known, but, this is probably an artefact due to physical cytoplasmic shrinkage involved in HCl digestion and collagenase splitting.

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Epithelial cell processes in the development of the secondary palate in the mouse

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Summary. Ultrastructural studies of palatal shelves of Tuck A mice embryos aged 12.25–14.25 days show discontinuities of the epithelial basement membrane traversed by epithelial cell processes before the onset of midline degenerative changes.

Interaction between ectoderm and mesoderm is a well-known embryogenic mechanism playing a crucial role in the development of many organ systems. The precise way in which mesoderm and ectoderm interact, although much studied, remains unclear.

During the development of the rodent secondary palate an epitheliomesenchymal interaction brings about appropriately timed cell death in a midline epithelial seam which is essential for mesenchymal union between apposed palatal shelves[1-3]. The basal lamina, across which any interaction must take place, is usually described as being continuous until degeneration of this epithelium is complete[4-7], although occasional discontinuities in the basal lamina of the developing mouse palate have been described on the 14th day in utero[8,9]. Epithelial cell processes (ECPs) of basal epithelial cells pass through these discontinuities into the mesenchymal cell compartment. At this stage of development extensive degenerative changes may be observed in the midline epithelial seam, including the appearance of abundant lysosomes[4,8], swelling of mitochondria and clearing of the mitochondrial matrix[5] and pyknosis[4]. The presence of ECPs antedating the degenerative processes has not been previously described.

Pregnant Tuck A females were killed by cervical dislocation at 12.25, 13.25 and 14.25 days of gestation. Fetuses were removed from the uterine horns and the palatal shelves dissected out, using the method of Goss and Avery[11], rinsed in phosphate buffer (pH 7.4), fixed in phosphate-buffered glutaraldehyde at 4 °C for 1.5 h, and postfixed in buffered osmium tetroxide for 1 h. Individual shelves were embedded in TAAB resin at 60 °C for 3 days. Ultrathin sections were cut on an LKB IV ultratome using a glass knife. Mounted sections were stained with uranyl acetate and lead citrate, and examined using an AEI EM6B electron microscope.

Although the basal lamina was generally continuous beneath the epithelium in the region of presumptive fusion at both 12.25 and 13.25 days, occasional discontinuities were seen. Processes from basal epithelial cells passing through these discontinuities into the mesenchymal compartment (figure 1) were very much more common at 13.25 than at 12.25 days. There was no evidence of lysosome

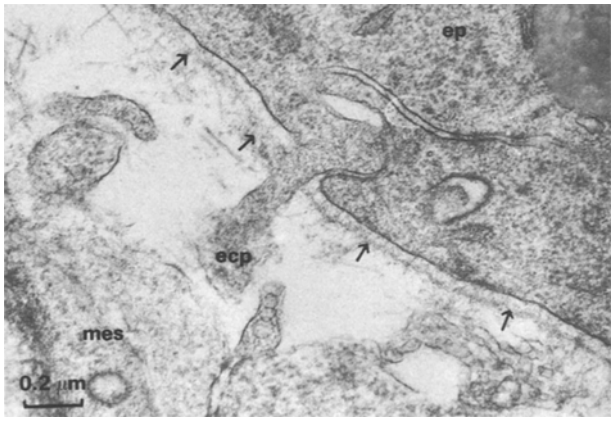

Fig. 1. Electron micrograph showing the basal lamina (arrowed) between epithelium (ep) and mesenchyme (mes) in the 13.25-day-old mouse palatal shelf. There is a discontinuity in the basal lamina which is traversed by an epithelial cell process (ecp). Bar 0.2 µm.

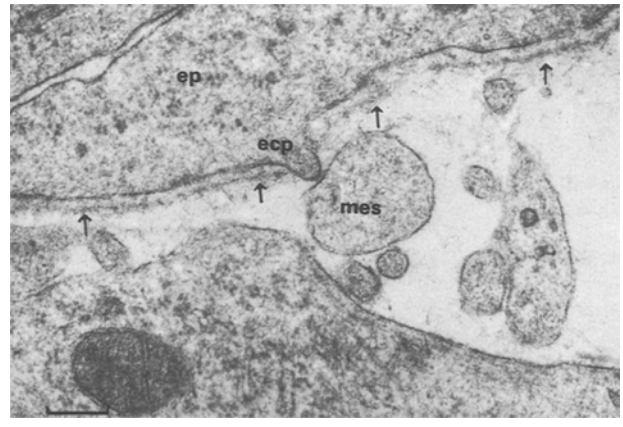

Fig. 2. Electron micrograph showing an epithelial cell process (ecp) in close contact with a subadjacent mesenchymal cell (mes). Bar 0.2 µm.

formation or mitochondrial changes at either age. Degenerative changes, including abundant lysosome formation and pyknosis, were first observed at 14.25 days.

If an epitheliomesenchymal interaction initiates changes leading to cell lysis, it is likely to take place shortly before the time when degenerative changes become apparent. The ECPs represent a potential pathway for communication and may play a crucial role in the epitheliomesenchymal interaction involved in normal palatogenesis.

The relationship between the terminal part of the ECP and any subjacent mesenchymal cell is of interest. 1 example was found of an ECP crossing the basal lamina and terminating close to the plasmalemma of a mesenchymal cell in the same plane of section (figure 2). It would be worthwhile following the progress of an ECP in serial sections to determine if such contacts commonly occur and the nature of junctions, if any, between ECP and mesenchymal plasmalemma.

The observation of cell processes uniting cells involved in embryogenetic interaction does not establish whether such processes are or are not necessary for such an interaction to take place, However, the fact that cell processes similar to ECPs have been described during the formation of the tooth[12] and the amphibian limb bud[13] suggests that ECPs could play a part in many epitheliomesenchymal interactions.

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Enhancement of the antitumor effect of illudin S by including it into liposomes

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Summary. Entrapping illudin S in liposome markedly enhanced life prolonging effect in ddN mice bearing Ehrlich ascites tumors, presumably by decreasing the side effects.

Chemical composition of illudin S, the toxic principle of *Lampteromyces japonicus,* was clarified by Nakanishi and others[2,3] in 1963, and its antitumor effects has been noted from early times[4]. However, illudin S showed fairly strong side effects, such as tissue damage in the liver, and hemorrhagic changes in digestive organs, lung and kidney[5]. Therefore, the problem was how to decrease this side effect without the loss of its antitumor effect. Based on such a concept, we examined the in vivo antitumor effect of illudin S in mice implanted with Ehrlich ascites tumor cells, using positively charged liposomes to decrease the side effects and to increase the antitumor effect of illudin S further.

Materials and methods. Illudin S was purified from *Lampteromyces japonicus* by the method of Nakanishi et al.[2]. Positively charged and sonicated liposomes entrapping illudin S were prepared by the method of Kimelberg[6], and the composition was lecithin (purified from chicken egg yolk by the method of Rhodes and Lea[7]), cholesterol (Nakarai Kagaku Co., Tokyo, Japan) and stearylamine (Tokyo Kasei Kogyo Co., Japan) in 3.2:2.2:1 molar ratio. Illudin S entrapped in liposomes and non-entrapped illudin S were separated by the method previously described[8]. Animals used for therapeutic experiments were male ddN mice (6 weeks old, weighing 20 g), divided into groups of 10 each. Mice in each group were inoculated i.p. with a suspension of Ehrlich ascites carcinoma cells (2×10^7 cells in 0.2 ml per animal) and, 24 h later, administered with aqueous solution of free illudin S or liposome-entrapped illudin S. The controls were given physiological saline. Survival time of the mice was then observed.

Results and discussion. In the group given free illudin S, average survival period of the mice with Ehrlich ascites tumor was longest in the group given 166 μg/kg of free illudin S in aqueous solution, and its percentage relative to the control (T over C) was 170%. In the group given liposome-entrapped illudin S, a more marked antitumor effect was observed, T over C being 225% in the group given 166 μg/kg, and 287% in the group given 333 μg/kg. More than one-half of the mice given 333 μg/kg of liposome-entrapped illudin S showed survival of over 25 days (table).

Komatsu et al.[4] reported that illudin S showed an antitumor effect against Ehrlich ascites carcinoma cells and mouse Sarcoma 180 cells. Illudin S in submicromolar concentrations inhibits the growth of cultured chicken

Chemotherapeutic effect of illudin S free or entrapped in liposomes

	Dose of illudin S (μg/kg)	Mean survival time (days ± SD)	Number of 25-day survivors per 10 mice
Free illudin S	83.0	10.1 ± 3.3	0
	166.0	15.8 ± 8.4	1
	333.0	14.5 ± 6.6	0
	800.0	8.5 ± 2.3	0
Illudin S entrapped in liposome	16.5	9.0 ± 2.8	0
	83.0	14.7 ± 3.3	0
	166.0	20.9 ± 7.4	2
	333.0	26.7 ± 11.1	6
	800.0	11.2 ± 4.0	0
Illudin S + liposome (mixture)	333.0	13.2 ± 1.3	0
Liposome only	0	10.7 ± 4.3	0
Control	0	9.3 ± 1.2	0

Mice were inoculated with 2×10^7 Ehrlich ascites carcinoma cells i.p. on day 0 and treated i.p. 24 h later with a single dose of the drug.