Feather-forming capacities of the avian extra-embryonic somatopleure

By DANIELLE DHOUAILLY

From the Laboratoire de Zoologie et Biologie Animale, Equipe de recherche associée au CNRS no. 621: 'Morphologie expérimentale', Université Scientifique et Médicale de Grenoble

SUMMARY

Blocks of 12·5- or 13·5-day embryonic mouse upper-lip dermis were introduced under the ectoderm of the extra-embryonic area of 2- to 3-day chick or duck embryos. Two kinds of ectopic cutaneous appendages were produced: either arrested feathers alone, or arrested feathers and full-grown feathers. The former developed in the ectoderm overlying the implanted mouse dermal cells, the latter formed in their close vicinity, but contained host dermal cells exclusively.

Thus, avian extra-embryonic somatopleure, both ectoderm and mesoderm, possesses the information for feather development: the extra-embryonic ectoderm, if it is brought in contact with an appendage-forming dermis, is able to respond to the dermal induction by initiating feather morphogenesis; the extra-embryonic mesoderm, if it is experimentally transformed into a dense dermis, can express its feather-forming capacity by specifying feather tract morphology and barb-ridge number, thus leading to the achievement of feather morphogenesis.

INTRODUCTION

Previous experimental studies have shown that chick ectoderm from extra-embryonic membranes is able to form a stratified epidermis (Bonetti, 1963; Mizuno, 1972) and to produce feathers (Kato & Hayashi, 1963; Kieny & Brugal, 1978), when it is brought into contact with an embryonic appendage-forming dermis of the same species.

Does this epidermal metaplasia of the extra-embryonic ectoderm correspond to its capacity of responding to an informative dermal 'feather' induction? Or does this ectoderm possess an intrinsic 'feather' information, the expression of which is simply triggered off by the presence of the associated dermis? If the latter hypothesis is correct, the presumptive ectoderm from amnion or chorion, like that from the chick embryo body (Dhouailly, 1973), should be able to express its feather-forming capacity even if associated with a non-feather-forming dermis, such as, for instance, mouse hair-forming dermis.

In order to verify either or both hypotheses, the following experiment was

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[1] *Author's address:* Laboratoire de Zoologie et Biologie Animale, Université Scientifique et Médicale de Grenoble, 38041 Grenoble Cedex, France.
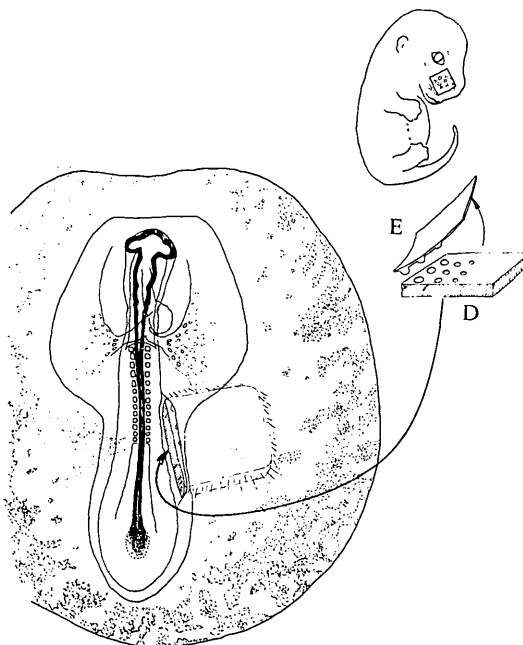
Fig. 1. Implantation of a 12·5-day mouse upper-lip dermis under the extra-embryonic ectoderm of a 2-day chick embryo. The mouse dermis (D) is isolated from the epidermis (E) after enzymic treatment.

carried out. A block of mouse upper-lip dermis was introduced beneath the ectoderm of the extra-embryonic area of 2- to 3-day chick or duck embryos and the subsequent morphogenesis of this patch of ectoderm was observed after a period of 1–2 weeks.

MATERIAL AND METHODS

White Leghorn chick embryos, Peking duck embryos and OF1 Swiss strain (albinos) mouse embryos were used in these experiments.

Mouse skin was cut from both sides of the upper-lip of 12·5-day and 13·5-day embryos. The dermis was separated from the epidermis after treatment with a solution of 1 % trypsin (Choay) and 1 % pangestine (Difco) (Fig. 1).

The host embryos ranged in developmental stages from 8 to 26 pairs of somites (chick) and from 12 to 27 pairs of somites (duck).

After staining with neutral red, a slit was cut in the ectoderm above the developing vitelline artery on the right side. The ectoderm was then lifted by means of two black glass needles to form a tunnel into which the mouse dermis was introduced (Fig. 1). The graft, given its size (1·5 × 1·5 mm), takes up a large part of the right extra-embryonic area and is located either above both the area pellucida and the area opaca, or entirely above the area opaca. The graft was

marked with carbon particles in order to facilitate its location 10–14 days after the operation, when the host embryos, surrounded by their membranes, were taken out of the shell. Once the graft had been located, the embryonic membranes surrounding it were excised, fixed in Bouin's fluid, photographed and then sectioned (5 μm) and stained with Ehrlich's haematoxylin and Biebrich scarlet for histological study. The dark nuclei of implanted mouse dermal cells were easy to distinguish from the clear nuclei of the neighbouring mesenchymal cells of the chick or duck host.

<div align="center">RESULTS</div>

Given its position and its weight, the implanted block of dermis caused the somatic mesoderm to bulge downward and to contact the splanchnic mesoderm of the developing vitelline vesicle (Figs. 2*a* and 5). Thanks to this contact, the graft was quickly vascularized by the vitelline blood supply. Twenty-four hours later, the amniotic or chorionic ectoderm (Fig. 2*b*) situated above the mouse dermis was transformed into a stratified epidermis (Fig. 6). The ultimate location of the graft was found to be variable, and is schematically visualized in Fig. 2(*b*)–(*e*).

Four hundred and fifty grafts were made. One hundred and twenty-six hosts survived beyond 12 days of incubation and were examined between 10 and 14 days after the operation. These 126 cases can be divided in the following way: in 71 cases, the implanted mouse dermis, which was attached to the vitelline sac by means of the splanchnic blood vessels, was isolated from the overlying ectoderm by the extension of the allantois, as early as 48–72 h after the operation (Fig. 2*c*). No appendages were produced in these cases. In the remaining 55 cases, the mouse dermis lost neither its contacts with the extra-embryonic ectoderm nor its vascular connexions with the vitelline mesoderm. The latter connexions were either short and wide when the graft was located in the amnion (30 cases), or long and extremely thin, when it was situated in the chorion (25 cases).

In 9 out of the latter 25 cases, the chorionic ectoderm was drawn downwards by the graft, which was then stuck at the bottom of a sort of well (Fig. 2*d*). No appendages were formed in these cases.

In the remaining 46 cases, the extra-embryonic ectoderm above the mouse dermis underwent an appendage-forming morphogenesis. A number (from two to six) of small buds developed. On histological examination it could be seen that these ectodermal formations comprised a mesodermal pulp containing mouse cells exclusively (Figs. 7 and 8) and an epidermal sheath, which, in some cases, was thrown into irregular inward protruding folds (Fig. 9).

In 26 out of these 46 cases, this kind of hypomorphic morphogenesis (small buds) alone was found. In the remaining 20 cases, however, two kinds of appendages were observed: in the neighbourhood of the small buds, a group of long feather filaments had formed (Figs. 3, 4 and 10).
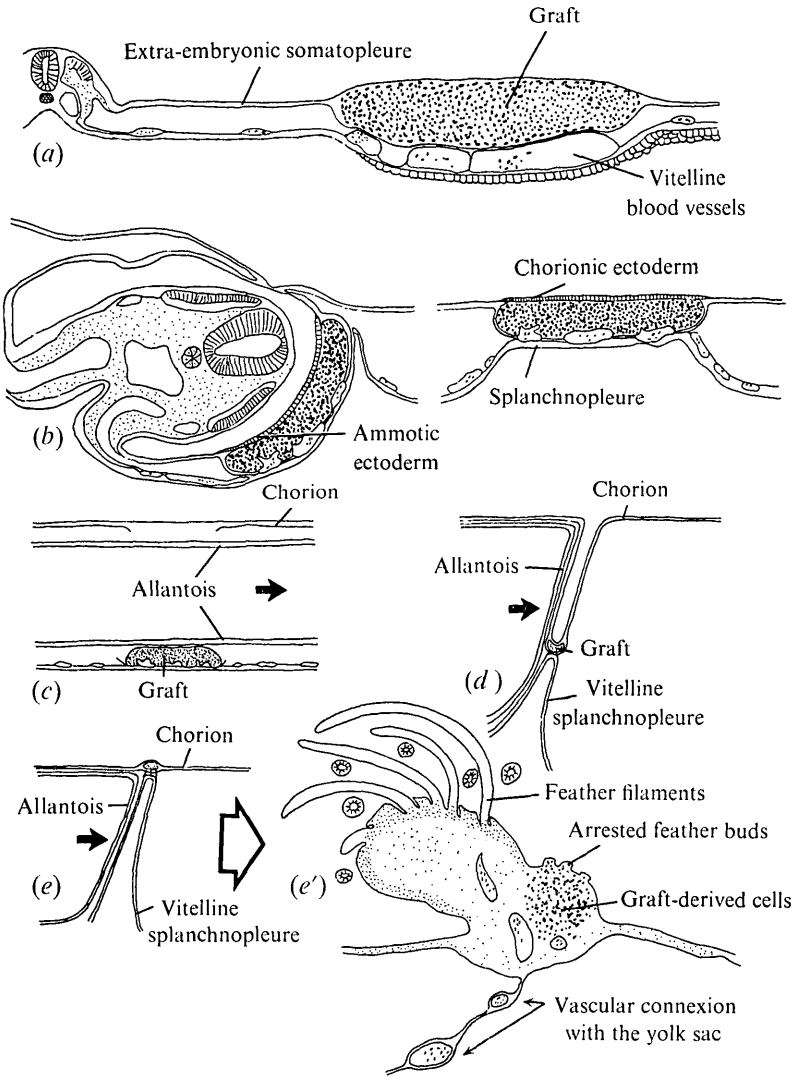
Fig. 2. Location of the graft 0–14 days after the implantation. (a) *Time zero:* relationship of the graft with the extra-embryonic membranes. (b) *Twenty-four hours later:* the graft is situated either within the developing amnion (left) or within the chorion (right), between ectoderm and mesoderm. It is vascularized by the vitelline blood supply. (c–e) *Seventy-two hours after implantation:* the three possibilities of ultimate location of the graft. (c) The outward extension of the allantois has isolated the graft from the ectoderm. (d) Both chorion and yolk sac have been stretched out by the abutting allantois (c and d: no appendages will be produced). (e) The yolk sac only has been stretched out by the presence of the graft, which remains at the outer surface of the embryonic membranes. (e′) *Fourteen days after implantation:* the extra-embryonic ectoderm has developed two kinds of appendages: small buds above the mouse dermis and long filaments in a piece of ectopic skin derived from the metaplastic transformation of the extra-embryonic membranes.

Figs. 3 and 4. Supernumerary pterylae formed by chick amniotic somatopleure (Fig. 3) or duck chorionic somatopleure (Fig. 4), 14 days after the implantation of mouse dermis in the extra-embryonic area. *A*, arrested feathers above the implanted mouse dermis; *C*, connexion with the yolk sac; *Pf*, praefiloplumae; *Pp*, praeplumulae. Bar: 0·6 mm.

These well-formed supernumerary feather filaments were situated in the amnion (independently of the umbilical cord), in 15 cases, at the junction of the amnion and the chorion (the amnion then remaining open), in one case, and in four cases in the chorion. Their number ranged from 2 to 60. In the cases where their number exceeded six (four cases), they were arranged in a more or less well recognizable pattern, either linear (one case) or hexagonal (three cases); in addition, each praepenna was surrounded respectively by praefiloplumae and praeplumulae in the case of a duck host (Fig. 4) or by praefiloplumae alone in the case of a chick host (Fig. 3). The length of the praepennae that had developed on the extra-embryonic membranes was equivalent to that of the praepennae of the host embryo. On histological examination, the cells of their dermal pulp were found to contain exclusively clear bird-type nuclei (Fig. 10). Their well-formed barb-ridges were arranged in a typical chick or duck fashion according to the host species (see Dhouailly, 1967).
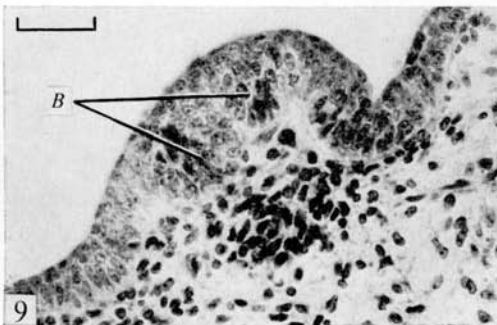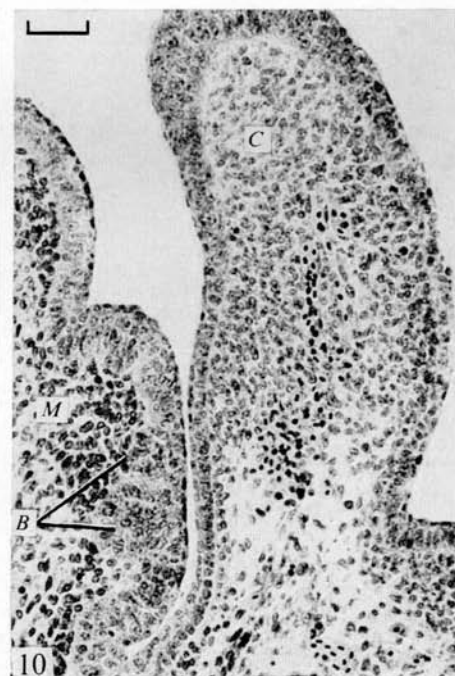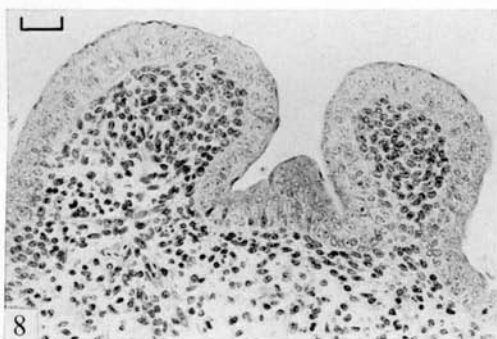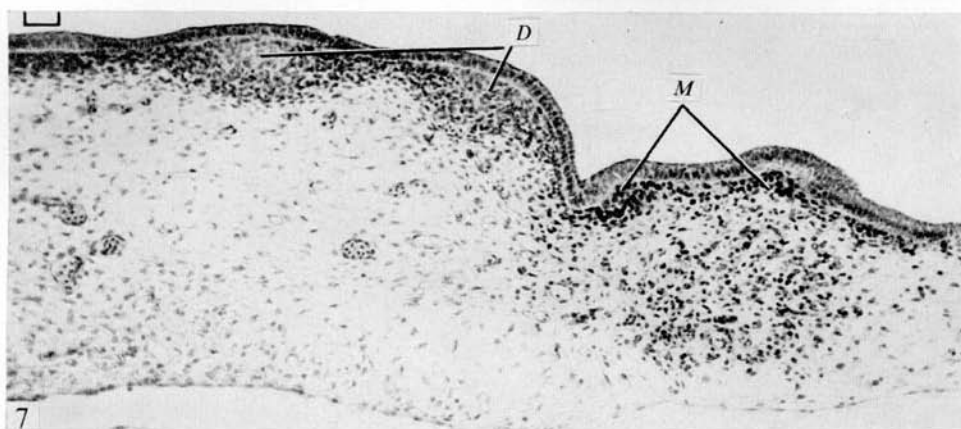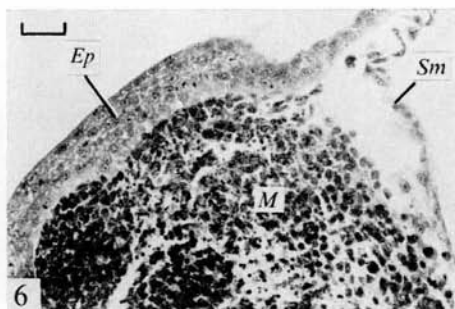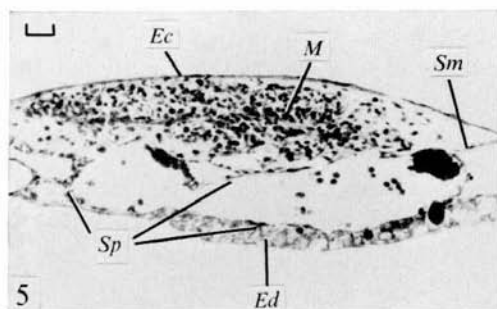
DISCUSSION

The implantation of mouse hair-forming dermis under avian extra-embryonic ectoderm causes the transformation of the latter into a typical stratified epidermis within 24 h and the subsequent development of two kinds of cutaneous appendages: small buds and typical long feather filaments.

The dermal component of the long feather filaments is exclusively of avian host origin. Grafted mouse cells do not participate in their formation. The morphogenetic events leading to their development are discussed below. Contrariwise, the small buds possess a dermal core exclusively formed by mouse cells. The epidermal sheath of these buds differentiates, in about 50 % of the cases, a number of irregular ridges, which can be interpreted as ill formed barb-ridges, similarly to those which were obtained in other types of chick epidermis/mouse dermis recombinations (Dhouailly, 1973). Accordingly, these buds can be taken as arrested feather filaments. This incomplete morphogenesis shows conclusively that the extra-embryonic ectoderm responds to an appendage-forming dermal induction in the same way as embryonic epidermis: it becomes involved in a type of morphogenesis in conformity to its avian class origin, i.e. it gives rise to feather placodes, and later to feather buds, which however, for want of the appropriate species-specific dermal information, cannot form well-shaped barb-ridges.

Contrariwise, the long feather filaments, whose dermal core is constituted solely by avian cells, form typical chick or duck barb-ridges; moreover, they may be arranged in a species-specific feather tract, according as they are produced in a chick or a duck embryo. They result from an autonomous morphogenetic activity of the extra-embryonic somatopleure, which is apparently

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Figs. 5–10. Differentiation of the avian extra-embryonic ectoderm after the implantation of mouse dermis (haematoxylin/Biebrich scarlet). Bar: 30 μm.

Fig. 5. *Time zero* (2 days incubation): the chick extra-embryonic ectoderm (*Ec*) is one cell thick. *M*, graft of mouse dermis; *Sm*, somatic mesoderm; *Sp*, splanchnic mesoderm with vitelline blood vessels; *Ed*, endoderm.

Fig. 6. *Twenty-four hours later:* formation of a pluristratified epidermis (*Ep*) above the implanted mouse dermis (*M*).

Fig. 7. *Ten days after implantation:* formation of two kinds of appendage primordia in the amnion of a duck host embryo. At left dermal condensations involve exclusively clear duck nuclei (*D*), at right mouse nuclei (*M*).

Figs. 8–10. *Fourteen days after implantation*. Figs. 8 and 9. Formation of arrested feather buds by chick (Fig. 8) and duck (Fig. 9) chorionic epithelium. Note the presence of ill-formed barb-ridges (*B*) (Fig. 9). Fig. 10. Formation of two kinds of appendages in the amnion of a chick embryo: an arrested feather with ill-formed barb-ridges (*B*) and a long feather filament with a dermal pulp exclusively constituted by clear chick nuclei (*C*).

caused by the mere presence of the graft, but requires neither its participation or its appendage-inducing capacity.

Similar ectopic feathers and feather tracts were obtained by Sengel & Kieny (1967 *a*, *b*) in the midventral apterium of the chick by implanting fragments of living tissues (neural tube, non-neural axial organs) or inanimate objects (agar, paraffin, aluminium) into the prospective area of the midventrum of 2-day chick embryos. The implants caused conspicuous perturbations of morphogenetic movements in the lateral plates and abnormal fusions between somatopleure and splanchnopleure. These in turn led to an ectopic increase in density of the prospective subectodermal mesenchyme of the midventral apterium, thus transforming it into a feather-forming dermis.

A similar situation appears to have prevailed in the present experiments. The graft, by becoming vascularized by and attached to the vitelline splanchnopleure, establishes a connective tissue link between both mesodermal layers. In addition, the graft may act as a mechanical obstacle to the centrifugal extension of the extra-embryonic tissues, thus causing an abnormal densification of the abutting somatopleural mesoderm.

This view and the analogous interpretation of Sengel & Kieny's results lead to the concept that the mere densification of the subectodermal mesenchyme above a certain threshold entails the acquisition of feather tract properties.

In conclusion, these and previous dermo-epidermal recombinations (Dhouailly, 1970, 1973, 1975) clearly show that the avian ectoderm all over the embryo and the extra-embryonic area is endowed with the same morphogenetic properties, namely the ability to build feather buds under the triggering influence of an underlying appendage-forming dermis. On the other hand, the subectodermal mesenchyme appears to be regionalized into feather-forming and bare embryonic and extra-embryonic areas. The latter do not possess the capacity to induce feather morphogenesis unless they are experimentally brought above a certain level of cell density, which by itself appears to be a sufficient condition for the acquisition of feather-inducing properties.

REFERENCES

BONETTI, D. (1963). L'action inductrice du derme de l'embryon de poulet sur l'épithélium chorionique en culture d'organe *in vitro*. *C. r. hebd. Séanc. Acad. Sci., Paris* D **249**, 1940–1941.

DHOUAILLY, D. (1967). Analyse des facteurs de la différenciation spécifique de la plume néoptile chez le Canard et le Poulet. *J. Embryol. exp. Morph.* **18**, 389–400.

DHOUAILLY, D. (1970). Détermination de la différenciation spécifique des plumes néoptiles et téléoptiles chez le Poulet et le Canard. *J. Embryol. exp. Morph.* **24**, 73–94.

DHOUAILLY, D. (1973). Dermo-epidermal interactions between birds and mammals: differentiation of cutaneous appendages. *J. Embryol. exp. Morph.* **30**, 587–603.

DHOUAILLY, D. (1975). Formation of cutaneous appendages in dermo-epidermal recombinations between Reptiles, Birds and Mammals. *Wilhelm Roux Arch. Devl Biol.* **177**, 323–340.

KATO, Y. & HAYASHI, Y. (1963). The inductive transformation of the chorionic epithelium into skin derivatives. *Expl Cell Res.* **31**, 599–602.

KIENY, M. & BRUGAL, M. (1978). Morphogenèse du membre chez l'embryon de poulet: compétence de l'ectoderme embryonnaire et extra-embryonnaire (submitted to Archives of Biology, 1978).

MIZUNO, T. (1972). Epidermal metaplasia of proamniotic epithelium induced by dorsal skin dermis in the chick embryo. *J. Embryol. exp. Morph.* **27**, 199–213.

SENGEL, P. & KIENY, M. (1967a). Production d'une ptéryle supplémentaire chez l'embryon de Poulet. I. Etude morphologique. *Archs Anat. microsc. Morph. exp.* **56**, 11–30.

SENGEL, P. & KIENY, M. (1967b). Production d'une ptéryle supplémentaire chez l'embryon de Poulet. II. Analyse expérimentale. *Devl Biol.* **16**, 532–563.