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Dermo-epidermal interactions between birds and mammals: differentiation of cutaneous appendages

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SUMMARY

1. The capacity of skin constituents to participate in feather and hair morphogenesis has been analysed in chick and mouse embryos.

2. Reconstituted homo- and heterospecific skin explants, consisting of epidermis and dermis from both species, were cultured for 8 days on the chorioallantoic membrane of the chick.

3. Recombinants of dorsal 11·5- and 12·5-day mouse epidermis and dorsal 7-day or tarsometatarsal 12-day chick dermis gave rise to stage 2 abnormally elongated hair prepapillae. Associations of plantar 14·5-day mouse epidermis with dorsal 7-day chick dermis formed stage 3 hair papillae.

4. The reverse combinations of dorsal 5- and 6-day chick epidermis and dorsal 11·5- to 14·5-day mouse dermis gave rise to arrested feather buds (with 11·5- and 12·5-day dermis) and to short and aberrant feather filaments (with 12·5-, 13·5- and 14·5-day dermis). These short filaments were characterized by the differentiation of easily recognizable but chaotically arranged barb-ridges. The same type of feather differentiation was obtained in recombinants of normally glabrous epidermis from the comb, midventral apterium, or tarsometatarsum from 10-day chick embryos and 13·5- and 14·5-day dorsal mouse dermis.

5. Control homospecific recombinations formed typical well organized feather filaments or stage 4–5 hair cone follicles. Heterospecific associations of feather- or hair-forming epidermis with dermis from glabrous regions did not differentiate any kind of cutaneous appendages.

6. When distribution of feather filaments was compared in recombinants of chick epidermis with either dorsal pelage hair dermis or upper-lip vibrissal dermis, it was found that the feather pattern conformed with the regional origin of the mouse dermis.

7. It was concluded that, during feather and hair development, the dermis transmits two kinds of morphogenetic messages: one that is apparently non-specific and can therefore be understood and expressed by a foreign epidermis from another zoological class, leading to the formation of feather or hair buds, in conformity with the origin of the epidermis; the other message contains specific cues necessary for specific morphological organization of feather and hair.

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INTRODUCTION

During embryogenesis, feather and hair primordia first appear as small whitish spots in the skin. These spots correspond to sites of increased opacity, which in turn are due to the formation of a thickened epidermal placode associated with an underlying dermal condensation of cells and fibrous material. In mammals, the dermal hair condensation is very small and inconspicuous at first; in birds, it is large, round and almost hemispherical. Earlier experiments have shown that the dermal component is responsible for the initiation of feather (Sengel, 1958) and hair (Kollar, 1970) formation. Without the dermal morphogenetic influence, no cutaneous appendages can form.

In brief, the early development of feather and hair can be described as follows. In mammals, epidermal buds extend from the placode into the dermis and form the hair plug by rapid proliferation of the stratum germinativum. The base of the plug then flattens, invaginates to form the papillar concavity, which is occupied by the condensation of dermal cells, the dermal papilla. Next the hair cone differentiates, pointing towards the surface of the skin. Inside the cone, the hair fibre is later formed. In birds, the feather bud bulges above the surface of the skin; it is constituted of an epidermal sheath and a dermal pulp. The elongation of feather bud gives rise to the feather filament. Within the inner part of the epidermal tube-shaped covering, barb-ridges differentiate in longitudinal rows. The core of the filament progressively withdraws towards the base and forms the permanent feather dermal papilla. Each barb-ridge gives rise to one barb and two rows of barbules of the neoptile feather.

The size, shape, number and architectural organization of barb-ridges are strictly dependent on the dermis. Thus, in heterospecific recombinations of dermis and epidermis between the duck and the chick, the dermis controls the specific morphology of the neoptile and teleoptile feather (Dhouailly, 1967, 1970). These results show that the dermis, even though it does not structurally participate in the construction of the feather, forces the epidermal cells to arrange themselves, by the means of some unknown mechanism, according to a specific pattern.

In order to gain a more precise knowledge of the role played by the dermis in the morphogenesis of cutaneous appendages, new recombination experiments were performed between epidermis and dermis of species less closely related than were the duck and the chick, namely the mouse and the chick. It was hoped, that in these more 'difficult' conditions, the morphogenetic activity of both dermis and epidermis could be analysed more accurately, particularly because the formation of the early feather and hair primordia differ markedly in their shape, unlike the morphologically indistinguishable duck and chick feather buds.

Several authors have already demonstrated the possibility of skin tissue associations between birds and mammals. Garber, Kollar & Moscona (1968) have obtained the formation of hair buds in co-aggregates of mouse epidermal

and chick skin cells. It was not possible from their experiments to decide whether hair rudiment formation might be due to continuation of processes initiated before explantation or whether mouse epidermis can respond to induction by chick dermis. In fact, in addition to experiments that failed to give rise to any cutaneous appendages (Mordoh & Lustig, 1966), two types of apparently contradictory results were observed, in which the epidermal differentiation either conformed to the specific origin of the epidermis (Jorquera & Pugin, 1971; Coulombre & Coulombre, 1971) or was under the specific control of the dermis (Propper, 1969).

The present research (where mouse and chick epidermis from naked, feathered or haired areas was combined with several types of dermal tissues) led to the conclusion that the morphogenetic activity of the dermis was dual and involved the transmission of both specific and non-specific factors, and that the type of cutaneous appendages conformed to the specific origin of the epidermis.

A preliminary and partial account of these results has already been published elsewhere (Dhouailly & Sengel, 1972).

MATERIAL AND METHODS

White Leghorn chick and mouse embryos of the OF 1 strain (Swiss originated) were used in these experiments.

Age was recorded as days following the inception of incubation for the chick. For the mouse, males were introduced into the cages with virgin females at 9 p.m. and removed again at 8 a.m. the next morning. The time of gestation was counted as starting 2 h after the male had been introduced into the cage with the females. Thus the nominal age is a maximum. In extreme cases embryos may be 12 h younger than the nominal age, i.e. if mating occurred late in the morning instead of approximately 2 h after beginning of cohabitation.

Blocks of skin ($2 \times 2{\cdot}5$ mm) were taken from different regions of the embryos: (1) *Chick:* dorsum of 5-, 6-, or 7-day, anterior tarsometatarsum, midventral apterium, comb of 10-, or 12-day. (2) *Mouse:* dorsum of 11·5-, 12·5-, 13·5-, or 14·5-day; upper lip of 12·5-day; plantar surface of the posterior foot-plate of 14·5-day.

These tissue fragments were treated with a solution of 0·5 % trypsin (Choay) and 1 % pangestine (Difco). Following this treatment, the epidermis and dermis were separated and stored until recombinations were performed.

Experimental and control tissue recombinations were prepared and cultured for 1 h to cohere on a medium composed of Tyrode's solution clotted with agar before they were transferred to the chorioallantoic membrane (CAM) of 10-day chick embryos. They were examined every day, and, unless stated otherwise, fixed after 8 days of culture and stained with Ehrlich's haematoxylin and Biebrich scarlet. Mouse nuclei stain darkly, chick nuclei more lightly (Wolff, 1954), and thus can easily be distinguished from one another in histological sections.

Stages of hair development were characterized according to the numeration and terminology of Hardy (1951): stage 0 = no hair primordia, epidermis of constant thickness; stage 1 = hair plug; stage 2 = pre-papilla; stage 3 = papilla; stage 4 = follicle with fibre cone; stage 5 = follicle with hair canal.

Stages of feather development were defined according to the terminology of Sengel (1971): *feather rudiment*, composed of a dermal condensation and an overlying epidermal placode, without measurable outgrowth; *feather bud*, consisting of an epidermal sheath and a dermal pulp, bulging measurably above the surface of the skin; *feather filament*, elongated structure characterized by the differentiation of barb-ridges in the epidermal tube-shaped sheath.

RESULTS

1. *Skin recombinations involving dorsal mouse epidermis* (*Table 1*)

In mice, pelage follicles begin their development at 14 days of gestation.

Homoplastic recombinants of dorsal mouse epidermis and dermis from 12·5-day embryos developed a majority of stage 4 hair follicles, characterized by the differentiation of a hair cone; in some explants, a few follicles formed a hair canal typical of stage 5 (Fig. 1).

Xenoplastic recombinants of 7-day dorsal chick dermis and 12·5-day dorsal mouse epidermis developed 7 to 12 hair pre-papillae (stage 2) after 3 days in culture; the epidermis extended into the underlying dermis by proliferation of the stratum germinativum, and was in contact with a typical chick dermal condensation (Fig. 2). After 8 days on the CAM, these primordia had elongated deeply into dermis (Fig. 3). They were then longer (0·15 mm) than wide (0·05 mm). However, they never formed either a papillar concavity or a hair cone and therefore could not be identified with any of the stages of normal development. Indeed, normal hair follicles of this length constantly show at least the formation of the papillar concavity (stage 3) or even the differentiation of a hair cone (stage 4).

A few xenoplastic recombinations using very thin and fragile 11·5-day dorsal mouse epidermis were also prepared. The results were the same as with the older epidermis of the previous series.

Xenoplastic recombinants of 12-day tarsometatarsal chick dermis and 12·5-day dorsal mouse epidermis developed hair pre-papillae (stage 2). These rudiments were somewhat longer than the normal stage 2 pre-papillae, were regularly spaced and separated by epidermal folds (Fig. 5). The chick dermis used for these recombinations had been removed from the anterior face of the foot at 12 days, when contours of future scales were already visible as distinct interscutellar furrows.

Of eleven chimaeric skin explants composed of 12-day chick dermis from the midventral apterium and 12·5-day dorsal mouse epidermis, three did not differentiate. In the eight remaining cases, one to four very short nodules (0·02 mm

in depth and diameter) consisting of about 20 cells had intruded into the dermis. Their volume was at least 6 times smaller than a normal stage 1 plug.

2. *Skin recombinations involving dorsal chick epidermis (Table 2)*

Dorsal skin from 6-day chick embryos is still undifferentiated and flat and contains no feather rudiments. Homoplastic recombinants of 6-day dorsal epidermis and dermis developed feather filaments of 3–6 mm in length, after 8 days on the CAM.

Xenoplastic recombinants of 5-, or 6-day dorsal chick epidermis and 13·5-, or 14·5-day dorsal mouse dermis developed dome-shaped buds after 3–4 days in culture. These consisted of an epidermal sheath bulging out above the surface of the skin, enclosing a mouse dermal papilla (Fig. 6). After 8 days, these buds formed very short and aberrant feather filaments. Their final length did not exceed 0·5 mm. Nevertheless, the epidermal sheath had differentiated numerous tortuous ridges (Fig. 7). Although their shape and orientation were not typical of normal feather morphology, the appearance and linear arrangement of their constituting cells (Fig. 8) were characteristic of barb-ridges (Fig. 9).

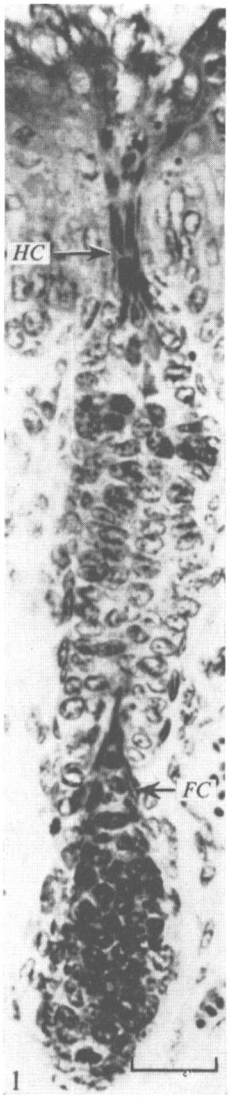
When dorsal mouse dermis was obtained from 12·5-day embryos and associated with 6-day dorsal chick epidermis, short aberrant feather filaments of the same type as before formed in some of the explants. In the others, feather buds did not elongate at all above the surface of the skin and gave rise to arrested feathers (Fig. 10), without any indication of barb-ridge differentiation. The latter type of feather-like structures arose from epidermal placodes that had appeared with a delay of 2–3 days (at days 5 or 6 of culture) as compared with the previous series of experiments, where the mouse dermis was taken at 13·5 or 14·5 days of gestation. When dorsal mouse pre-dermal mesenchyme was obtained from 11·5-day embryos, in three out of four cases, the epidermis remained flat and formed no placodes; in one case arrested feather-like structures were formed, comparable with that shown in Fig. 10.

In xenoplastic recombinants of 14·5-day mouse plantar dermis and 6-day chick dorsal epidermis, no rudiments of cutaneous appendages were obtained. The epidermis became very thick and large amounts of keratin were formed (Fig. 14).

3. *Skin recombinations involving non-hair- or non-feather-forming epidermis (Table 3)*

The mouse plantar epidermis of the posterior foot plates does not develop hair follicles at any time. Removed at 14·5 days of gestation and associated with 7-day dorsal chick dermis, it produced hair papillae with a flattened base (Fig. 4) or with a well-formed papillar cavity filled exclusively with dermal chick cells.

Similarly, epidermis, which does not form any feathers under normal conditions, such as obtained from the tarsometatarsum, the comb or the midventral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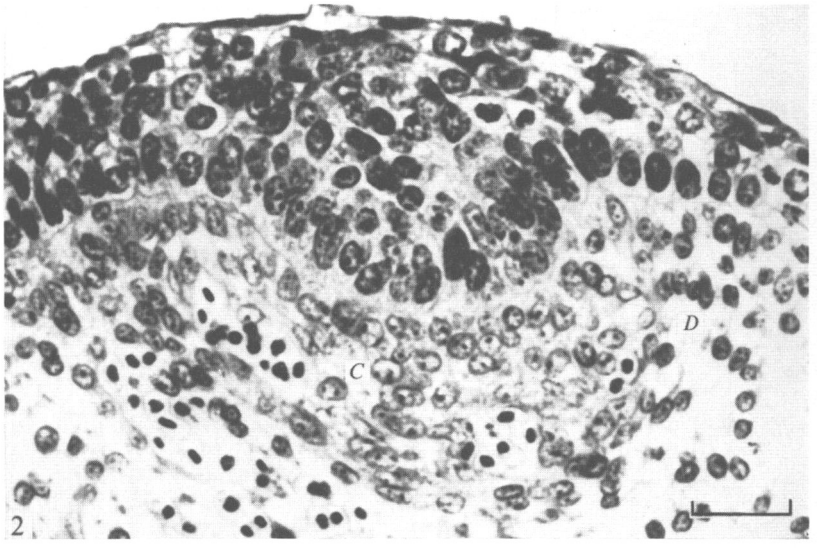
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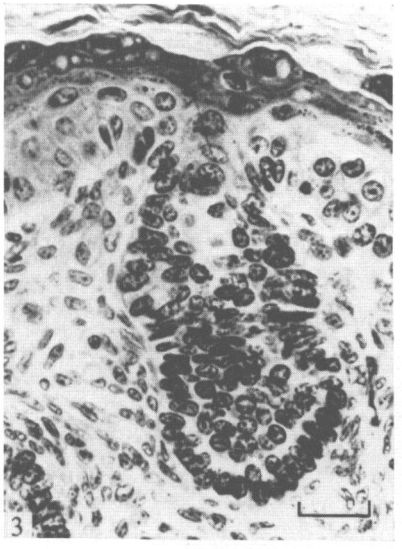
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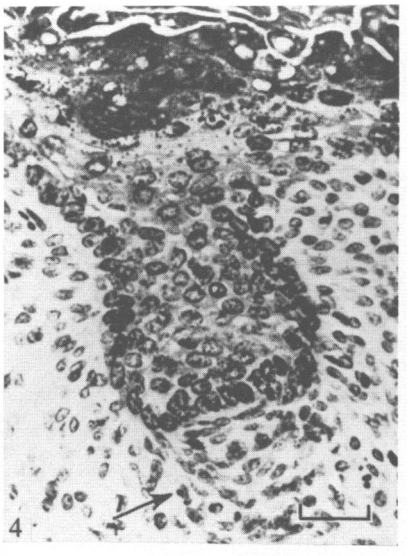
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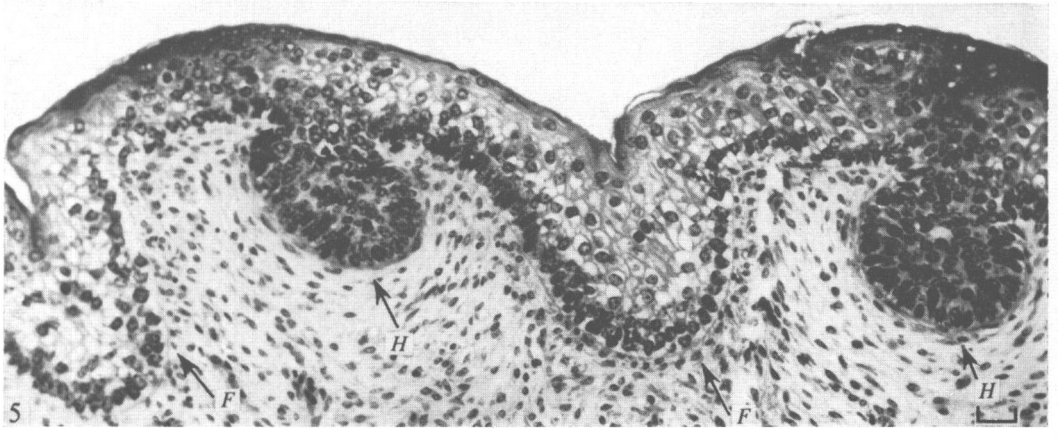
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Table 1. *Cutaneous appendages formed in skin recombinations involving dorsal mouse epidermis, after 8 days of culture on the CAM*

Origin of dermis	Origin of epidermis	No. of cases	Cutaneous appendages: Mean no. per explant	Cutaneous appendages: Type
Mouse dorsal 12·5 days	Mouse* dorsal 12·5 days	5	25	Stage 4 or 5 pelage hair follicles
Chick dorsal 7 days		23	10	Stage 2 abnormally elongated pelage hair pre-papillae
Chick tarsometatarsal 12 days		17	7	Stage 2 slightly elongated pelage hair pre-papillae
Chick midventral apterium 12 days		11 (3)	0	None
		11 (8)	3	Small atypical epidermal nodules
Chick dorsal 7 days	Mouse* dorsal 11·5 days	5	12	Stage 2 abnormally elongated pelage hair pre-papillae

* Stage 0 of pelage hair development.

FIGURES 1–5

Skin recombinants involving mouse epidermis, cultured on the chorioallantoic membrane of the chick. Haematoxylin–Biebrich scarlet: darkly staining mouse nuclei are easily distinguished from lighter staining chick nuclei.

Fig. 1. Homoplastic recombinant of 12·5-day dorsal mouse epidermis and dermis, that developed after 8 days of culture. Example of hair follicle of stage 5. Note the formation of a hair canal (*HC*) and a fibre cone (*FC*).

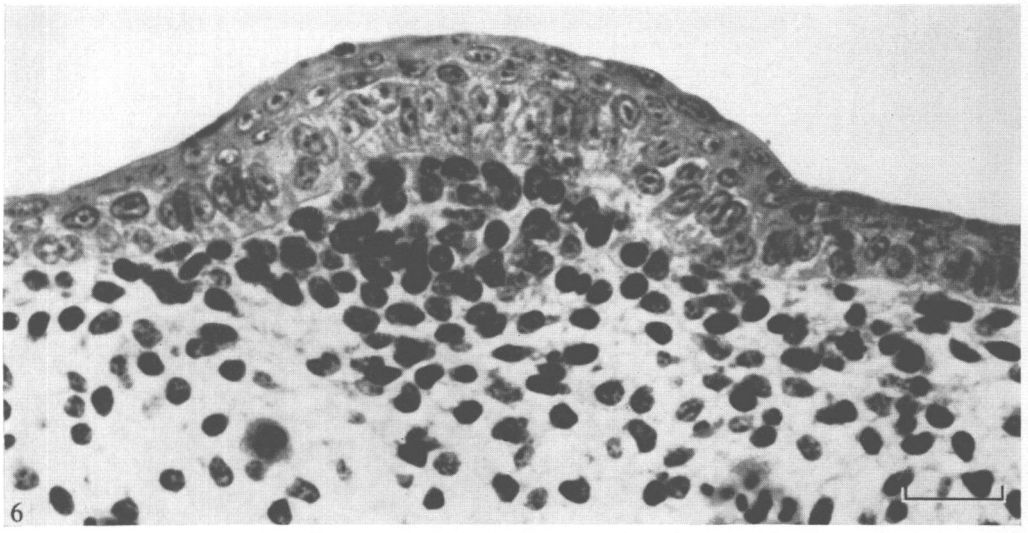
Fig. 2. Xenoplastic recombinant of 12·5-day mouse epidermis and 7-day dorsal chick dermis, after 3 days of culture. Hair pre-papilla (stage 2) that developed in contact with a chick dermal condensation, typically constituted by central cells with big and clear nuclei (*C*) surrounded by cells with small and dark nuclei (*D*).

Fig. 3. Same type of recombinant as Fig. 2, after 8 days of culture. The hair pre-papilla is abnormally elongated, but did not differentiate any fibre cone or papillar cavity.

Fig. 4. Xenoplastic recombinant of 14·5-day plantar mouse epidermis and 7-day dorsal chick dermis. In this normally glabrous epidermis, stage 3 hair papillae developed after 8 days of culture. They were flattened or even cavitated at their base, where they were in close contact with a dermal papilla formed exclusively by chick cells (arrow).

Fig. 5. Xenoplastic recombinant of 12·5-day dorsal mouse epidermis and 12-day tarsometatarsal chick dermis. Note the differentiation of hair pre-papillae (*H*) between epidermal scale folds (*F*).

Bars = 20 μm.



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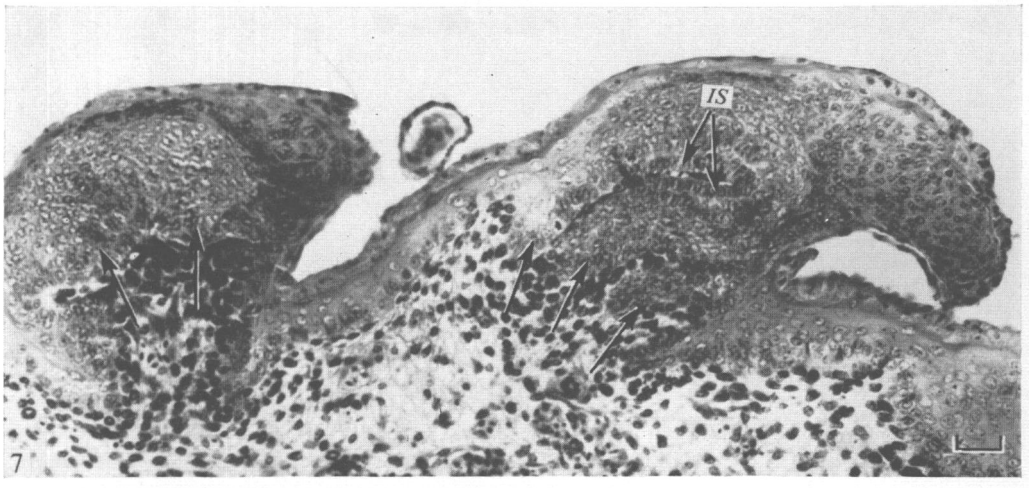

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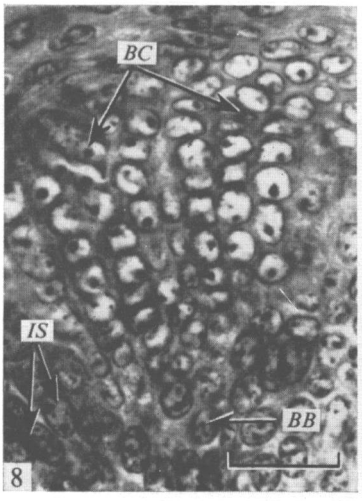

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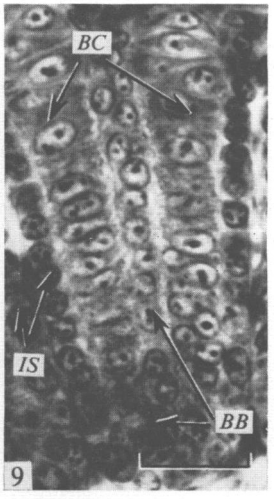

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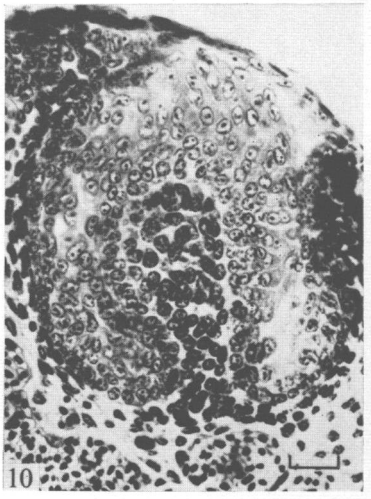

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Table 2. *Cutaneous appendages formed in skin recombinations involving dorsal chick epidermis, after 8 days of culture on the CAM*

Origin of dermis	Origin of epidermis	No. of cases	Cutaneous appendages: Mean no. per explant	Cutaneous appendages: Type
Chick dorsal 6 days	Chick* dorsal 6 days	5	18	Feather filaments
Mouse pre-dermal mesenchyme 11·5 days	Chick* dorsal 6 days	4 (3)	0	None
		4 (1)	5	Arrested feather buds
Mouse dorsal 12·5 days	Chick* dorsal 6 days	12 (5)	6	Arrested feather buds
		12 (7)	20	Short and aberrant feather filaments
Mouse dorsal 13·5 and 14·5 days	Chick* dorsal 6 days	8	30	Short and aberrant feather filaments
Mouse plantar 14·5 days	Chick* dorsal 6 days	5	0	None
Mouse dorsal 13·5 and 14·5 days	Chick* dorsal 5 days	4	22	Short and aberrant feather filaments

* Stage 0 of feather development.

FIGURES 6–10

Skin recombinants involving chick epidermis, cultured on the chorioallantoic membrane of the chick.

Fig. 6. Xenoplastic recombinant of 6-day dorsal chick epidermis and 14·5-day dorsal mouse dermis, after 4 days of culture. Example of a chimaeric feather bud, composed of an epidermal sheath of chick cells overlying a dermal condensation of mouse cells.

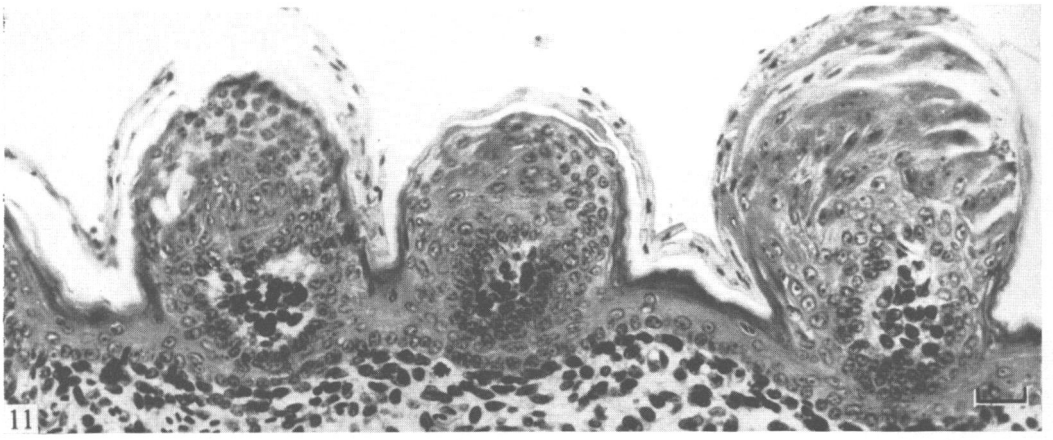
Fig. 7. Same type of recombinant as Fig. 6, after 8 days of culture. The feather buds developed into short feather filaments (the one at right is cut longitudinally in its entire length). The apex is filled exclusively by chick epidermal cells. Chaotic feather barb-ridges differentiated (arrows), with well recognizable inner sheath cells (*IS*).

Fig. 8. Cross-section of feather filament that developed in the previous combination (Fig. 7) reveals typical, although somewhat hyperplastic, barb-ridges. Note the characteristic linear arrangement of epidermal barbule cells (*BC*), with their large and clear nuclei. *BB*, barb cells; *IS*, inner sheath cells. Compare with a normal barb-ridge (Fig. 9).

Fig. 9. Cross-section of a barb-ridge from a normal feather filament that differentiated in a homospecific recombinant of chick epidermis and dermis. *BB*, barb cells; *BC*, barbule cells; *IS*, inner sheath cells.

Fig. 10. Xenoplastic recombinant of 6-day dorsal chick epidermis and 12·5-day dorsal mouse epidermis. Differentiation of an arrested feather bud.

Bars = 20 μm.



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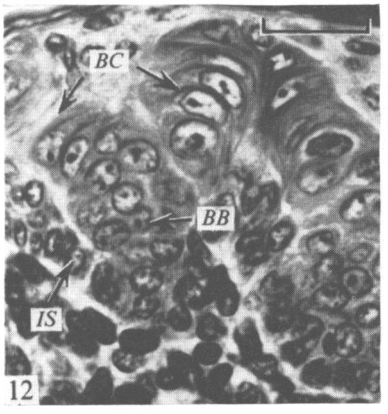

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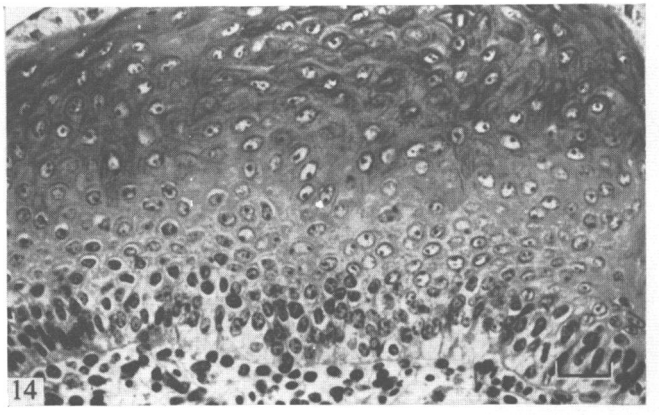

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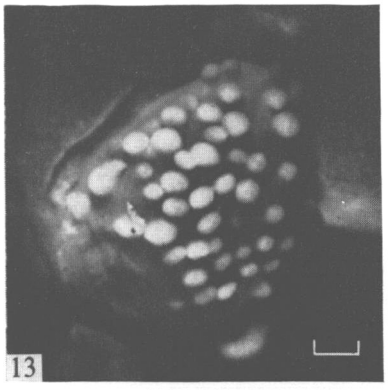

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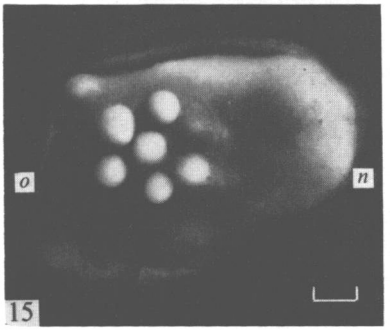

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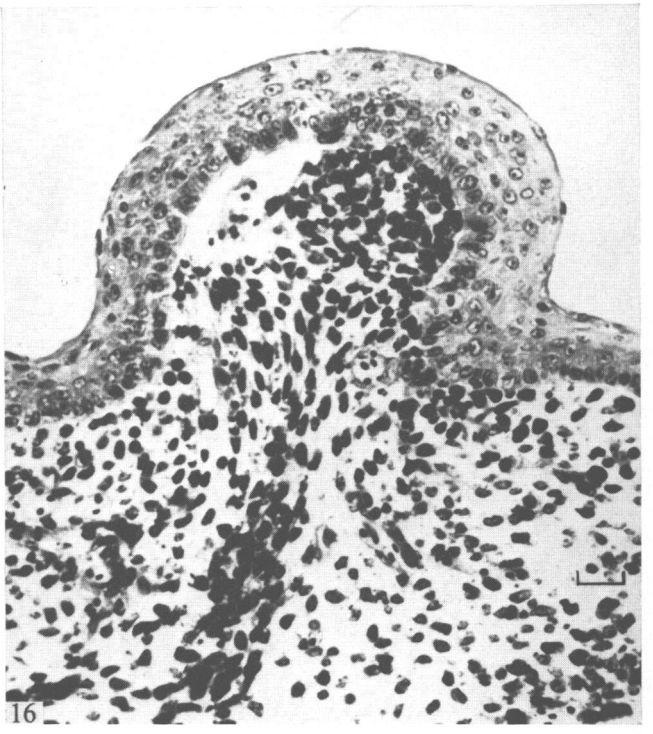

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Table 3. *Cutaneous appendages formed in skin recombinations involving non-hair-forming or non-feather-forming epidermis, after 8 days of culture on the CAM*

Origin of dermis	Origin of epidermis		No. of cases	Cutaneous appendages: Mean no. per explant	Cutaneous appendages: Type
Chick dorsal 7 days	Mouse plantar 14·5 days		15	13	Stage 3 hair papillae
Mouse dorsal 12·5 days	Chick 10 days	Tarsometatarsum	13	0	None
		Midventral apterium	12		
		Comb	2		
Mouse upper-lip 12·5 days	Chick 10 days	Tarsometatarsum	9	6	Large aberrant feather filaments (0·2 mm in diameter)
		Midventral apterium	8		
		Comb	14		
Mouse dorsal 14·5 days	Chick 10 days	Tarsometatarsum	9	35	Small aberrant feather filaments (0·1 mm in diameter)
		Midventral apterium	5		
		Comb	9		

apterium at 10 days of incubation and associated with 14·5-day dorsal mouse dermis, gave rise to short aberrant feather filaments whose pulpar cavity was filled exclusively with mouse dermal cells (Fig. 11). In a few cases, typical barbule cells differentiated (Fig. 12). However, the epidermis of these recombinants keratinized early, rapidly and formed desquamating sheets of cells. The thickness of the living part of that epidermis remained roughly constant and never formed barb-ridges.

FIGURES 11–16

Skin recombinants involving chick epidermis and mouse dermis from three different body regions.

Figs. 11–13. Xenoplastic recombinants of 10-day comb chick epidermis and 14·5-day dorsal mouse dermis. Differentiation of numerous and short feather filaments (Figs. 11, 13). Note the presence of 15 larger feather filaments intermingled with 30 smaller ones. In a few cases, the epidermal sheath formed barb-ridges with their typical elements (Fig. 12): barb cells (*BB*), barbule cells (*BC*), and inner sheath cells (*IS*). The pulp cavity is filled by mouse dermal cells.

Fig. 14. Xenoplastic recombinant of 6-day dorsal chick epidermis and 14·5-day plantar mouse dermis. No cutaneous appendages formed. The epidermis is unusually thick and consists of numerous strata of keratinizing cells.

Figs. 15, 16. Xenoplastic recombinant of 10-day comb chick epidermis and 12·5-day upper-lip mouse dermis. Differentiation of a limited number of short and large feather filaments. Compare their disposition and diameter with those of Figs. 11 and 13 (same magnifications). They are restricted to the ocular half of the dermal component (*o*, ocular side; *n*, nasal side). Note typical vibrissal pattern of these feather filaments (cf. Fig. 17).

Black bars = 20 μm; white bars = 0·3 mm.

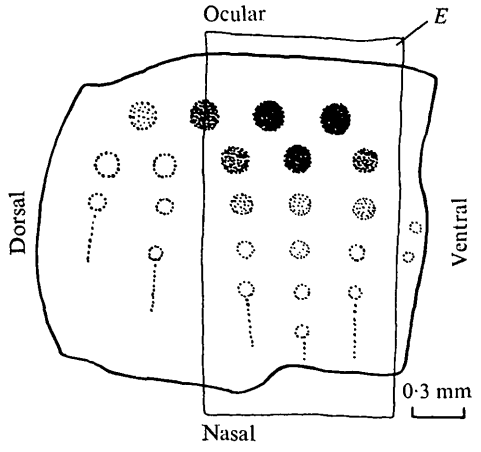

Fig. 17. Pattern of vibrissae in mouse left upper-lip dermis recombined with chick non-feather-forming epidermis (*E*) (from comb, tarsometatarsum or midventral apterium). *Stippled circles*, dermal primordia of vibrissae present at explantation at 12·5-day of gestation (the darkest ones are the first to appear); *dotted circles*, site of prospective vibrissae normally appearing at 13 days of gestation; no feather filaments developed over these dermal primordia which are not yet formed at explantation.

When non-feather-forming epidermis was associated with a 12·5-day dorsal mouse dermis, cutaneous appendages were always lacking. The epidermis became highly keratinized and wrinkled.

4. *Xenoplastic recombinants and pattern of cutaneous appendages* (*Table 3*)

In the mouse, the primary X follicles appear widely spaced at approximately equal distances from one another. New follicles are formed later in the spaces between them (Hardy, 1951). In the chick, the feather buds are arranged in a strict hexagonal pattern (Sengel, 1971).

In the preceding experiments, where chick epidermis was combined with mouse dermis from the dorsal pelage hair region, explants gave rise to 20–50 feather filaments, among which two kinds could, in some cases, be distinguished: large ones (0·15 mm in diameter), irregularly intermingled with small ones (not exceeding 0·1 mm in diameter) (Figs. 11, 13). The reciprocal combination of epidermis from dorsal pelage hair region and dorsal chick dermis formed seven to twelve hair follicles. The distribution of these chimaeric cutaneous appendages on the surface of the explant was however too irregular and random to be assimilated to any patterned configuration.

In consequence, a new experimental series was performed, using pieces of mouse upper-lip and chick featherless skin. The vibrissae follicles, which are larger than pelage follicles, arise in rows starting next to the eye and proceeding towards the olfactory pit in a gradient of developmental stages (Fig. 17 and Kollar, 1966). Upper-lip dermis from 12·5-day mouse embryos was associated

with 10-day chick epidermis from the comb, the tarsometatarsum or the midventral apterium. Explants had approximately the same size as in the preceding experiments (2 × 1·5 mm). Five to nine feather buds, 0·2 mm in diameter (Fig. 15), developed on each of the recombinants. They were strictly localized in the region of the explant where the epidermis was in contact with that portion of the dermis which would have given rise to the first vibrissae. In other words, they were restricted to the ocular half of the dermal component. Their arrangement and number seemed to correspond to the vibrissae pattern (Fig. 17).

DISCUSSION

The present experiments show that hair rudiments can develop in mouse epidermis combined with dorsal chick dermis. The formation of these rudiments, which do not develop beyond the stage of hair-papilla, does not result from autonomous differentiation of the mouse epidermis. Indeed, the latter, when obtained from early 11·5-day or 12·5-day embryos, is unable to differentiate any cutaneous appendages in combination with non-feather-forming chick dermis from the midventral apterium or with non-hair-forming mouse dermis from the plantar region (Kollar, 1970). The hair rudiments, then, which are formed in recombinants of dorsal mouse epidermis and dorsal chick dermis, result from a morphogenetic influence exerted by the feather-forming chick dermis. This morphogenetic activity is furthermore clearly demonstrated by the production of hair rudiments in recombinations of normally glabrous plantar mouse epidermis with dorsal chick dermis. Chick dermis from the tarsometatarsum appears to be endowed with a similar morphogenetic capacity, since it is also able to elicit the formation of hair rudiments in dorsal mouse epidermis.

Similarly, feather structures can be induced in chick epidermis by dorsal mouse dermis. These feathers do not develop beyond the formation of barb-ridges and barbule cell differentiation, the organization of which however is more or less chaotic, resulting in short aberrant feather filaments. This morphogenesis is not due to autonomous differentiating capacities of the epidermis, because the epidermis is unable, when obtained at 5 or 6 days of incubation, to form feathers in combination with dermis from glabrous regions like the midventral apterium of the chick (Sengel, Dhouailly & Kieny, 1969) or the plantar region of the mouse. Consequently, the feather rudiments that are formed in recombinants of dorsal chick epidermis and dorsal mouse dermis develop under an inductive influence emanating from the mouse dermis. The morphogenetic capacity of the latter is also clearly demonstrated by its ability to induce feather filaments in normally featherless chick epidermis from the comb, the tarsometatarsum, the midventral apterium, and also, as shown by Coulombre & Coulombre (1971), from the cornea. The morphogenetic activity of the associated dermis is further attested by the number and size of the feather filaments, which depend on the regional origin of the dermis: they are numerous and

small with dorsal dermis (pelage hair), few and big with upper-lip dermis (vibrissae).

The results of these xenoplastic combinations thus lead to the conclusion that the epidermis always responds according to its specific (feather-forming or hair-forming) properties. The dermis appears to be the source of a non-specific triggering factor, without which the epidermis is unable to express its morphogenetic capacities. Previous experiments, where initiation of hair formation was obtained in chimaeric limbs composed of chick mesoderm and rat ectoderm (Jorquera & Pugin, 1971), and where feather filaments developed in chick cornea associated with mouse dermis (Coulombre & Coulombre, 1971), are in line with this conclusion. The opposite conclusion was reached by Propper (1969) in experiments where mammary type glandular invaginations were formed in chick epidermis combined with rabbit dermis from the pectoral region. In some of these recombinations, however, the chick epidermal cells that gave rise to glandular invaginations lost their normal aspect and acquired a morphology and staining properties similar to those of rabbit cells, a transformation which was never observed in the present experiments, where chick and mouse cells maintained their original specific morphology and stainability throughout the cultivation period.

The development of the cutaneous appendages obtained with heterospecific dermis does not proceed to the accomplishment of either feather or hair, suggesting that, once morphogenesis has started in the epidermis under the influence of the non-specific dermal factor, its continuation is dependent on other and more specific cues also originating from the dermis. The necessity of a homospecific dermal influence for the sustained elongation of feather filaments was also illustrated by Garber *et al.* (1968), who obtained either arrested feather buds when epidermis was in contact with a pure population of mouse dermal cells, or elongated feather filaments with well differentiated barb-ridges when the associated dermis was a mixed population of mouse and chick cells. Similar conditions appeared to prevail in combinations of mouse epidermis and chick dermis to which a small proportion of mouse dermal cells had been added (unpublished data). It was then observed that whenever a few mouse cells came to lie adjacent to the ingrowing hair plug, the latter would develop further and reach a typical hair cone stage. In the same explant, however, those hair rudiments which happened to be surrounded by chick cells only did not differentiate beyond the pre-papilla or papilla stage.

Thus, late specific inductive factors are required to control and sustain continued elongation of the feather bud and harmonious patterning of barb-ridges, as well as differentiation of hair cone and individualization of hair and inner root sheath.

In birds, taking advantage of the complexity of the specific architecture of the neoptile and teleoptile feathers, it was possible to demonstrate that such specific factors were indeed at work during feather elongation and differentiation of

barb-ridges: feathers developing from the heterotopic (wing and thigh tracts, Cairns & Saunders, 1954) or heterospecific (chick and duck, Dhouailly, 1967, 1970) combination of epidermis and dermis always conform in their gross morphology (arrangement, size and number of barbs) to the origin of the dermis.

Besides its non-specific triggering influence and its specific morphogenetic action, the dermis appears to exert a third type of organizing effect on the epidermis, namely on the spatial distribution of cutaneous appendages. By comparing the density and pattern of feather rudiments which were induced in chick epidermis by either dorsal or upper-lip mouse dermis, it became evident that the arrangement (and also size) of the appendages was determined by the regional origin of the mouse dermis. These results are in line with the data obtained in the chick by Sengel & Novel (1970) and by Linsenmayer (1972), who showed that polarity and regional specificity of feathers and scales was strictly governed by the dermis.

In conclusion, the results of the present experiments may be interpreted in the following way: the formation of a feather or hair results from a continued morphogenetic influence exerted by the dermis on the epidermis. This influence contains at least two messages: one of them is non-specific and may be transmitted, interpreted and morphologically translated by any epidermis of heterotopical or heterospecific origin. The response of the epidermis to this message leads to the formation of a rudimentary cutaneous appendage, whose specific morphology depends exclusively on intrinsic properties of the epidermis. The site and distribution of the rudiments, however, are determined by the dermis. The other message transmitted from the dermis is a specific one and contains determinants for the specific arrangement of epidermal keratinizing cells. This message can only be used and interpreted by an epidermis of the same zoological class of vertebrates; it is inadequate for an epidermis of another class, which is already in the process of building its own type of appendage and unable to translate the foreign message into its own language. Under these circumstances, the epidermis that is associated with dermis from another zoological class is unable to give rise to the hair cone (in the case of mouse epidermis) or to correctly organized barb-ridges (in the case of chick epidermis).

The differentiation of barb-ridges and barbule cells in the latter case deserves some additional discussion. From the experiments, where ill-organized barb-ridges were formed in combinations of chick epidermis and mouse dermis, it can be assumed that the epidermis possesses the intrinsic capacity of constructing barb-ridges but needs the specific dermal information to organize them in a particular pattern. In intra-class combinations between chick and duck (Dhouailly, 1967, 1970) this information is correctly understood and expressed morphologically by the foreign epidermis. In inter-class combinations of mouse and chick, the mouse dermis does not deliver this type of information, hence the chaotic arrangement of barb-ridges.

RÉSUMÉ

1. L'aptitude des constituants cutanés, derme et épiderme, à participer à la morphogenèse de la plume et du poil a été étudiée chez l'embryon de poulet et de souris.

2. Les fragments de peau reconstituée homo- et hétérospécifique, comprenant derme et épiderme des deux espèces, ont été cultivés pendant 8 jours sur la membrane chorio-allantoïdienne du poulet.

3. Les associations d'épiderme dorsal de souris de 11,5 et 12,5 jours et de derme dorsal de poulet de 7 jours ou tarsométatarsien de 12 jours ont donné naissance à des bourgeons pileux de stade 2 anormalement longs. Les combinaisons d'épiderme plantaire de souris de 14,5 jours et de derme dorsal de poulet de 7 jours ont formé des bourgeons pileux de stade 3.

4. Les associations inverses d'épiderme dorsal de poulet de 5 et 6 jours et de derme dorsal de souris de 11,5 à 14,5 jours ont formé soit des ébauches plumaires ne s'élevant pas au-dessus de la surface de la peau (avec du derme de 11,5 et 12,5 jours), soit de très courts filaments plumaires (avec du derme de 12,5, 13,5 et 14,5 jours). Ces filaments sont caractérisés par la présence de crêtes barbaires chaotiques. Une différenciation plumaire semblable a été obtenue dans le cas d'épiderme de région aptère (crête, tarsométatarse, aptérie médioventrale) de poulet de 10 jours associé à un derme dorsal de souris de 13,5 et 14,5 jours.

5. Les associations homospécifiques témoins de peau dorsale de poulet et de souris ont formé respectivement de longs filaments plumaires conformes au développement normal de la plume néoptile ou des follicules pileux de stade 4 et parfois 5. Aucun phanère ne s'est différencié à partir d'explants xénoplastiques comportant du derme d'une région glabre de l'embryon de poulet ou de souris.

6. Le patron plumaire des associations d'épiderme de poulet et de derme de souris est conforme à la qualité régionale du derme employé, derme dorsal (pelage) ou derme de la lèvre supérieure (vibrisses).

7. En conclusion, pendant le développement de la plume et du poil, le derme transmet à l'épiderme deux catégories de message morphogène. L'un est non-spécifique et peut être compris et interprété par un épiderme d'une autre classe zoologique; il aboutit à l'édification de bourgeons plumaires ou pileux, conformément à l'origine de l'épiderme. L'autre contient des informations spécifiques nécessaires à l'organisation morphologique spécifique de la plume et du poil.

This paper represents a portion of the thesis that will be submitted by the author to the Université scientifique et médicale de Grenoble for the degree of Doctorat d'Etat ès Sciences. The results in this paper were presented at the Third Meeting of the European Society for Dermatological Research, Amsterdam, 25–26 April 1973.

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