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Connective tissue is involved in adult epithelial development of the small intestine during anuran metamorphosis in vitro

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Summary. The role of connective tissue in metamorphic changes of the small intestinal epithelium in *Xenopus laevis* tadpoles was investigated by using organ culture techniques and electron microscopy. Tissue fragments isolated from various parts of the small intestine at stage 57 were cultivated. Larval cell death of the epithelium was induced by thyroid hormone in all fragments, whereas adult epithelial development was observed only in fragments isolated from the anterior intestinal region containing the typhlosole where most of the larval connective tissue was localized. The epithelium was then cultivated in recombination with homologous or heterologous non-epithelial components. The adult epithelium developed only in recombinants containing a thick connective tissue layer from the typhlosole. There was no regional difference in the developmental potency of the epithelium itself. In all explants where adult epithelium developed, the connective tissue increased in cell density just beneath the epithelium, which was rapidly proliferating and forming typical islets. At the same time, fibroblasts possessing well-developed rough endoplasmic reticulum differentiated close to epithelial cells and often made contact with them. These results indicate that the connective tissue originating from the typhlosole plays an important role in adult epithelial development of the anuran small intestine, probably via direct cell-to-cell contacts or some factor(s) synthesized by the fibroblasts.

Key words: Intestinal epithelium – Anuran metamorphosis – Organ culture – Tissue interaction – Regional difference

Introduction

The epithelium of the anuran small intestine transforms from larval to adult type, accompanying a change in feeding habits, during spontaneous metamorphosis; larval epithelial cells degenerate, whereas adult cells rapidly proliferate, differentiate, and replace the larval cells (Dournon and Chibon 1974; Hourdry and Dauca 1977; McAvoy and Dixon 1977). Although this phenomenon is interesting from the standpoint of organogenesis, its mechanism remains unknown.

Our previous morphological observations on the normal development of the small intestine of *Xenopus* tadpoles have indicated that the epithelial transition from the larval to the adult type is closely related spatio-temporarily to metamorphic changes in the connective tissue. We thus suggested some active roles for connective tissue in the epithelial transition (Ishizuya-Oka and Shimozawa 1987a, b, 1990). Recently, in the anuran skin, the leading role of the connective tissue in epithelial changes during metamorphosis has been shown by transplantation experiments (Kinoshita et al. 1986, 1989; Yoshizato 1989). Although the importance of the mesenchyme in morphogenesis and epithelial differentiation has been amply shown in the avian and mammalian small intestine (Mathan et al. 1972; Ishizuya-Oka and Mizuno 1984; Yasugi and Mizuno 1990), we have very little information on tissue interactions in the amphibian intestine. In a preceding paper, as a first step in examining experimentally the nature of such tissue interactions, we have established an in vitro organ culture system where the epithelium of intact intestinal fragments at stage 57 can undergo the transition from the larval to the adult type as observed during spontaneous metamorphosis (Ishizuya-Oka and Shimozawa 1991). In this system, the most dramatic epithelial changes were induced by thyroid hormone treatment on the fifth day of cultivation; the larval cells rapidly decreased in number, whilst the adult cells rapidly proliferated and formed islets which would later differentiate into a typical absorptive epithelium possessing a brush border. At the same time, interestingly, the connective tissue dramatically changed, as observed during spontaneous metamorphosis. However, the possible effects of the connective tissue on the epithelium have not yet been directly shown. Therefore, the present study aims to clarify the

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role of the connective tissue in the epithelial transition during metamorphosis, using this culture system.

It is known that almost all of the connective tissue of the larval small intestine of *Xenopus* tadpoles is localized in a longitudinal fold, the typhlosole, restricted to the anterior region of the intestine (Kordylewski 1983; Marshall and Dixon 1978; Ishizuya-Oka and Shimozawa 1987a). Thus, it is presumed that this localization of the larval connective tissue may affect metamorphic changes of the epithelium in vitro. In the present paper, we first compared epithelial changes of intact tissue fragments isolated from the anterior region containing the typhlosole with those of fragments isolated from the other region where there is little connective tissue. Next, recombination experiments were done to examine whether differences in the epithelial changes in vitro are really caused by the connective tissue and not by the epithelium itself.

Materials and methods

Preparation of tissue fragments. Tadpoles of the South African clawed frog (*Xenopus laevis*) were purchased from a commercial source in Hamamatsu, Japan. Tadpoles at stage 57 (Nieuwkoop and Faber 1967), when the total length of the small intestine was maximum, were immersed in tap water containing 100 IU/ml of penicillin and 100 μg/ml of streptomycin (GIBCO, Grand Island, USA) overnight and were then dissected for organ cultivation. Tubular fragments, about 3 mm in length, were isolated from various parts of the small intestine (No. 1–6) as shown in Fig. 1, and split open lengthwise with fine forceps. Some of the fragments isolated from part 1 (No. 1 fragments) were further divided with forceps into 1a fragments including the typhlosole and 1b fragments excluding it. Thus, 1 (which were not divided into 1a and 1b), 1a, 2, and 3 fragments contained a thick connective tissue layer of typhlosole, while 1b and 4–6 fragments had very little connective tissue. Some 1 and 5 fragments were treated with dispase (Godo, Tokyo, Japan; 1000 Protease Unit/ml) in Freed's modified solution (Freed and Mezger-Freed 1970) at 32° C for 30 or 45 min and were separated with sharp tweezers into epithelial components (E components) and the remainders, the latter consisting mainly of connective tissue and muscles (non-E components). E components were then recombined with homologous or heterologous non-E components as shown in Fig. 2.

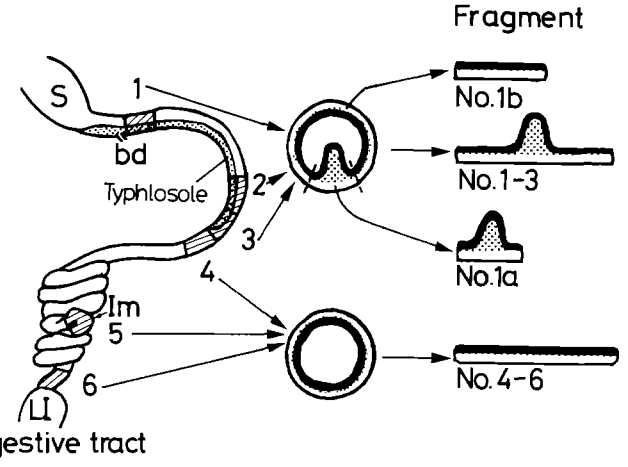


Fig. 1. Isolation of tissue fragments from stage 57 small intestine. Tubular fragments, about 3 mm in length, were cut from parts 1–6 (▨) of the intestine. *S*, Stomach; *bd*, bile duct; *Im*, intestinal portion between outer and inner spirals; *LI*, large intestine; ■, epithelium; ▨, connective tissue

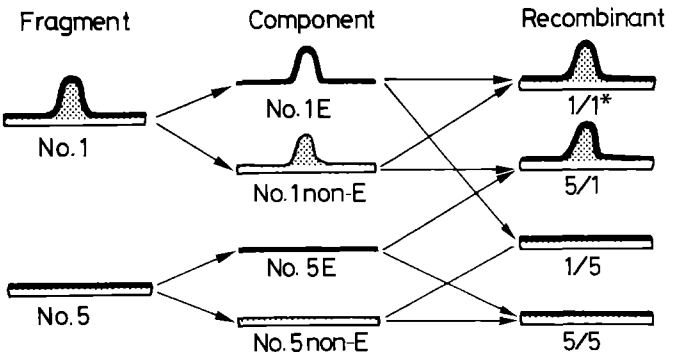


Fig. 2. Types of recombination. ■, Epithelium; ▨, connective tissue. * No. of E (epithelial) component/No. of non-E (=mesodermal) component

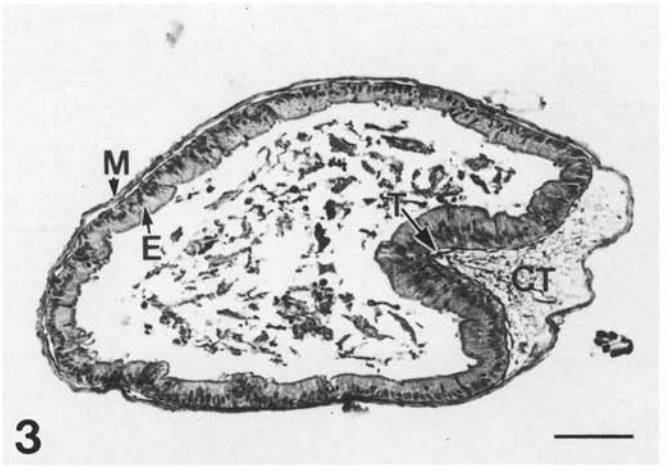
Organ culture. The intact tissue fragments and the recombinants were cultured by the organ culture method described in our previous paper (Ishizuya-Oka and Shimozawa 1991). Briefly, they were placed on membrane filters with a pore size of 1.2 μm (Millipore Corp., Bedford, USA; Type HAWP) laid on stainless steel grids in culture dishes by a modified Trowell's technique (1959). Culture medium was based on 60% Leibovitz-15 medium diluted by sterile water supplemented with 100 IU/ml of penicillin, 100 μg/ml of streptomycin, and 10% fetal bovine serum which had been treated with activated charcoal (Sigma, St. Louis, USA) according to the method of Yoshizato et al. (1980) to remove endogenous thyroid hormones (CTS medium). To induce metamorphosis, 3,3',5-triiodo-L-thyronine (T_3), insulin, and hydrocortisone (Sigma) were added to CTS medium at 10^{-8} M, 5 μg/ml, and 0.5 μg/ml, respectively (Ishizuya-Oka and Shimozawa 1991). Explants were incubated in the culture medium, which was changed every other day, for 5 days at 26° C.

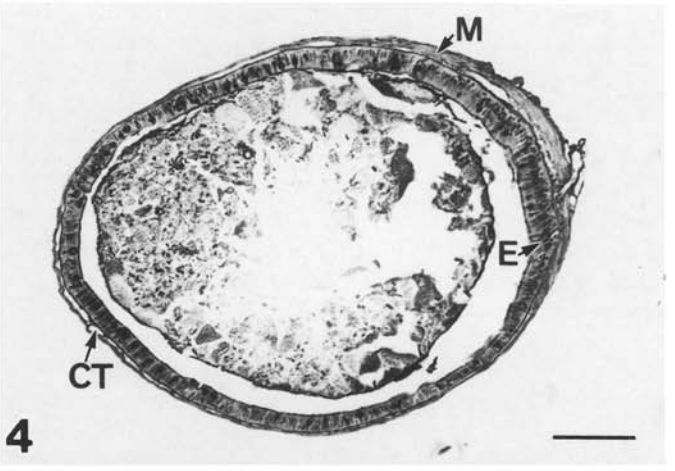
Measurements of the epithelial mitotic index and the cell density of connective tissue. Procedures for measurement of the mitotic index of epithelial cells and the density of connective tissue cells were the same as described in our previous paper (Ishizuya-Oka and Shimozawa 1991). Briefly, the explants were treated with 2 μg/ml of Colcemid (GIBCO) for 4 h and fixed with Bouin's solution. Paraffin sections cut at 4 μm were stained with haematoxylin and eosin. The epithelial mitotic index (%) was calculated as the average of the ratio, metaphase-arrested nuclei/total epithelial nuclei, in more than ten sections randomly selected from each explant. The cell density of the connective tissue was calculated as the average of the number of connective tissue cells/unit areas (0.002 mm^2) just beneath the epithelium in more than five sections randomly selected from each explant. At least five explants were examined for each experiment.

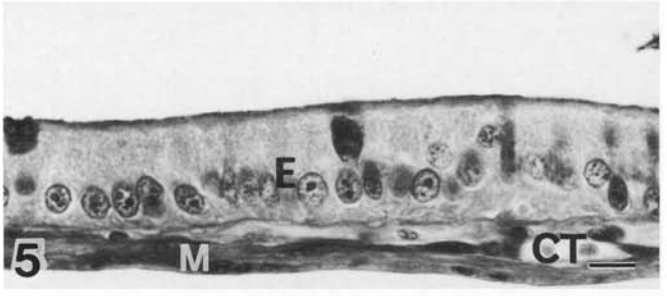
Electron microscopy. The explants were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) at 4° C for 2 h and post-fixed with 1% osmium tetroxide in the same buffer for 2 h at 4° C. They were then stained en bloc with uranyl acetate and embedded in epoxy resin. Ultra-thin sections were stained with lead citrate and examined with a JEOL 200CX electron microscope.

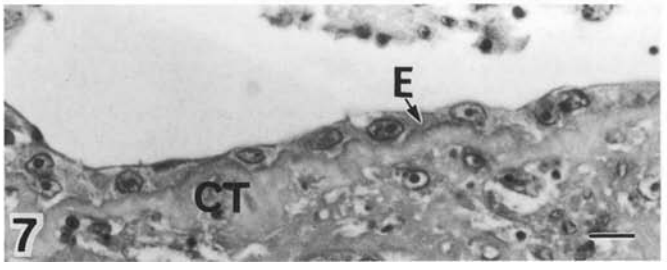
Results

In intact tissue fragments cultured in the presence of T_3 in vitro, the results of the epithelial changes fell into two groups. In 1, 1a, 2, and 3 fragments (Group I) which contained a thick layer of connective tissue at the start









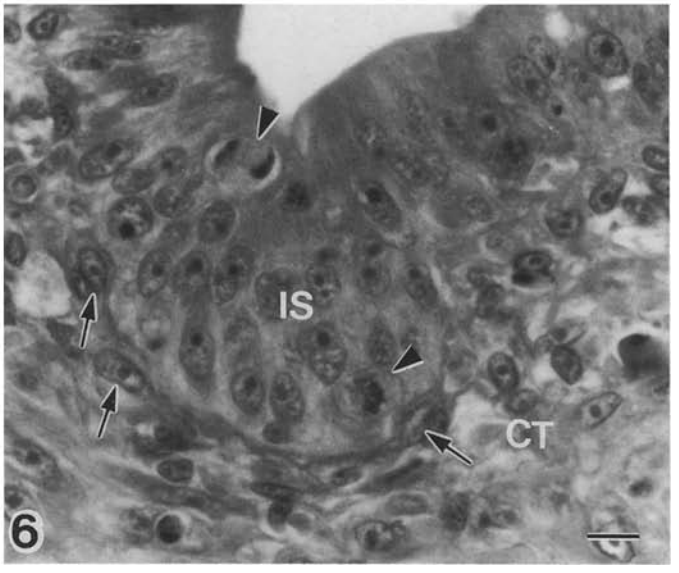


Fig. 3. Cross section of No. 1 fragment before cultivation. The connective tissue (*CT*) is localized in a typhlosole (*T*). *E*, Epithelium; *M*, muscle. *Scale bar:* 100 μm

Fig. 4. Cross section of No. 5 fragment before cultivation. The connective tissue is very thin. *Scale bar:* 100 μm

Fig. 5. Cross section of control No. 5 fragment cultured in CTS medium for 5 days. A simple columnar structure of the larval epithelium remains. Connective tissue is undeveloped. *Scale bar:* 10 μm

Fig. 6. Cross section of No. 1 fragment cultured for 5 days with several hormones including T_3 (see Material and methods). An islet (*IS*) containing mitotic cells (*arrowheads*) develops among the larval epithelium. Connective tissue cells (*arrows*) surround the islet. *Scale bar:* 10 μm

Fig. 7. Cross section of No. 5 fragment cultured for 5 days with hormones added. No islets are observed. The simple squamous epithelium consists of a small number of cells. Connective tissue cells just beneath the epithelium are few. *Scale bar:* 10 μm

of cultivation (Fig. 3), the larval epithelium degenerated, and the adult epithelium developed. However, in 1 b and 4–6 fragments (Group II) which contained very little connective tissue (Fig. 4), only larval cell death was observed as described below.

In all control fragments cultured in CTS medium, neither larval epithelial degeneration nor adult epithelial development was observed. The larval epithelium remained in good condition and the connective tissue remained undeveloped throughout the cultivation (Fig. 5).

Development of adult epithelium

Rapid proliferation of epithelial cells was observed on the fifth day of cultivation with T_3 only in Group I. Proliferating cells were localized in islets which were the primordia of adult intestinal epithelium (Fig. 6). Whenever typical islets were observed, the epithelial mitotic index was high (>2%).

In Group II, the mitotic index of the epithelium remained low throughout the cultivation, whereas the larval epithelial cells degenerated. As a result, the epithelial cells decreased in number and became squamous by the fifth day. In this case, the islets were small and few if forming at all (Fig. 7). These differences in epithelial proliferation and islet-formation between Group I and II are summarized in Fig. 8.

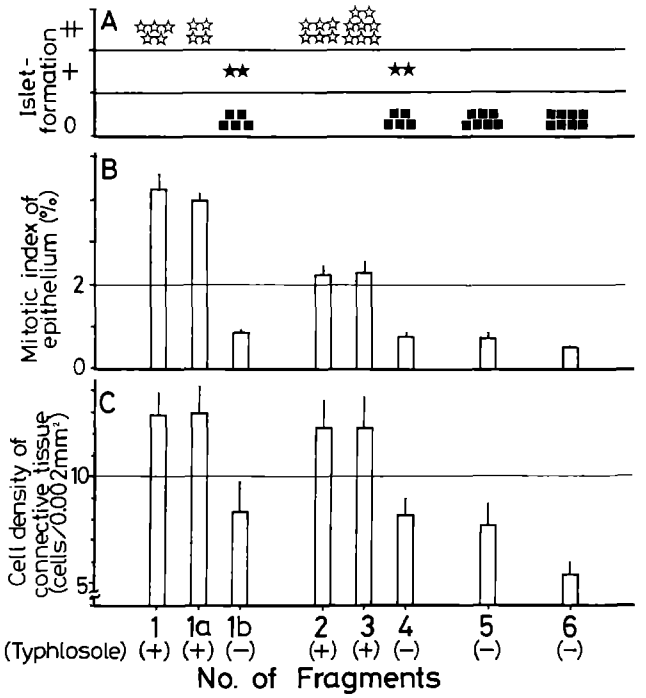


Fig. 8 A–C. Islet-formation in the nascent adult epithelium (**A**), epithelial mitotic index (**B**), and cell density of the connective tissue (**C**) among intact fragments (for numbering see Fig. 1) cultured for 5 days. **A** Each symbol indicates one explant. *O*, explants without islets; +, explants with only small islets consisting of less than five cells per cross section; ++, explants with definite islets. **B** and **C** Each value represents the mean+SD of more than five specimens

Cell death of larval epithelium

With respect to the progress of larval cell death, there was no regional difference in the small intestine. In both Group I and II, larval epithelial cells began to decrease in number on the third day of cultivation and more rapidly around the fifth day. On and after the third day, spherical or ovoid cell bodies of various sizes, which sometimes contained nuclear fragments possessing condensed chromatin close to nuclear membranes, were often observed within the larval epithelium (Fig. 9).

Development of connective tissue

At the start of cultivation in Group I, cell density of the connective tissue was always low (<10 cells/0.002 mm^2) and almost all identifiable cells were immature fibroblast-like cells which were irregular or slender in shape and had large nuclei and poorly-developed cell organelles (Fig. 10). The connective tissue began to increase in cell number on the third day of cultivation and more rapidly on the fifth day when mitotic cells were often observed in the connective tissue (Fig. 11). In all explants of Group I, the connective tissue cells increased in density, especially just beneath the epithelium, and often were seen to surround developing islets (Fig. 6). Most of these connective tissue cells close to the epithelium were mature fibroblasts which contained a well-developed rough endoplasmic reticulum (Fig. 12).

However, in Group II, the number of connective tissue cells remained small throughout the cultivation, and cell density was low (<10 cells/0.002 mm^2), even just beneath the epithelium (Fig. 7). The difference in cell density of the connective tissue between Group I and II is summarized in Fig. 8.

Epithelial-connective tissue interface

Basal lamina. In both Group I and II, the basal lamina began to fold into accordion-like pleats by the third day of cultivation as has been observed at the onset of spontaneous metamorphic climax (Ishizuya-Oka and Shimozawa 1987b). On the fifth day, the thick folded basal lamina was observed in all regions beneath the epithelial cells (Fig. 13). Its thickness was variable but often reached 2 μm or more. In control explants, in contrast, the basal lamina remained thin throughout the cultivation (Fig. 14).

Cell contacts. In Group I, some of the connective tissue cells came close to the epithelium on and after the third day of cultivation. On the fifth day, direct contacts between the epithelial cells and mature fibroblasts possessing the well-developed rough endoplasmic reticulum were first and most frequently observed through the thick basal lamina (Fig. 12). Amorphous materials sometimes existed in a gap between the apposed cell membranes. These observations on cell contacts are consistent with those made during the early period of spontaneous metamorphic climax (Ishizuya-Oka and Shimozawa 1987b). In contrast, in Group II, direct cell contacts between these two tissues were not detected in the present investigation.

Metamorphic changes in recombinants

The results of recombination experiments are summarized in Table 1. Both epithelial proliferation and islet-formation were primarily dependent on non-E components, but not on E components; number 5E components, which could not form islets when recombined with homotopical number 5 non-E components, rapidly proliferated and formed typical islets when recombined with number 1 non-E components (Fig. 15) as did number 1 homotopical recombinants. In contrast, number 1E components, which could form islets when recombined with homotopical number 1 non-E components, proliferated insufficiently and failed to form islets when recombined with number 5 non-E components (Fig. 16) as did number 5 homotopical recombinants. In addition, a close relationship between epithelial mitotic index, islet-formation and cell density of the connective tissue was found to exist in the recombinants as in the intact fragments (Table 1). The connective tissue cells surrounding the islets were also mature fibroblasts possessing a well-developed rough endoplasmic reticulum and

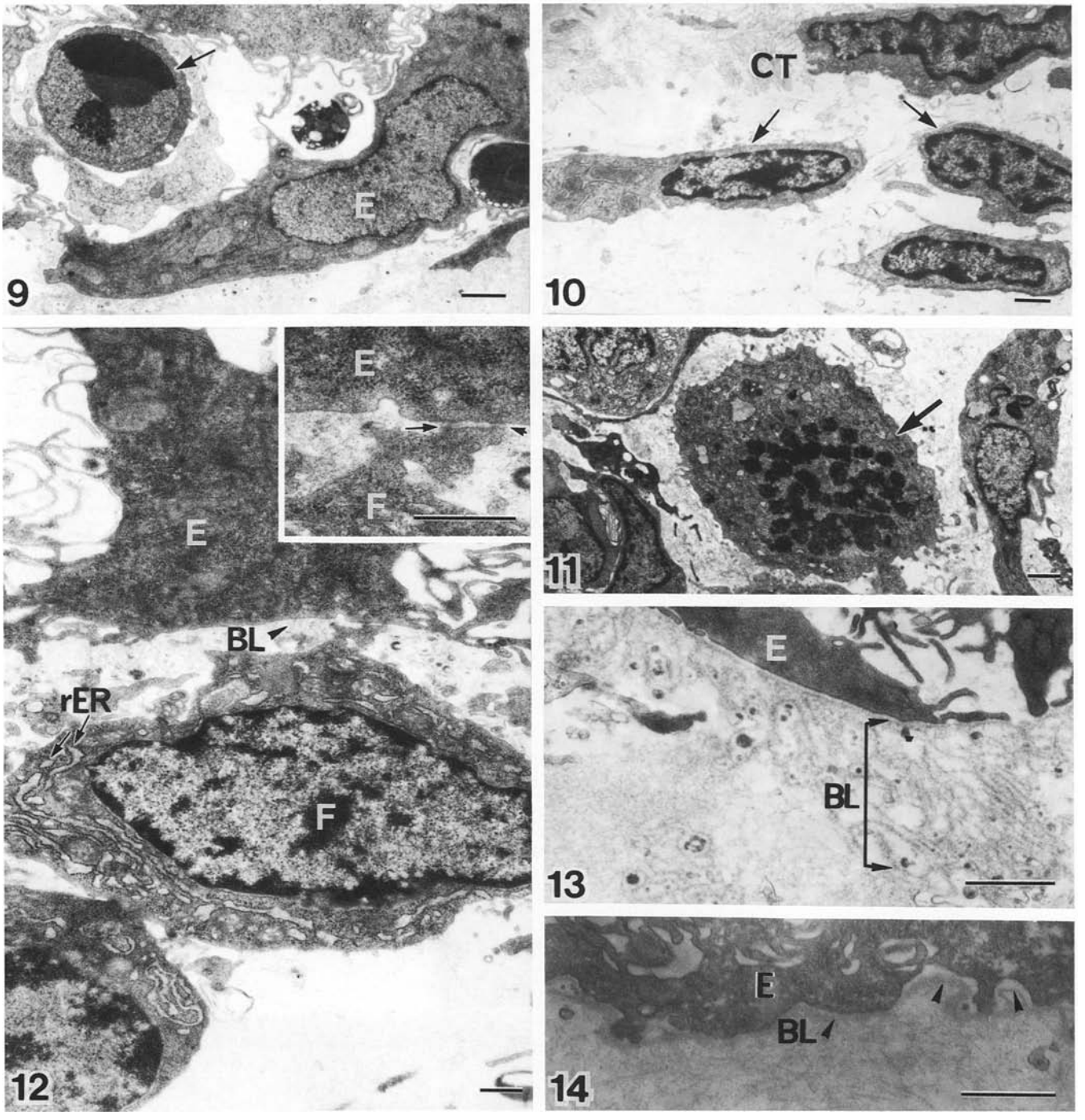


Fig. 9. Larval epithelium (*E*) of No. 1 fragments cultured for 5 days with hormones added. One cell body contains a nuclear fragment with condensed chromatin close to the nuclear envelope (*arrow*). *Scale bar:* 1 μm

Fig. 10. Connective tissue (*CT*) in the typhlosole of No. 1 fragment before cultivation. Immature fibroblasts (*arrows*) have large nuclei and poorly-developed cell organelles. *Scale bar:* 1 μm

Fig. 11. Connective tissue of no. 1 fragment cultured for 5 days with hormons added. A mitotic cell (*arrow*) is observed. *Scale bar:* 1 μm

Fig. 12. Epithelial-connective tissue interface of No. 1 fragment cultured for 5 days with hormons added. A fibroblast (*F*) possessing the well-developed rough endoplasmic reticulum (*rER*) contacts an epithelial cell through the basal lamina (*BL*). *Scale bar:* 1 μm. *Inset* Higher magnification of the contact site. Amorphous materials exist in a gap between the apposed cell membranes (*arrows*). *Scale bar:* 1 μm

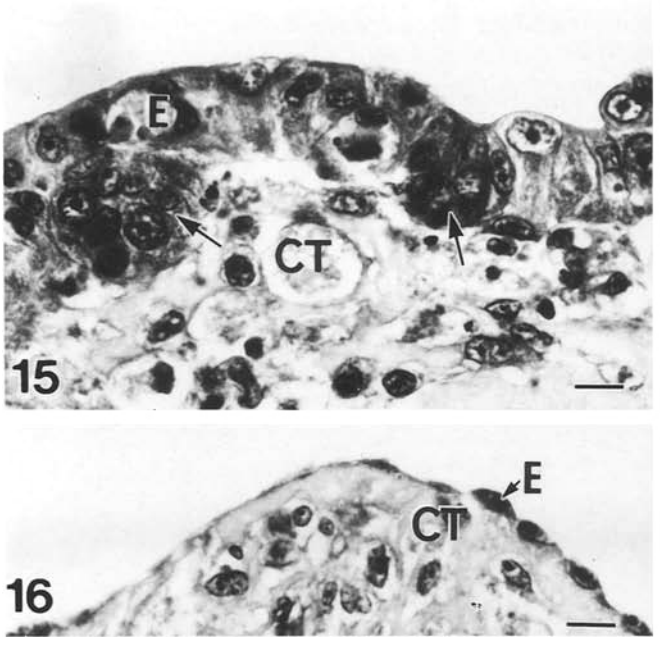
Fig. 13. Epithelial-connective tissue interface of No. 1 fragment cultured for 5 days with hormones added. The basal lamina is thick. *Scale bar:* 1 μm

Fig. 14. Epithelial-connective tissue interface of control No. 1 fragment in CTS medium cultured for 5 days. The basal lamina (indicated by *arrowheads*) is thin. *Scale bar:* 1 μm

Table 1. Metamorphic changes in the recombinants cultured in vitro for 5 days

E[a] component (No.)	Non-E[b] component (No.)	Epithelial mitotic index (%)	Cell density of connective tissue (cells/0.002 mm^2)	Islet-formation		
				0	+	++[cd]
1	1	1.98 ± 0.20^{ef}	11.2 ± 0.9^{h}	0/6	2/6	4/6
5	1	1.97 ± 0.37^{f}	10.8 ± 0.3^{h}	0/5	2/5	3/5
1	5	0.71 ± 0.07^{g}	7.1 ± 1.0^{i}	5/5	0/5	0/5
5	5	0.73 ± 0.15^{g}	7.2 ± 0.9^{i}	5/5	0/5	0/5

[a] Epithelial (=endodermal) component
[b] Non-epithelial (=mesodermal) component
[c] For classes see Fig. 8
[d] Figures represent number of recombinants/total number of recombinants
[e] Mean ± SD
[f–i] The differences between f and g, and between h and i are significant ($P < 0.001$) according to Student's t-test. The differences between values marked by the same letters are not significant



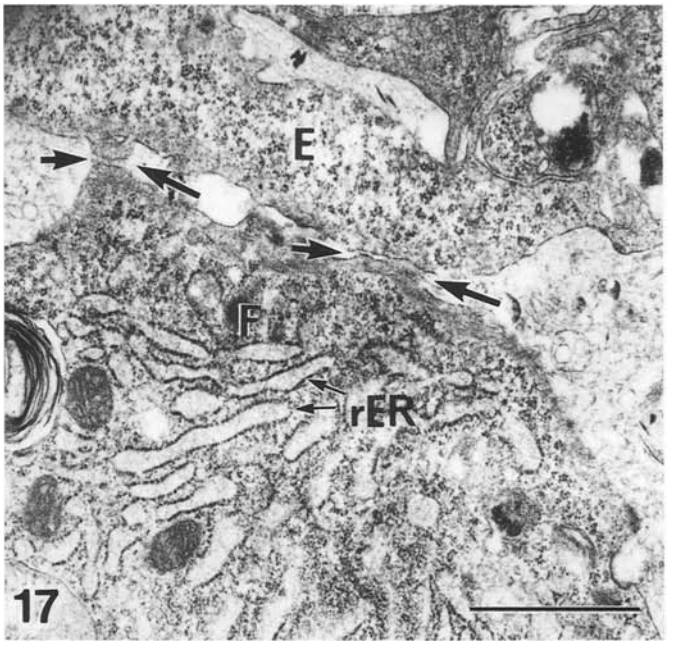


Fig. 15. Recombinant of No. 5 E (epithelial) component and No. 1 non-E component cultured for 5 days with hormones added. Islets (*arrows*) develop among the larval epithelium (*E*). *CT*, Connective tisue. *Scale bar:* 10 μm

Fig. 16. Recombinant of No. 1 E component and No. 5 non-E component cultured for 5 days. The epithelium consists of a small number of cells. No islets are observed. *Scale bar:* 10 μm

Fig. 17. Cell contacts (*arrows*) between a fibroblast (*F*) and an epithelial cell in a recombinant of No. 5 E component and No. 1 non-E component cultured for 5 days. The fibroblast possesses a well-developed rough endoplasmic reticulum (*rER*). Note amorphous materials (*arrows*) between apposed cell surfaces. *Scale bar:* 1 μm

these cells sometimes made contacts with the epithelial cells (Fig. 17).

Discussion

Although there is a remarkable regional difference in the anuran larval small intestine with respect to the amount of connective tissue, previous in vitro studies did not mention precisely what part of the intestine was used for cultivation (Pouyet and Hourdry 1977, 1980). The present study, for the first time, paid attention to this regional difference in the small intestine and obtained some important information on mesodermal/endodermal interaction in intestinal organogenesis during anuran metamorphosis.

The most important finding of the present study is that the connective tissue which is localized in the typhlosole of the larval small intestine is essential for the development of adult epithelium but not for cell death of larval epithelium during thyroid hormone-induced metamorphosis in vitro. The recombination experiments have indicated directly that non-E (=mesodermal) components containing the connective tissue of the typhlo-

sole offer a microenvironment that stimulates adult epithelial development, and that a failure of epithelial development in Group II is not due to a lack of develomental potency of the epithelium itself but to the absence of some factor(s) produced by the connective tissue. During spontaneous metamorphosis, typical islets form in the epithelium following an increase in the number of connective tissue cells along the entire small intestine (Ishizuya-Oka and Shimozawa 1987a). Therefore, it seems likely that in the posterior intestinal region where initially the amount of connective tissue is very small, the increase in non-E cell number may be at least partly explained by migration of connective tissue cells from the other region, probably from the typhlosole. However, it still remains uncertain whether only the connective tissue in the typhlosole is definitely essential for the development of the adult epithelium, or whether the connective tissue derived from other regions can replace it if only the amount of connective tissue is enough. This problem is worth further study.

Furthermore, the present study has shown close relationships between epithelial proliferation, islet-formation and cell density of fibroblasts just beneath the epithelium on the fifth day of cultivation. At this time, fibroblasts which were active in protein synthesis made contacts with the epithelial cells just as at the onset of spontaneous metamorphic climax (Ishizuya-Oka and Shimozawa 1987b). These results strongly suggest that the fibroblasts close to the epithelium play an important role in proliferation and/or differentiation of adult epithelial cells. It is an interesting future problem to study whether the fibroblasts exert their effects by direct cell-to-cell contacts, as has been shown in organogenesis of kidney (Lehtonen et al. 1975) and tooth (Thesleff and Hurmerinta 1981), or by producing some diffusible factor(s) as has been suggested for the pancreas (Rutter et al. 1978) and mammary gland (Enami et al. 1983).

Rapid degeneration of larval epithelium was observed in all explants cultured in the hormone-containing medium, but was not seen in control explants cultured in hormone-free CTS medium. Therefore, it seems likely that larval cell death is induced by thyroid hormone directly or via some factor(s) present in the small intestine. Furthermore, the present result that thickening of the basal lamina was always observed whenever the larval epithelium degenerated suggests a role for the basal lamina in larval cell death.

It has been argued that cell death can be classified into "necrosis", which results from injury and passive degeneration, and "apoptosis" which is programmed and active self destruction (Kerr et al. 1973). Although the classification can be made by morphological criteria, numerous previous morphological studies on epithelial changes of the small intestine during anuran metamorphosis did not distinguish between "necrosis" and "apoptosis" (Bonneville 1963; Hourdry and Dauca 1977; Pouyet and Hourdry 1977; Fox 1983). Ultrastructural features of cell bodies observed within the larval epithelium in the present study agree well with those of apoptotic bodies, which are spherical or ovoid in shape and often contain nuclear fragments characterized by condensed chromatin close to nuclear membranes (Wyllie et al. 1980). Therefore, the larval cell death induced by thyroid hormone can be classified as "apoptosis". This is especially significant in the in vitro system where cell death is often suspected to be "necrosis".

In the avian and mammalian digestive tract, it has been established that the mesenchyme and connective tissue are important for epithelial differentiation of embryos (Le Douarin 1964; Yasugi and Mizuno 1990) and for epithelial cell renewal of adults (Marsh and Trier 1974; Parker et al. 1974), respectively. However, we have only limited information as to which cells of the mesenchyme or the connective tissue are really effective, because we can hardly detect the effective cells unless they show remarkable characteristics during the course of tissue interactions. In the present study, the effective cells are probably a special group of mature fibroblasts that possess the well-developed rough endoplasmic reticulum. They appear at a certain time (at the onset of metamorphic climax) in a certain area (just beneath the epithelium) and thus can be easily detected. In addition, their cytodifferentiation and effects on the epithelium can be completely reproduced in vitro within a short period whenever thyroid hormone is added to the medium. Therefore, the present culture system seems very useful for clarifying the mechanisms of tissue interactions in the digestive tract at the cellular and molecular levels.

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References

Bonneville MA (1963) Fine structural changes in the intestinal epithelium of the bullfrog during metamorphosis. J Cell Biol 18:579–597

Dournon C, Chibon P (1974) Influence de la température, de l'âge et des conditions hormonales (thyroxine) sur la prolifération cellulaire chez la jeune larve et pendant la métamorphose du crapaud *Bufo bufo* L. (Amphibien Anoure). Roux's Arch Dev Biol 175:27–47

Enami J, Enami S, Koga M (1983) Growth of normal and neoplastic mouse mammary epithelial cells in primary culture: stimulation by conditioned medium from mouse mammary fibroblasts. Gann 74:845–853

Fox H (1983) Amphibian metamorphosis. Humana Press, Clifton, pp 119–126

Freed J, Mezger-Freed L (1970) Culture methods for anuran cells. In: Prescott DM (ed) Methods in cell physiology, vol 4. Academic Press, New York, pp 19–47

Hourdry J, Dauca M (1977) Cytological and cytochemical changes in the intestinal epithelium during anuran metamorphosis. Int Rev Cytol [Suppl] 5:337–385

Ishizuya-Oka A, Mizuno T (1984) Intestinal cytodifferentiation in vitro of chick stomach endoderm induced by the duodenal mesenchyme. J Embryol Exp Morphol 82:163–176

Ishizuya-Oka A, Shimozawa A (1987a) Development of the connective tissue in the digestive tract of the larval and metamorphosing *Xenopus laevis*. Anat Anz 164:81–93

Ishizuya-Oka A, Shimozawa A (1987b) Ultrastructural changes in the intestinal connective tissue of *Xenopus laevis* during metamorphosis. J Morphol 193:13–22

Ishizuya-Oka A, Shimozawa A (1990) Changes in lectin-binding pattern in the digestive tract of *Xenopus laevis* during metamorphosis: II. Small intestine. J Morphol 205:9–15

Ishizuya-Oka A, Shimozawa A (1991) Induction of metamorphosis by thyroid hormone in anuran small intestine cultured organotypically in vitro. In Vitro Cell Dev Biol 27A:853–857

Kerr JFR, Wyllie AH, Currie AR (1972) Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26:239–257

Kinoshita T, Sasaki F, Watanabe K (1986) Regional specificity of anuran larval skin during metamorphosis: Dermal specificity in development and histolysis of recombined skin grafts. Cell Tiss Res 245:297–304

Kinoshita T, Takahama H, Sasaki F, Watanabe K (1989) Determination of cell death in the developmental process of anuran larval skin. J Exp Zool 251:37–46

Kordylewski L (1983) Light and electron microscopic observations of the development of intestinal musculature in *Xenopus*. Z Mikrosk-anat Forsh 97:719–734

Le Douarin N (1964) Étude expérimentale de l'organogènese du tube digestif et du foie chez l'embryon de Poulet. Bull Biol Fr Belg 98:543–676

Lehtonen E, Wartiovaara J, Nordling S, Saxen L (1975) Demonstration of cytoplasmic processes in Millipore filters permitting kidney tube induction. J Embryol Exp Morphol 33:187–203

Marsh MN, Trier JS (1974) Morphology and cell proliferation of subepithelial fibroblasts in adult mouse jejunum: I. Structural features. Gastroenterology 67:622–635

Marshall JA, Dixon KE (1978) Cell specialization in the epithelium of the small intestine of feeding *Xenopus laevis* tadpoles. J Anat 126:133–144

Mathan M, Hermos JA, Trier JS (1972) Structural features of the epithelio-mesenchymal interface of rat duodenal mucosa during development. J Cell Biol 52:577–588

McAvoy JW, Dixon KE (1977) Cell proliferation and renewal in the small intestinal epithelium of metamorphosing and adult *Xenopus laevis*. J Exp Zool 202:129–138

Nieuwkoop PD, Faber J (1967) Normal table of *Xenopus laevis* (Daudin). North-Holland, Amsterdam

Parker FG, Barnes EN, Kaye GI (1974) The pericryptal fibroblast sheath: IV. Replication, migration, and differentiation of the subepithelial fibroblasts of the crypt and villus of the rabbit jejunum. Gastroenterology 67:607–621

Pouyet JC, Hourdry J (1977) Effet de la thyroxine sur la structure des épithéliocytes intestinaux en culture organotypique, chez la larve de *Discoglossus pictus* Otth (Amphibien Anoure). Biol Cell 29:123–134

Pouyet JC, Hourdry J (1980) Effet de la thyroxine sur la prolifération des épithéliocytes intestinaux en culture organotypique, chez la larve du Crapaud-accoucheur (*Alytes obstetricans* Laurenti). Biol Cell 38:237–242

Rutter WJ, Pictel RL, Harding JD, Chirgwin JM, MacDonald RJ, Przybyla AE (1978) An analysis of pancreatic development: Role of mesenchymal factor and other extracellular factors. In: Papaconstantinou J, Rutter WJ (eds) Molecular control of proliferation and differentiation. Academic Press, New York, pp 205–277

Thesleff I, Hurmerinta K (1981) Tissue interactions in tooth development. Differentiation 18:75–88

Trowell OA (1959) The culture of mature organs in a synthetic medium. Exp Cell Res 16:118–148

Wyllie AH, Kerr JFR, Currie AR (1980) Cell death: The significance of apoptosis. Int Rev Cytol 68:251–306

Yasugi S, Mizuno T (1990) Mesenchymal-epithelial interactions in the organogenesis of digestive tract. Zool Sci 7:163–174

Yoshizato K (1989) Biochemistry and cell biology of amphibian metamorphosis with a special emphasis on the mechanism of removal of larval organs. Int Rev Cytol 119:97–149

Yoshizato K, Kikuyama S, Shioya N (1980) Stimulation of glucose utilization and lactate production in cultured human fibroblasts by thyroid hormone. Biochim Biophys Acta 627:23–29