Epidermal metaplasia of proamnionic epithelium induced by dorsal skin dermis in the chick embryo

By TAKEO MIZUNO

From the Laboratoire d'Embryologie Expérientale du Collège de France, Nogent-sur-Marne, France (Directeur: Professeur Et. Wolff)

SUMMARY

Proamnionic epithelium of the chick embryo cultivated directly on Wolff and Haffen's medium in the absence of mesenchymes fails to differentiate. Cultivation of the dorsal dermis of 6-5-day chick embryos in the absence of epithelium also results in lack of differentiation of dermal cells.

When proamnionic epithelium taken from embryos before the 10-somite stage is cultivated combined with dorsal dermis of 6-5-day embryos for 6 days, the epithelium invariably undergoes metaplastic changes, forming stratified epidermis, sometimes with keratinized superficial layer. The underlying dermal cells are condensed and this often leads to the formation of feather germ-like structures.

The competence of the epithelium for changing into the epidermis is gradually lost after the 10-somite stage, and the dorsal dermis from 8-5-day embryos is not very effective in inducing the epidermal metaplasia.

Proamnionic epithelium cultivated on heat-killed dorsal dermis seems healthy but shows no sign of differentiation. Dorsal dermis combined with heat-killed proamnionic epithelium spreads and remains almost undifferentiated. These observations suggest that reciprocal induction mechanisms are involved in the epithelial and dermal differentiation.

Cultivation of proamnionic epithelium with various heterologous mesenchymes or fragments of embryonic organs shows that this epithelium is only competent for epidermal differentiation when combined with dorsal dermis.

When proamnion (proamnionic epithelium plus hypoblast) is directly combined with 6-5-day dorsal dermis it undergoes metaplastic changes. The same result is obtained when inverted (upside-down) proamnion is combined with the dermis. Hypoblast does not seem to affect the inductive interaction between the epithelium and the dorsal dermis.

INTRODUCTION

The importance of stromal factors in the differentiation of the epidermis has been noticed by many authors, as in the cases of the development of scales and claws (Cairns & Saunders, 1954; Dodson, 1963; Rawles, 1963), uropygium (Gomot, 1959), and mucous epithelium (McLoughlin, 1961), and also in certain transfilter systems (Wessells, 1962). The case of feather germ differentiation in

1 Author's address: The Zoological Institute, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan.
the chick embryo seems somewhat complicated: (1) the dermis can induce differentiation of typical epidermis in simple embryonic ectoderm; (2) axial organs beneath the dermis, such as neural tube, notochord, myotomes or sclerotomes, induce feather rudiments in the dermis; (3) the dermal feather rudiments exert an inductor effect on the overlying epidermis, which results in the primary outgrowth of the epidermis; (4) the epidermis, in turn, induces the dermal cells to colonize the epidermal sheath and fixes the orientation of feather germs according to cephalocaudal polarity (Sengel, 1958a, b). Also, it was reported that chick chorionic epithelium can be transformed into typical scale epidermis when it is combined with the tarsometatarsal dermis and cultured on the chorioallantoic membrane (Kato & Hayashi, 1963; Kato, 1969).

The present study was performed in order to know (a) whether chick proamnionic epithelium can undergo metaplastic change into the epidermis when it is combined with the dermis and (b) whether the proamnionic epithelium can also contribute to the dermal differentiation.

A preliminary report of this work has appeared elsewhere (Mizuno, 1970).

MATERIALS AND METHODS

Tissues were obtained from White Leghorn (*Gallus domesticus*) embryos and the diagram of the experimental methods is shown in Fig. 1.

Isolation of tissue fragments

Proamnionic epithelium was dissociated from the proamnion of the embryos at definitive streak to 10-somite stage in a Ca-, Mg-free Tyrode's solution or in a cold solution of trypsin, Difco 1: 250 (Fig. 2). The amnionic epithelium was obtained from the amnion (from 22-somite stage to 5-day embryos) with the aid of trypsin. The dermis was separated from the dorsal skin of the 6-5-day and 8-5-day embryos and from the tarsometatarsal skin of the 10-day and 13-5-day embryos in a 0-5% cold solution of trypsin in a Ca-, Mg-free Tyrode's solution. The time required for the separation depends on the ages and the sources of the skin. Ten to twenty minutes were usually found sufficient. The mesenchymes of proventriculus, gizzard, trachea, and lung were obtained with the aid of trypsin or in the Ca, Mg-free Tyrode's solution. The fragments of the other organs, such as heart, bulbus cordis, liver, chorda with sclerotome, and mesonephros, were used without isolation of their mesenchymes. Tissues isolated with the aid of trypsin were rinsed thoroughly in a series of Tyrode's solution containing embryo extract and horse serum to eliminate the excess of trypsin, and then in a fresh Tyrode's solution.

Recombination of the separated tissues

A fragment of mesenchyme or a piece of organ thus obtained was wrapped in a folded proamnionic or amnionic epithelium and cultured on Wolff & Haffen's
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Diverse mesenchymes

Fig. 1. Diagram showing the mode of combinations between the proamnionic epithelium (or hypoblast) from head-process stage embryos and the dorsal dermis (or other mesenchymes) from 6-5-day embryos.

(1952) medium with the addition of horse serum instead of Tyrode’s solution:
7 parts of 1 % Bactoagar (Difco) in Gey’s solution + 3 parts of filtrated horse serum (Institut Pasteur) + 3 parts of 50 % chick embryo extract in Tyrode’s solution + 1 part of Tyrode’s solution containing Penicilline G (20000 i.u./cm³), at 38 °C. On the 6th day of the culture the explants were fixed in Bouin’s fluid, and sectioned in paraffin at 5 μm thick and stained with Carazzi’s glycéralum and eosin.

RESULTS

Cultivation of dissociated proamnionic or amnionic epithelium and of dissociated dorsal dermis

In order to test the self-differentiating capacity of proamnionic or amnionic epithelium and of dorsal dermis, the following experiments were carried out. When a sheet of proamnionic or amnionic epithelium is cultured for 6 days on the medium without combination of dermis or other mesenchymes, the epithelial cells form an undifferentiated cell mass (see Table 1). Likewise, when a piece of dorsal dermis of 6-5-day embryos is explanted alone, the dermal cells spread and do not show any sign of differentiation. These facts indicate that isolated proamnionic or amnionic epithelium and isolated dorsal dermis cannot differentiate by themselves under the conditions of the present experiment.
Table 1. Differentiation of the proamnionic or amnionic epithelium when combined with the dermis of the dorsal skin and cultured for 6 days in vitro

<table>
<thead>
<tr>
<th>Stage of proamnion or amnion (Hamburger &amp; Hamilton, 1951)</th>
<th>Stage of dermis (days)</th>
<th>No. of exp.</th>
<th>Differentiation of feather germ</th>
<th>Differentiation of epithelium</th>
<th>Keratinization of epithelium</th>
<th>Differentiation of dermis</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-26</td>
<td>—</td>
<td>10</td>
<td>0</td>
<td>10: no differentiation</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>—</td>
<td>6-5</td>
<td>9</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>9: Spread, no differentiation</td>
</tr>
<tr>
<td>4-10</td>
<td>6-5</td>
<td>22</td>
<td>7</td>
<td>{ 11: epidermal transformation</td>
<td>4(++)</td>
<td>Very well</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11: stratified epithelium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14-15</td>
<td>6-5</td>
<td>2</td>
<td>0</td>
<td>2: stratified epithelium</td>
<td>1(+)</td>
<td>Well</td>
</tr>
<tr>
<td>25-27</td>
<td>6-5</td>
<td>6</td>
<td>0</td>
<td>6: epidermoid</td>
<td>0</td>
<td>Insufficient</td>
</tr>
<tr>
<td>4-10</td>
<td>8-5</td>
<td>11</td>
<td>0</td>
<td>{ 5: stratified epithelium</td>
<td>0</td>
<td>Dead</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6: no differentiation</td>
<td>0</td>
<td>Dedifferentiation</td>
</tr>
<tr>
<td>4-5</td>
<td>6-5 (killed at 60 °C for 10 min)</td>
<td>5</td>
<td>0</td>
<td>5: no differentiation</td>
<td>0</td>
<td>Spread, no differentiation</td>
</tr>
<tr>
<td>5-8 (killed at 60 °C for 10 min)</td>
<td>6-5</td>
<td>5</td>
<td>0</td>
<td>5: dead</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
Figures 2-4

Fig. 2. Longitudinal section of the anterior part of the head-process stage embryo. Dotted lines indicate the area used for culture. × 150. PE, Proamnionic epithelium; HYP, hypoblast; Y, yolk.

Fig. 3. Six days after combination of proamnionic epithelium from definitive streak stage embryo with 6-5-day dorsal dermis. Note the keratinized stratified epithelium with mitoses and well-differentiated dermal cells. × 200. K, Keratin.

Fig. 4. Six days after combination of proamnionic epithelium from head-process stage embryo with 6-5-day dorsal dermis. × 250. K, Keratin.
Combination of proamnionic epithelium and 6·5-day dorsal dermis

Proamnionic epithelium isolated from embryos from definitive streak stage to 10-somite stage was explanted together with 6·5-day dorsal dermis. The information sought concerns the course of differentiation that the proamnionic epithelium (which would normally give rise only to the amnionic epithelium) will follow in response to the possible inductive stimuli coming from the 'foreign' stroma, i.e. the dorsal dermis. The results show that the single layered proamnionic epithelium, when combined with dermis, always differentiates into stratified epithelium, and often into definite epidermal structure (11 out of 22 cases) and sometimes produces keratin (Figs. 3, 4). The dermal cells always gather together forming a lenticular mass. In some explants, these cells form the dermal part of feather germ-like structures (7 out of 22 cases – Figs. 5, 6). From these results it is clear that the proamnionic epithelium has a competence for differentiating into epidermis and that this epithelium conversely supports the differentiation of the dermis, these two processes leading to the feather germ formation.

Combination of amnionic epithelium and 6·5-day dorsal dermis

When the amnionic epithelium from embryos from 22- to 24-somite stages is combined with 6·5-day dorsal dermis, the epithelium usually differentiates into a stratified epithelium and produces a small amount of keratin. The differentiation of the dermis is incomplete and the feather germ structure fails to develop (Fig. 7).

However, when the amnionic epithelium from later stages (4·5–5·5 days of incubation) is combined with 6·5-day dorsal dermis, the epithelium tends to an epidermoid structure but to neither stratification nor keratinization, and the dermis shows little differentiation. These facts indicate that the competence of the epithelium for differentiating into epidermis is gradually lost as the stage advances, and that the dermis combined with such epithelium becomes incapable of further differentiation, and feather germ formation becomes no longer observed.

Combination of proamnionic epithelium and 8·5-day dorsal dermis

In the dorsal skin of 8·5-day chick embryos the rows of feather germ make their appearance. The fibroblasts in the dermis gather together to form colonies of cell groups. The proamnionic epithelium was combined with the 8·5-day dorsal dermis to examine whether this dorsal dermis could still be effective in producing metaplasia in the proamnionic epithelium as in the case of the 6·5-day dorsal dermis, and whether the epithelium could affect the development of the dorsal dermis of this stage. It was found that in 5 out of 11 explants, the epithelium exhibited transformation into stratified transitional epithelium with dermal differentiation, though feather germ-like structures were never observed. In the other 6 cultures, however, no differentiation of the epithelium was observed and
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FIGURES 5-7

Figs. 5, 6. Six days after combination of proamnionic epithelium from head-process stage embryo with 6-5-day dorsal dermis. Feather germ-like structures are prominent. × 200.

Fig. 7. Six days after combination of amnionic epithelium of 22-somite stage embryo with 6-5-day dorsal dermis. The epithelium is stratified but shows no typical epidermal structure. The differentiation of the dermis is also incomplete. × 200.
in the dermis fibroblasts were seen dispersed as in the 6-5-day dorsal skin cultured alone, showing a sign of dedifferentiation. This process was also seen when 8-5-day dorsal dermis was cultivated under similar conditions without combination with the epithelium. It seems therefore that the dermis from 8-5-day embryos is less effective in producing definite metaplasia in the proamnionic epithelium, and this is reflected in the lower grade of differentiation of the dermal cells.

The results hitherto described show that reciprocal relationships exist between the occurrence of metaplasia of the proamnionic epithelium and the differentiation of the dermis and that both take place readily when these two tissues are obtained from embryos of younger stages.

**Combination of proamnionic epithelium and heat-killed 6-5-day dorsal dermis**

In order to test whether or not the existence of living dermis is essential for the induction of metaplastic changes in the proamnionic epithelium, the living epithelium was then combined with dorsal dermis which had been killed in Tyrode’s solution at 60 °C for 10 min. After 6 days’ incubation the epithelial cells were healthy but showed no indication of differentiation and consequently no feather germ was obtained. This indicates that for differentiation of the proamnionic epithelium, some factors contained in the living dermis must be needed.

**Combination of heat-killed proamnionic epithelium and 6-5-day dorsal dermis**

A reverse experiment was next carried out to see whether the killed proamnionic epithelium could bring about differentiation of the dermis. The epithelium was treated at 60 °C for 10 min in Tyrode’s solution and then combined with the living 6-5-day dorsal dermis. Results show that the killed epithelium is not effective in inducing dermal differentiation after 6 days’ incubation (Fig. 8).

These results, together with those of the preceding sections, seem to indicate that both the metaplastic changes in the proamnionic epithelium and differentiation of the dermis take place only when living cells act on competent tissues.

**Combination of proamnionic epithelium and diverse mesenchymes or fragments of other organs**

The question arises as to whether diverse mesenchymes other than the dorsal dermis could be equally effective in changing the direction of histo-differentiation in the proamnionic epithelium. To answer this question, proamnionic epithelium was cultivated on various mesenchymes other than dorsal dermis, or on fragments of other organs. The results are summarized in Table 2.

When the epithelium is associated with the dermis derived from 10-day tarsometatarsus, a stratified transitional epithelium is induced, true epidermal or scale structure being never formed (Fig. 9). Even 13-5-day tarsometatarsal
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Figures 8-10

Fig. 8. Six days after combination of heat-killed proamnionic epithelium with living dorsal dermis. Note the dead epithelium (DE) and necrotic dermis (ND). × 200.

Fig. 9. Six days after combination of proamnionic epithelium from head-process stage embryo with 10-day tarsometatarsal dermis. No scales. × 200.

Fig. 10. Six days after combination of proamnionic epithelium from the streak stage embryo with 4-day ventricle. The epithelium (PE) shows no particular differentiation, but a rhythmically pulsating ventricle (V) is fully differentiated. × 200.
Table 2. Differentiation of the proamnionic epithelium combined with various mesenchymes or organs of chick embryos

<table>
<thead>
<tr>
<th>Stage of proamnion (Hamburger &amp; Hamilton, 1951)</th>
<th>Combined mesenchymes or organs</th>
<th>No. of exp.</th>
<th>Differentiation of epithelium</th>
<th>Differentiation of stroma</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-5</td>
<td>10-day tarsometatarsal dermis</td>
<td>3</td>
<td>Stratified epithelium</td>
<td>Very well</td>
</tr>
<tr>
<td>4-8</td>
<td>13.5-day tarsometatarsal dermis</td>
<td>12</td>
<td>No differentiation</td>
<td>Well</td>
</tr>
<tr>
<td>4-9</td>
<td>6-day proventricular mesenchyme</td>
<td>13</td>
<td>4: thick epithelium</td>
<td>Well</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9: no differentiation</td>
<td></td>
</tr>
<tr>
<td>5-8</td>
<td>6.5-day gizzard mesenchyme</td>
<td>4</td>
<td>No differentiation</td>
<td>Very well</td>
</tr>
<tr>
<td>4-9</td>
<td>5-day lung mesenchyme</td>
<td>11</td>
<td>No differentiation</td>
<td>Well</td>
</tr>
<tr>
<td>4-5</td>
<td>13.5-day trachea stroma</td>
<td>4</td>
<td>No differentiation</td>
<td>Very well</td>
</tr>
<tr>
<td>5</td>
<td>4-day heart</td>
<td>3</td>
<td>No differentiation</td>
<td>Very well</td>
</tr>
<tr>
<td>4</td>
<td>4-day bulbus cordis</td>
<td>1</td>
<td>No differentiation</td>
<td>No differentiation</td>
</tr>
<tr>
<td>5</td>
<td>4-day liver</td>
<td>3</td>
<td>No differentiation</td>
<td>Well</td>
</tr>
<tr>
<td>4-6</td>
<td>5- to 6-day chorda + sclerotome</td>
<td>5</td>
<td>No differentiation</td>
<td>Very well</td>
</tr>
<tr>
<td>6-9</td>
<td>6-day mesonephros</td>
<td>4</td>
<td>No differentiation</td>
<td>Well</td>
</tr>
</tbody>
</table>
dermis fails to induce such an epithelial differentiation. There are some discrepancies between our results and Kato's (Kato & Hayashi, 1963; Kato, 1969), but this might be due to the differences of the experimental conditions employed. The mesenchymes of digestive tube, such as 5-5- to 6-5-day proventriculus and gizzard, and the mesenchymes of respiratory organs, such as 5-day lung and 13-5-day trachea, cannot cause metaplasia of the epithelium either. The fragment of other organs, such as 4-day heart, bulbus cordis and liver, 5- to 6-day chorda plus sclerotome and mesonephros, are also not effective in inducing organ-specific differentiation of the epithelium (Fig. 10). It does not appear therefore that morphological differentiation of the proamnionic epithelium is always induced in accord with every type of mesenchymes applied except in the case of the dermis.

**Combination of proamnionic hypoblast and 6-5-day dorsal dermis**

Next, experiments were done to examine whether the hypoblast which lies under the proamnionic epiblast would differentiate into some specific structures when it is combined with 6-5-day dorsal dermis. After 6 days of incubation of the combined tissues, the hypoblast forms a loose, non-differentiated cell mass and the underlying dermis remains dispersed showing little sign of differentiation (5 out of 5 cases – Fig. 11). Thus, no interactions whatever appear to occur between the proamnionic hypoblast and the dermis.

**Combination of proamnion and 6-5-day dorsal dermis**

Proamnion (proamnionic epithelium plus underlying hypoblast) was obtained from embryos of definitive streak stage to 10-somite stage and cultivated alone or on the 6-5-day dorsal dermis for 6 days. When the proamnion is cultivated alone, the epithelium spreads and the hypoblast forms a cell mass and both tissues remain undifferentiated. When proamnion is cultivated on the 6-5-day dorsal dermis, the proamnionic epithelium undergoes metaplastic changes into epidermal structures and often shows keratinizing differentiation, the cells of the hypoblast becoming no longer recognized between the well differentiated epithelium and dermis (Fig. 12). It seems therefore that proamnionic hypoblast may not interrupt morphogenetic interactions occurring between the epithelium and the dorsal dermis.

Similar experiments were also performed combining inverted proamnion (superficial hypoblast plus underlying proamnionic epithelium) with the 6-5-day dorsal dermis, so that the free surface of the epithelium was placed directly on the dermis. After 6 days of incubation, the proamnionic epithelium thickens and differentiates into the epidermis, basal side inwards, and keratinizing cells outwards. The superficial layer of the newly orientated epithelium becomes keratinized, but the basal layer, which was originally the free surface of the epithelium, differentiates into the germinal layer. The cells of the hypoblast form an undifferentiated cell mass remaining on the top of the keratinized
Figures 11–13

Fig. 11. Six days after combination of proamnion hypoblast (HYP) with 6-5-day dorsal dermis. × 200.

Fig. 12. Six days after combination of proamnion from definitive streak stage embryo with 6-5-day dorsal dermis. × 200.

Fig. 13. Six days after combination of inverted (upside-down) proamnion from 7-somite stage with 6-5-day dorsal dermis. × 200. HYP, Hypoblast; KPE, keratinized proamnionic epithelium; D, dorsal dermis.
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epithelium (Fig. 13). These results indicate that the metaplastic differentiation of the proamnionic epithelium takes place even when the free surface of the epithelium is placed on the dermis and that the proamnionic hypoblast is again unaffected by dermal influences.

DISCUSSION

It is well known that epithelio-mesenchymal interactions exist in the histodifferentiation of avian and mammalian skin (McLoughlin, 1961; Wessells, 1962; Dodson, 1963; 1967a, b) and of its integumentary appendages such as feathers (Sengel, 1958a, b; Rawles, 1963), scales (Cairns & Saunders, 1954; Sengel & Abbott, 1963), mammary gland (Propper & Gomot, 1967; Propper, 1968, 1969; Kratochwil, 1969), uropygial gland (Gomot, 1958, 1959), and hairs and vibrissae (Jacobson, 1966; Kollar, 1966). Most of the experimental findings indicate that differentiation of presumptive epidermis is usually determined by morphogenetic stimuli coming from the underlying mesenchyme and that no predetermined regional specificity can be demonstrated in the embryonic ectoderm in general. Examples of atypical modifications of the epidermis due to heterologous mesenchymes or agents have also been shown including the case of metaplasia of the epidermis into mucus-secreting epithelium (Fell & Mellanby, 1953; Moscona, 1961; McLoughlin, 1961). However, it is to be noted that heterologous stromal factors are not always effective in determining the differentiation of the embryonic epidermis (Kollar, 1966; Propper, 1968; Sengel, Dhouailly & Kieny, 1969).

The work reported here (and briefly elsewhere, Mizuno, 1970) demonstrated that the developmental pathways of the undifferentiated proamnionic epithelium can be modified by combining it with the dorsal skin dermis, the epithelium changing into stratified epidermis, although other heterologous mesenchymes fail to exert any influences on the differentiation of this epithelium. In this connexion it is interesting to note that atypical keratinization of the chick chorionic epithelium is induced in vitro by exposure to air (Moscona, 1959) or by the combination with dermal tissues (Bonetti, 1959; Kato & Hayashi, 1963; Kato, 1969).

In the present study it is also shown that isolated proamnionic epithelium or dorsal dermis fails to differentiate when cultured alone and that heat-killed proamnionic epithelium or dermis loses its capacity for inducing differentiation when combined with the living partner tissue, suggesting that reciprocal induction mechanisms might be involved in the differentiation of both the proamnionic epithelium and the dermis: (1) the dermis induces the epidermal metaplasia of the proamnionic epithelium, and (2) the epithelium reciprocally induces the formation of the mesenchymal condensation of a feather germ-like structure. Similar mechanisms have also been reported in the case of feather germ differentiation in the normal skin of embryonic chick (Sengel, 1958a, b).

It has also been shown in the present study that if amnionic epithelium from
older embryos (4-5–5-5 days) is combined with 6-5-day dorsal skin dermis, no differentiation whatsoever is observed, indicating that its competence for responding to dermal inductor becomes gradually lost as development advances.

Summing up, the significance of the present work will be in providing evidence that such apparently neutral, inert epithelium as proamnionic can differentiate into stratified epidermis when combined with the dermis of 6-5-day embryonic skin, and that there exists a reciprocal relationship between the differentiation of the epithelium and that of the dermis even under such conditions as the present experiment.

RESUME

La métaplasie épidermique de l'épithélium proamniotique induit par le derme de la peau dorsale chez l'embryon de Poulet

Lorsque l'épithélium proamniotique de l'embryon de Poulet est cultivé directement sur le milieu sans association de mésenchyme, les cellules épithéliales forment une masse indifférenciée. Quand le derme de peau dorsale chez l'embryon de 6-5 jours est cultivé seul, ses cellules s'étalent et ne montrent aucun signe de différenciation.

Quand l'épithélium proamniotique prélevé avant le stade de 10 somites est associé au derme de la peau dorsale d'embryon de 6-5 jours, l'épithélium subit des changements métaplastiques et devient stratifié et parfois kératinisé. Les cellules du derme se différencient et des germes plumaires apparaissent.

Cette compétence de l'épithélium est graduellement perdue après le stade de 10 somites et le derme de la peau dorsale d'embryon de 8-5 jours n'est pas susceptible de métaplasie épidermique.

L'épithélium proamniotique cultivé sur le derme tué par la chaleur (60 °C pendant 10 min) est sain, mais ne présente aucun signe de différenciation. Le derme de la peau du dos associé avec l'épithélium proamniotique tué par la chaleur ne se différencie pas. On peut donc conclure que des mécanismes d'induction réciproque sont responsables de la différenciation de l'épithélium et du derme.

L'étude de l'association de l'épithélium proamniotique et de divers mésenchymes hétérologues ou fragments d'organes embryonnaires montre que cet épithélium n'est pas compétent pour se différencier dans le sens correspondant à chaque mésenchyme ou fragment.

Quand le proamnios (l'épithélium proamniotique plus l'hypoblaste) est associé directement au derme de la peau dorsale d'embryon de 6-5 jours, il subit des changements métaplastiques. Le même résultat est obtenu quand le proamnios retourné (le haut en bas) est associé au derme. L'hypoblaste n'interrompt pas l'interaction inductive de l'épithélium et du derme dorsal.

The author wishes to express his sincere gratitude to Professor Etienne Wolff, Director of the Laboratoire d'Embryologie Expérimentale du Collège de France, for his constant encouragement and guidance throughout the course of this work. He is also indebted to the staff and the technicians at the laboratory for their kind help and warm hospitality. He also expresses his thanks to Professor Emeritus T. Fujii of Tokyo University for his kind help in preparing the manuscript.

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