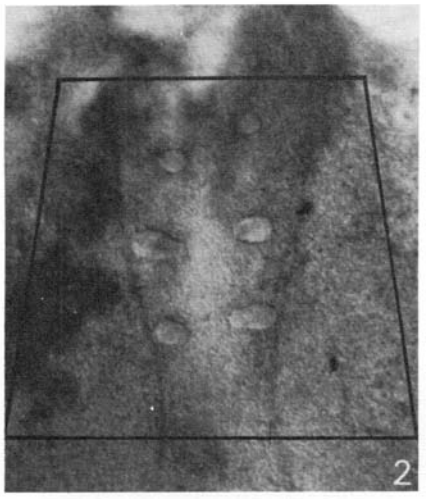
(Nogawa, 1978). In the present study we examined whether the elongating morphogenesis of quail anterior submaxillary originated from the nature of the epithelium or whether it was directed by the mesenchymal component. This was investigated in experiments involving recombination of the epithelium and mesenchyme between the elongating-type (quail anterior submaxillary) and branching-type (quail anterior lingual and mouse submaxillary) salivary glands.

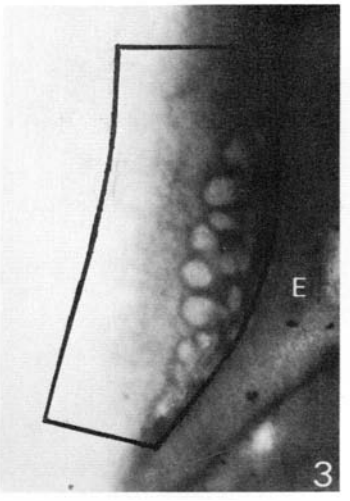
MATERIALS AND METHODS

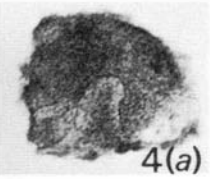
Isolation of salivary rudiments

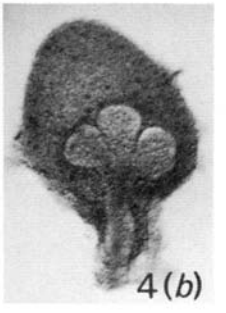
Eggs of Japanese quail (*Coturnix coturnix japonica*) were incubated at 38 °C. Anterior submaxillary rudiments were isolated from the lower jaw of 8-day embryos: a piece of the floor of the mouth in front of the intersection of the ventral side of the tongue and the floor was isolated from the mandibular bone and the underlying muscular layer (Fig. 2). Anterior lingual rudiments were isolated from the tongues of 8- and 9-day embryos: two tongue fragments were cut off, one on each side of the entoglossal cartilage (Fig. 3).

ICR mice were mated during the night, and the morning of the discovery of the vaginal plug was counted as day 0. Submaxillary rudiments, free from an









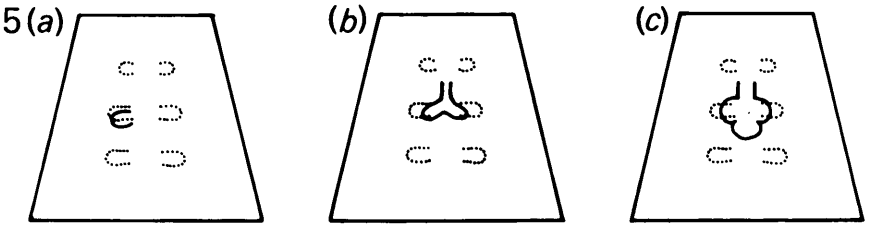


Fig. 2. The floor of an 8-day quail mouth. The region within a solid line including anterior submaxillary rudiments was isolated. × 40.

Fig. 3. A ventral view of the right half of a 9·5-day quail tongue. The region within a solid line including anterior lingual rudiments was cut off along the entoglossal cartilage (E). × 40.

Fig. 4. A 12- (*a*) and a 13-day (*b*) mouse submaxillary gland. The 13-day epithelium consists of three lobes, and one lobe is as large as the 12-day epithelium. × 40.

Fig. 5. Orientation of epithelia recombined with quail anterior submaxillary mesenchyme. ....., Former positions of quail anterior submaxillary epithelia as observed in Fig. 2; ——, an explanted epithelium: (*a*) an 8-day quail anterior submaxillary, an 8-day quail anterior lingual, a 12-day mouse submaxillary or one lobe of a 13-day mouse sub-maxillary epithelium, (*b*) a 9-day quail anterior lingual epithelium, and (*c*) a 13-day mouse submaxillary epithelium.

accompanied sublingual rudiment, were isolated from 12- and 13-day foetuses according to the procedure of Borghese (1950) (Fig. 4 *a*, *b*).

Separation of epithelia and mesenchymes

Each isolated salivary rudiment was exposed to collagenase (Worthington Biochemical Corporation, New Jersey, U.S.A.; CLSPA, 0·03% in Tyrode's solution) at 38 °C for 60 min. Epithelia and mesenchymes of quail salivary

rudiments were separated with fine forceps, and those of mouse submaxillary rudiments were separated after gentle teasing with a small-bore pipette. Separated tissue fragments were rinsed twice in a mixture of Tyrode's solution and horse serum (1:1), and stored in a solution consisting of Tyrode's solution, chick embryo extract and horse serum (7:3:3) at room temperature until they were used.

Organ culture

Explants were cultivated according to the method of Wolff & Haffen (1952). The medium comprised seven parts of agar (1 % in Gey's solution), three parts of digestive-tract-free and salivary-gland-free 12-day chick embryo extract (50 % in Tyrode's solution) and three parts of horse serum (Flow Laboratories, Virginia, U.S.A.), and contained penicillin G potassium (300 units/ml).

Epithelia and mesenchymes were recombined with particular attention to two points. The first was the volume of the mesenchyme and epithelium. Lawson (1974) reported that the volume of mesenchyme influenced the morphogenetic activity of epithelium. To equalize the volume of mesenchyme in a recombinant, one isolated 8-day quail anterior submaxillary mesenchyme, an assemblage of two isolated 9-day quail anterior lingual mesenchymes or that of three isolated 13-day mouse submaxillary mesenchymes was used. As for epithelia, the volume was made equal as far as possible. The second point was the orientation of the recombined epithelium. In normal development quail anterior submaxillary rudiment elongates obliquely backwards away from the midline (Fig. 1). To place an epithelium on the anterior submaxillary mesenchyme in the same orientation as *in vivo*, the epithelium was placed on the mesenchyme as shown in Fig. 5. In the case of the other branching-type salivary mesenchymes, an epithelium was placed on the centre of the mesenchymal mass.

Estimation of morphology

It was difficult to observe the detailed morphology of epithelia in living recombinants with quail salivary mesenchymes because of the low contrast between the epithelium and mesenchyme. In such cases the morphology of the recombinants was estimated both from the outline sketches of living explants and from the reconstruction of serial frontal sections, cut at 5 μm and stained with haematoxylin and eosin or with azan.

The morphology of epithelia was classified into three types: 'branching', 'elongating' and 'round'. Among non-branching epithelia, the epithelium the length of which exceeded twice the width was classified as 'elongating', and that the length of which was less than twice the width was classified as 'round'. At the beginning of the cultivation, 8-day quail salivary epithelia, 12-day and a lobe of 13-day mouse submaxillary epithelia were round.

RESULTS

Morphogenesis of quail anterior submaxillary epithelium recombined with homo- and heterotypic mesenchymes

In most recombinants of 8-day quail anterior submaxillary epithelium and the homotypic mesenchyme, the epithelium elongated without branching (Table 1, Fig. 6). Since an elongating epithelial head reached the periphery of the explant within 2 cultivation days, further elongation rarely occurred on the 3rd day *in vitro*. A few of the recombinants showed branching morphogenesis, but the number of branches was less than three.

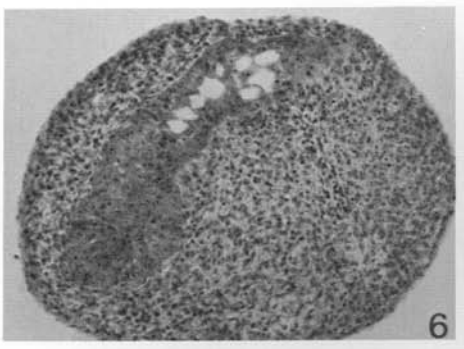
Quail anterior submaxillary epithelium (elongating type) came to branch when recombined with salivary mesenchymes of branching type (Table 1). Quail anterior lingual mesenchyme (branching type) caused the epithelium to branch on day 1 of cultivation, and elongating epithelium without branches was not observed on day 2 (Fig. 7). Mouse submaxillary mesenchyme (branching type) also caused the epithelium to branch: the epithelium sprouted several spherical buds without forming stalk regions on day 2 (Fig. 8*a*, *b*). This branching pattern, however, was different from that of the quail anterior submaxillary epithelium recombined with quail anterior lingual mesenchyme (Fig. 7) or of the mouse submaxillary epithelium homotypically recombined (Fig. 15) which branched forming stalks in a tree-fashion.

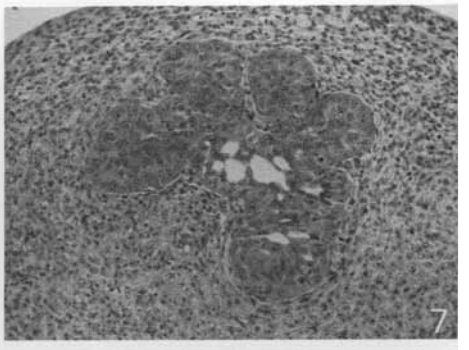
The question then arose as to whether quail anterior submaxillary mesenchyme could influence the other salivary epithelia and alter their morphogenesis.

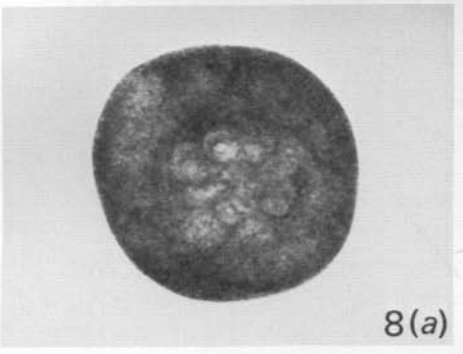
Influence of quail anterior submaxillary mesenchyme on the morphogenesis of quail anterior lingual epithelium

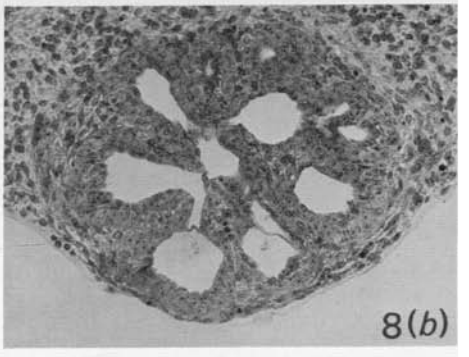
Eight-day quail anterior lingual epithelium was cultivated recombined with quail anterior lingual or submaxillary mesenchyme (Table 2). In the homotypic recombinants, the epithelium branched (Fig. 9) in half the cases on day 2 of cultivation. In contrast, when recombined with the anterior submaxillary mesenchyme, the epithelium elongated (Fig. 10) in half the cases and branched in only a few cases on day 2.

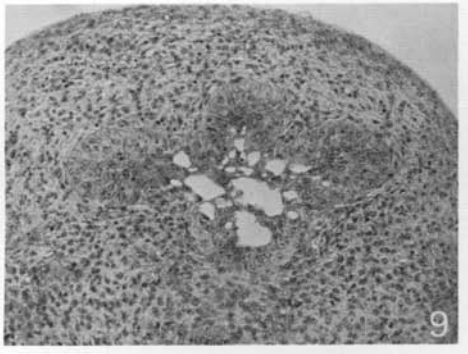
We then examined whether the quail anterior submaxillary mesenchyme could affect the morphogenesis of 9-day quail anterior lingual epithelium (Table 2): the epithelium of the 9-day gland began to fork into two branches (Fig. 3). On day 2 of cultivation the epithelium branched further in the homotypic recombinants (Fig. 11), while it failed to branch further in most recombinants with the quail anterior submaxillary mesenchyme and two existing branches elongated in half the cases (Fig. 12).

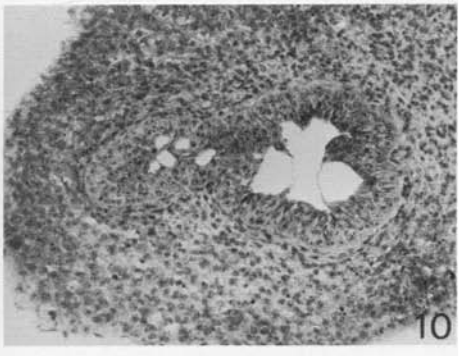

6

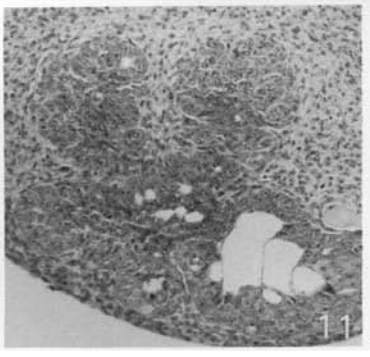

7


8(*a*)


8(*b*)


9


10


11

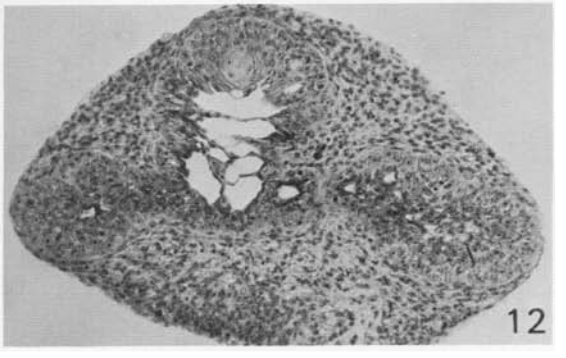

12

Table 1. *Morphogenesis of 8-day quail anterior submaxillary epithelium recombined with homo- or heterotypic mesenchyme*

Mesenchyme	Days in culture	Morphology			Total no. of recombinants
		Branching	Elongating	Round	
Quail anterior submaxillary	1	2	14	4	20
	2	3	15	4	22
	3	3	15	3	21
Quail anterior lingual	1	10	4	2	16
	2	13	0	4	17
Mouse submaxillary	1	4	0	7	11
	2	13	0	0	13

Influence of quail anterior submaxillary mesenchyme on the morphogenesis of mouse submaxillary epithelium

Since quail anterior submaxillary epithelium showed branching morphogenesis in response to mouse submaxillary mesenchyme, the morphogenesis in the reverse recombination was then examined (Table 3). In the present paper, three or four lobes initially formed as shown in Fig. 4(*b*) were named 'primary' branches, and the further branches were called 'secondary'.

When 12-day mouse submaxillary epithelium was recombined with the homospecific, homotypic mesenchyme, it remained round on day 1 of cultivation, but it formed primary branches on day 2, and branched secondarily on day 3 (Fig. 13). When recombined with quail anterior submaxillary mesenchyme, the epithelium remained round in three-quarters of the cases on day 2, but on day 3

Fig. 6. A section of the 8-day quail anterior submaxillary epithelium cultured in combination with the homotypic mesenchyme for 2 days. × 150.

Fig. 7. A section of the 8-day quail anterior submaxillary epithelium cultured in combination with quail anterior lingual mesenchyme for 2 days. × 150.

Fig. 8. The 8-day quail anterior submaxillary epithelium cultured in combination with mouse submaxillary mesenchyme for 2 days. (*a*) A living recombinant (× 40), and (*b*) a section of it (× 150). The epithelium forms several lobes lacking stalk regions.

Fig. 9. A section of the 8-day quail anterior lingual epithelium cultured in combination with the homotypic mesenchyme for 2 days. × 150.

Fig. 10. A section of the 8-day quail anterior lingual epithelium cultured in combination with quail anterior submaxillary mesenchyme for 2 days. × 150.

Fig. 11. A section of the 9-day quail anterior lingual epithelium cultured in combination with the homotypic mesenchyme for 2 days. × 150.

Fig. 12. A section of the 9-day quail anterior lingual epithelium cultured in combination with quail anterior submaxillary mesenchyme for 2 days. Two lobes having been already formed at the beginning of the cultivation elongate without branching. × 150.

Table 2. *Morphogenesis of 8- and 9-day quail anterior lingual epithelia recombined with homo- or heterotypic mesenchyme*

Epithelium	Mesenchyme	Days in culture	Morphology			Total no. of recombinants
			Branching	Elongating	Round	
8-day	Quail anterior lingual	1	5	3	7	15
		2	7	3	4	14
	Quail anterior submaxillary	1	0	10	9	19
		2	3	11	6	20
9-day	Quail anterior lingual	2	10	0	2*	12
	Quail anterior submaxillary	2	1	6	5*	12

* These values include the number of recombinants in which epithelium did not further branch nor elongate though having two branches, because the 9-day epithelium had two branches at the beginning of the cultivation.

it elongated in half the cases (Fig. 14), though primary branches were formed in a few cases. These results show that the quail anterior submaxillary mesenchyme can also affect the morphogenesis of the mouse submaxillary epithelium.

Once 13-day mouse submaxillary epithelium was separated from the capsular mesenchyme with partially crude collagenase, it took a round shape with disappearance of clefts within 6 h in cultivation with mesenchymes as was reported by Bernfield, Banerjee & Cohn (1972). Thereafter, the epithelium branched well in recombination with the homotypic mesenchyme (Fig. 15*a*, *b*). On the other hand, when recombined with quail anterior submaxillary mesenchyme, the

Fig. 13. A section of the 12-day mouse submaxillary epithelium cultured in combination with the homotypic mesenchyme for 3 days. × 150.

Fig. 14. A section of the 12-day mouse submaxillary epithelium cultured in combination with quail anterior submaxillary mesenchyme for 3 days. × 150.

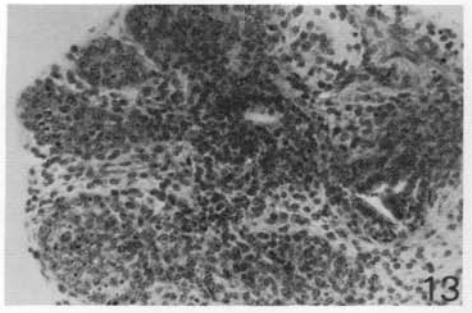
Fig. 15. The 13-day mouse submaxillary epithelium cultured in combination with the homotypic mesenchyme for 3 days. (*a*) A living recombinant (× 40), and (*b*) a section of it (× 150).

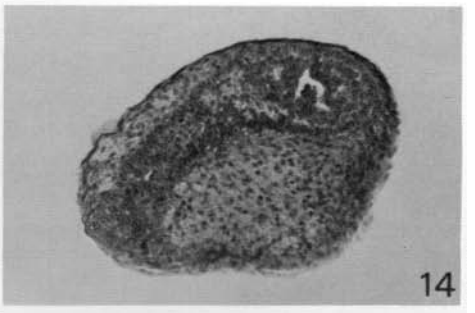
Fig. 16. A section of the 13-day mouse submaxillary epithelium cultured in combination with quail anterior submaxillary mesenchyme for 3 days. Three epithelial lobes elongate without branching in contrast to Fig. 17. × 150.

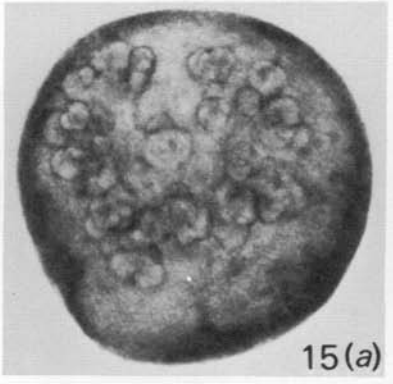
Fig. 17. A section of the 13-day mouse submaxillary epithelium cultured in combination with quail anterior submaxillary mesenchyme for 3 days. × 150.

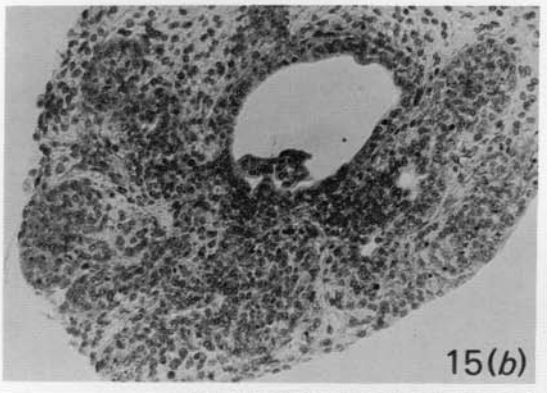
Fig. 18. A section of one lobe of 13-day mouse submaxillary epithelium cultured in combination with the homotypic mesenchyme for 3 days. × 150.

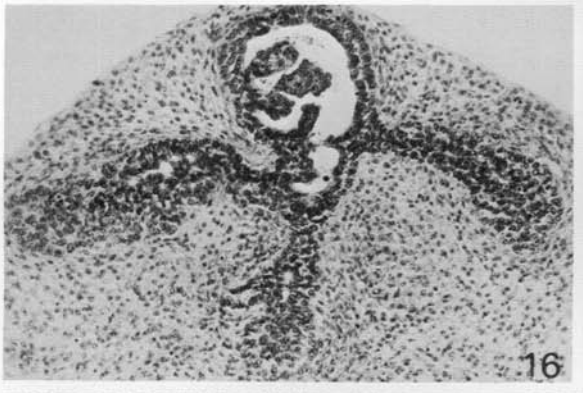
Fig. 19. A section of one lobe of 13-day mouse submaxillary epithelium cultured in combination with quail anterior submaxillary mesenchyme for 3 days. × 150.

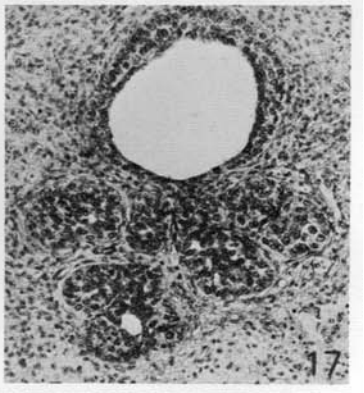

13

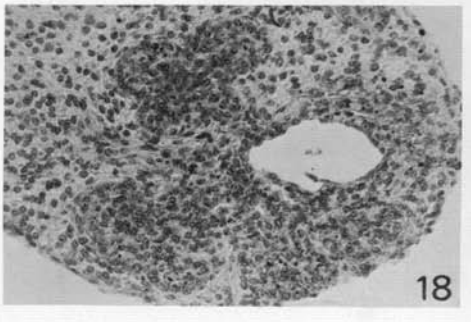

14


15(*a*)


15(*b*)


16


17


18

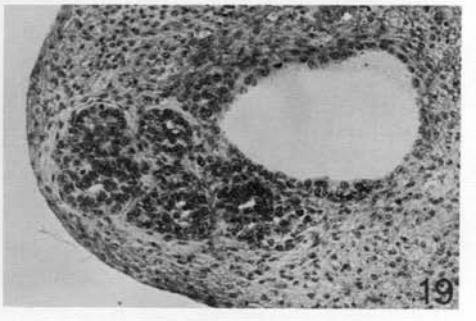

19

Table 3. *Morphogenesis of 12- and 13-day mouse submaxillary epithelia recombined with homo- or heterotypic mesenchyme*

Epithelium	Mesenchyme	Days in culture	Morphology: Branching S	Morphology: Branching P	Morphology: Elongating	Morphology: Round	Total no. of recombinants
12-day whole	Mouse submaxillary	1	0	4	0	8	12
		2	3	9	0	2	14
		3	14	0	0	0	14
	Quail anterior submaxillary	1	0	0	0	9	9
		2	0	3	2	15	20
		3	0	2	7	5	14
13-day whole	Mouse submaxillary	1	0	10	0	2	12
		2	14	0	0	0	14
		3	12	0	0	0	12
	Quail anterior submaxillary	1	0	14	0	0	14
		2	2	10	0	0	12
		3	5	8	0	0	13
13-day* lobe	Mouse submaxillary	1	0		0	12	12
		2	14		0	0	14
		3	20		0	0	20
	Quail anterior submaxillary	3	8		4	1	13

S, The number of recombinants branching secondarily; P, the number of recombinants branching primarily.

* Branches in recombinants of the 13-day lobe were regarded as secondary, because the lobe itself was formed by primarily branching.

epithelium formed primary branches similarly to the homotypic recombinants on day 1 of cultivation, then the primary branches in some recombinants elongated without branching (Fig. 16) and those in some others continued to branch (Fig. 17) on day 2 and 3. These results show that the quail anterior submaxillary mesenchyme can influence the morphogenesis of the 13-day mouse submaxillary epithelium to a lesser extent than that of the 12-day epithelium. Since such a difference might be due to the fact that the 13-day epithelium is larger than the 12-day epithelium (cf. Fig. 4*a*, *b*), one lobe of the 13-day epithelium which was as large as a whole 12-day epithelium was used (Table 3). The lobe recombined with the homospecific, homotypic mesenchyme did not branch on day 1 of cultivation but branched normally thereafter (Fig. 18). When recombined with quail anterior submaxillary mesenchyme, the lobe branched in 8 out of 13 cases on day 3 (Fig. 19). These results suggest that the 13-day mouse submaxillary epithelium may have been determined to branch, and can branch even under the influence of the mesenchyme of elongating-type gland.

DISCUSSION

Experiments involving recombination of the epithelium and mesenchyme between quail anterior submaxillary (elongating type) and quail anterior lingual (branching type) glands *in vitro* showed that the elongating-type epithelium recombined with the branching-type mesenchyme came to branch, and conversely that the elongating-type mesenchyme did not permit the branching-type epithelium to branch further but caused it to elongate. These results suggest that the elongating or branching morphogenesis of quail salivary glands is directed by the mesenchyme, and agree with the results of recombination experiments between trachea and bronchus of mouse lung (Alescio & Cassini, 1962; Wessells, 1970) or mouse mammary and salivary glands (Kratochwil, 1969; Sakakura *et al.* 1976).

In the present study it was also demonstrated that quail anterior submaxillary epithelium could branch in response to the mesenchyme of mouse submaxillary gland (branching type). However, its branching pattern was not typical of normal mouse submaxillary gland: the quail epithelium did not have any stalk regions (Fig. 8), while mouse submaxillary epithelium recombined with its own mesenchyme developed branches with stalk regions (Figs. 13, 15, 18). Quai anterior lingual epithelium recombined with mouse submaxillary mesenchyme also branched without forming stalk regions (data not shown). To explain the difference between quail and mouse salivary epithelia, we might postulate that mouse submaxillary epithelium is able to form stalk regions *per se* and its mesenchyme has no capacity of inducing stalk regions, or that quail salivary epithelium possesses no competence to form stalk regions although mouse submaxillary mesenchyme has the capacity of inducing stalk regions. The former may be more probable, because branches which were formed in quail salivary epithelia recombined with quail anterior lingual mesenchyme had stalk regions (Figs. 7, 9 and 11 in this paper), and both mesenchymes of stalk and bulb regions of mouse submaxillary gland can support the branching morphogenesis of mouse submaxillary epithelium (Wessells, 1970).

In the present study, it was further demonstrated that quail anterior submaxillary mesenchyme could cause the 12-day mouse submaxillary epithelium to elongate, but it mostly allowed the 13-day epithelium to branch. Since the mesenchyme of the elongating-type gland does not seem to have the capacity to induce branches, the 13-day epithelium may have been determined to branch and may be able to branch even under the influence of the mesenchyme of the elongating-type gland. In any case there is a significant difference between the 12- and 13-day mouse submaxillary epithelia. In rat submaxillary glands, it was reported that isolated 15-day epithelium (not yet branched) failed to attach to the culture dish and degenerated in culture, while isolated 16-day epithelium (primarily branched) could attach to the dish and underwent cytodifferentiation (Cutler, 1980).

Sherman (1960) has reported that chick submandibular gland, which seems to correspond to quail anterior submaxillary gland, elongated *in vivo* and *in vitro*, and that its mesenchyme partially supported the branching morphogenesis of mouse submaxillary epithelium, while chick submandibular epithelium recombined with mouse submaxillary mesenchyme showed no sign of morphogenesis. His result with chick submandibular epithelium recombined with mouse submaxillary mesenchyme is not consistent with the present result that the 8-day quail anterior submaxillary epithelium similarly recombined comes to branch. This may be because the 12-day chick submandibular epithelium used in his experiments was too old to respond to mouse submaxillary mesenchyme.

The present study shows that the elongating morphogenesis of quail anterior submaxillary gland is controlled by the mesenchyme. How then does the mesenchyme control epithelial elongating morphogenesis? Observations *in vivo* indicate that the mesenchyme does not specifically stimulate the epithelial cell proliferation of the distal end of the rudiment nor lengthen epithelial cells along the long axis of the rudiment (Nogawa, 1981). Although there is a hypothesis that localized epithelial cell proliferation which is stimulated by the mesenchyme determines the morphogenetic pattern (Goldin & Opperman, 1980), this is not the case in the morphogenesis of this salivary gland. We also reported in the previous paper that the basement membrane was more obscure in the distal part than in the rest of the quail anterior submaxillary rudiment, and that mesenchymal cells encircled the epithelial cord perpendicularly to its long axis *in vivo* (Nogawa, 1981). Since an important role of the basement membrane in maintaining epithelial morphology has been postulated by Banerjee, Cohn & Bernfield (1977), the epithelial morphology will change in the part where the basement membrane is weak. If such a weak part continues to be limited to the distal end of growing rudiments, the rudiments will elongate. Mesenchymal cells of the quail anterior submaxillary rudiment might control the epithelial elongating morphogenesis by regulating the formation of the basement membrane or by encircling the epithelial cord so as to maintain the morphology of parts other than the distal end.

Salivary glands of quail embryos are suitable materials for the investigation of morphogenetic mechanisms, and we hope to elucidate the distribution of the basement membrane and collagen fibrils, and the behaviour of the epithelial and mesenchymal cells during elongating and branching morphogenesis, by electron microscopy or microcinematography in future work.

REFERENCES

ALESCIO, T. & CASSINI, A. (1962). Induction *in vitro* of tracheal buds by pulmonary mesenchyme grafted on tracheal epithelium. *J. exp. Zool.* **150**, 83–94.

BANERJEE, S. D., COHN, R. H. & BERNFIELD, M. R. (1977). Basal lamina of embryonic salivary epithelia: production by the epithelium and role in maintaining lobular morphology. *J. Cell Biol.* **73**, 445–463.

BERNFIELD, M. R., BANERJEE, S. D. & COHN, R. H. (1972). Dependence of salivary epithelial morphology and branching morphogenesis upon acid mucopolysaccharide-protein (proteoglycan) at the epithelial surface. *J. Cell Biol.* **52**, 674–689.

BORGHESE, E. (1950). The development *in vitro* of the submandibular and sublingual glands of *Mus musculus*. *J. Anat., Lond.* **84**, 287–302.

CUTLER, L. S. (1980). The dependent and independent relationships between cytodifferentiation and morphogenesis in developing salivary gland secretory cells. *Anat. Rec.* **196**, 341–347.

GOLDIN, G. V. & OPPERMAN, L. A. (1980). Induction of supernumerary tracheal buds and the stimulation of DNA synthesis in the embryonic chick lung and trachea by epidermal growth factor. *J. Embryol. exp. Morph.* **60**, 235–243.

KRATOCHWIL, K. (1969). Organ specificity in mesenchymal induction demonstrated in the embryonic development of the mammary gland of the mouse. *Devl Biol.* **20**, 46–71.

LAWSON, K. A. (1974). Mesenchyme specificity in rodent salivary gland development: the response of salivary epithelium to lung mesenchyme *in vitro*. *J. Embryol. exp. Morph.* **32**, 469–493.

NOGAWA, H. (1978). The development of the salivary glands in the Japanese quail. *J. Fac. Sci., Univ. Tokyo IV* **14**, 95–103.

NOGAWA, H. (1981). Analysis of elongating morphogenesis of quail anterior submaxillary gland: absence of localized cell proliferation. *J. Embryol. exp. Morph.* **62**, 229–239.

SAKAKURA, T., NISHIZUKA, Y. & DAWE, C. J. (1976). Mesenchyme-dependent morphogenesis and epithelium-specific cytodifferentiation in mouse mammary gland. *Science, N.Y.* **194**, 1439–1441.

SHERMAN, J. E. (1960). Description and experimental analysis of chick sub-mandibular gland morphogenesis. *Trans. Wis. Acad. Sci.* **49**, 171–189.

WESSELLS, N. K. (1970). Mammalian lung development: interactions in formation and morphogenesis of tracheal buds. *J. exp. Zool.* **175**, 455–466.

WOLFF, ET. & HAFFEN, K. (1952). Sur une méthode de culture d'organes embryonnaires "in vitro". *Tex. Rep. Biol. Med.* **10**, 463–472.

(Received 18 May 1981, revised 3 July 1981)