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Special Review

Pattern formation in skin development

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Introduction

Chick embryo skin is a morphogenetic system easily accessible to experimental analysis. Like most other organs it is composed of two tissues, one epithelial, the other mesenchymal, of distinct embryological origin. The advantage of skin over other organogenetic systems is that its two components, epidermis and dermis, become superimposed upon each other at the periphery of the body early in development and stay in this situation throughout life, so that they remain readily attainable for experimental manipulation. Epidermis is a pluristratified keratinizing epithelium of ectodermal origin, while dermis arises from mesoderm and acquires a typical mesenchymal (connective tissue) structure. The two tissues are linked to each other by extracellular matrix, part of which, the basement membrane, mostly of epidermal origin, develops underneath the basal pole of basal epidermal cells, the rest of it being of mesenchymal origin and constituting the interstitial dermal matrix. Skin produces various types of adnexa, such as cutaneous glands and keratinized appendages. The latter in birds comprise feathers and scales, in addition to beak, comb, spur, claws and wattle. In the present brief review, discussion will be restricted to the development of feathers and scales.

Several fundamental questions have been asked regarding the development of those two types of cutaneous appendages. Some of them have received partial answers. They refer to the origin of skin and appendages, to the establishment of the feather or scale pattern, to the mechanisms whereby individual feathers or scales are constructed (Sengel, 1976a, b).

The explanation of the origin of skin is pretty straightforward, although not entirely solved. During gastrulation and neurulation, presumptive mesoderm reaches an inside location where the outer part of its somitic and somatopleural components becomes associated with the overlying ectoderm. Interspecific quail/chick transplantation experiments have shown that in the dorsal and dorso-lateral regions, presumptive dermal cells arise from the dermatomal part of the somitic mesoderm, while in the lateral and ventral body parts they originate from the somatopleural layer of the lateral plates (Mauger, 1972a). In the head region, the majority of dermal cells arise from neural crest cells.

Once the entire previously cell-empty space beneath the ectoderm is populated by prospective dermal cells, the integument has acquired morphogenetic properties, the expression of which can lead to the formation of cutaneous appendages. By this time – corresponding in the chick embryo to approximately 4 days of incubation – although skin as such is not yet individualized, particularly because dermis is not yet histologically distinguishable from the underlying subcutaneous mesenchyme, the presumptive integument, made up of the ectodermal cover, the ecto-mesodermal junction and the predermal mesenchyme, is able to give rise to cutaneous appendages when isolated in a suitable nutritive environment, such as the chorio-allantoic membrane of the chick. Thus, some time between gastrulation and 4 days of incubation, the prospective skin tissues acquire appendage-forming properties and become independent from underlying tissues.

By what mechanism and through what messages does prospective skin acquire these properties? This leads us to the problem of feather or scale pattern formation. Indeed, in chicks as in most birds, feathers and scales are not evenly distributed on the surface of the body (Fig. 1). They are restricted to certain areas called feather tracts (pterylae) or scale tracts. Between these tracts exist appendageless or appendage-poor regions, the so-called apteria or partial apteria, respectively. Tracts, apteria and partial apteria are disposed in a very regular, genetically controlled, fashion. The existence of feather-forming, scale-forming, and glabrous regions raises the question of their embryonic determination.

Furthermore, within each tract, individual feathers or scales are also arranged in a regular, although somewhat variable, manner. Feathers are arranged in a hexagonal pattern, while scales are disposed on the foot in proximo-distally oriented rows.

Finally, each appendage rudiment is an organogenetic system of its own, the development of which has been analyzed mainly by dermal-epidermal recombination experiments, where the role of each of the two component tissues can be determined and the contents of the exchanged morphogenetic messages can be, at least in part, deciphered.

The formation of pterylae and apteria

Between 5 and 6 days of incubation, typical skin is formed at first only in certain restricted areas, which correspond to the future pterylae - the feather-forming areas. Outside these zones, skin differentiates later at a much slower rate. Feather-forming areas are characterized by a dense dermis (2.60 nuclei/1000 μm^3), readily distinguishable from the underlying less dense (1.96 nuclei/1000 μm^3) subcutaneous mesenchyme (Wessells, 1965), and by an epidermis the basal layer of which acquires its permanent columnar epithelial structure. In prospective glabrous regions, these morphological features are not attained until very much later in development.

The question of the choice between feather-forming skin and glabrous skin has been approached by trying to produce featherless skin within the boundaries of a pteryla, or conversely to induce ectopic feathers in regions where they normally do not form.

The production of experimental apteria

The production of featherless skin can be achieved by several microsurgical operations performed early in development, at about 2 days of incubation. Excision of a portion of spinal cord (Fig. 2a), the length of which equals that of 3 - 10 somites, frequently results in the formation of a transverse band of featherless skin spanning the whole width of the spinal pteryla (Fig. 2b) (Sengel and Kieny, 1963; Mauger, 1972b). Likewise the bilateral X-irradiation of a chain of 3 - 6 somites, the spinal cord being either shielded or not, and the lateral plates being shielded from the irradiation by an

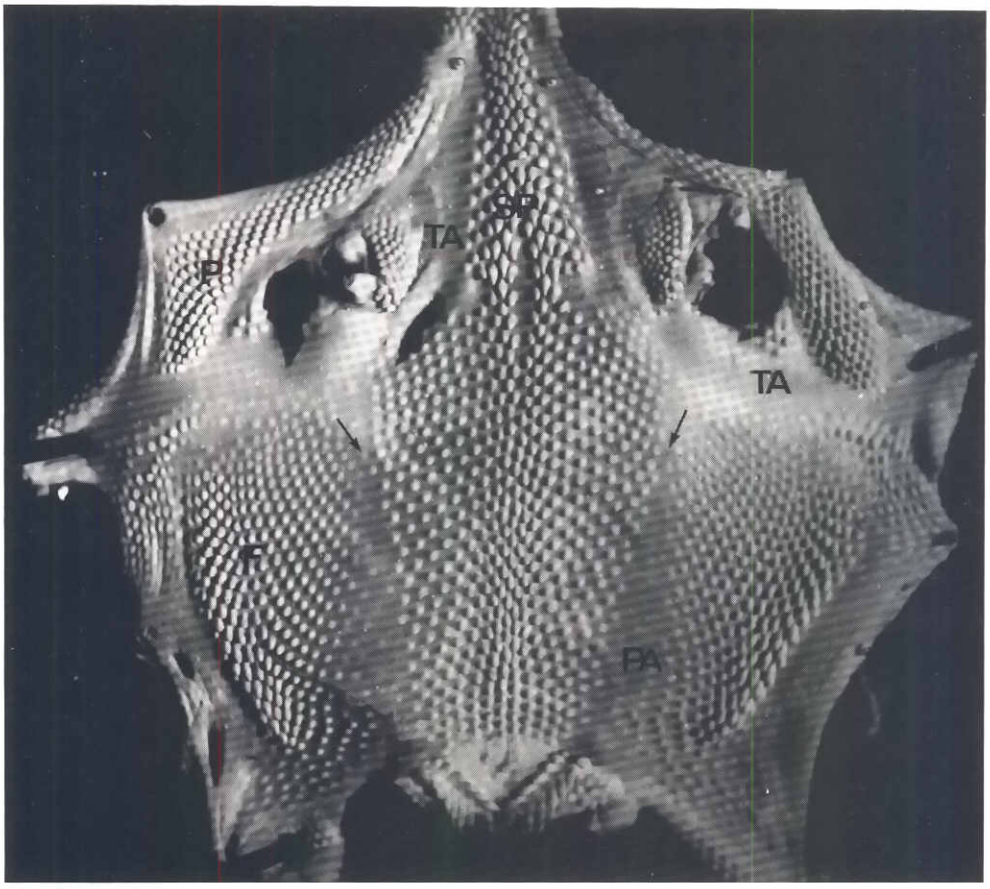


Fig.1. Part of the pterylose of an 11-day chick embryo. *The anterior cervical part of the spinal pteryla (Sp) runs out of the picture at the top. Note the presence of a small but characteristic mid-dorsal apterium in the spinal pteryla, slightly below the shoulder level. Partial apteria (PA) separate the femoral pterylae (F) from the spinal pteryla except at one anterior point (arrow). Total apteria (TA) are seen between the spinal and the pectoral (P) pterylae, and between the base of the wing and the femoral pteryla. Observe the decreasing length of the feather buds, in mediolateral direction in the spinal pteryla, in opposite distal-proximal direction in the femoral pterylae. These directions are evidence of the sequence in which the rows of feather buds were laid down.*

adequately shaped tantalum screen, leads to the formation of a transverse apterium (Fig. 3a, b) (Sengel and Mauger, 1967; Mauger, 1970). Unilateral X-irradiation of a chain of somites results in the formation of a bare indentation of the spinal pteryla at the level of the irradiation (Fig. 3c). Interestingly, excision of somites, even bilaterally, never results in the formation of an apterium; the

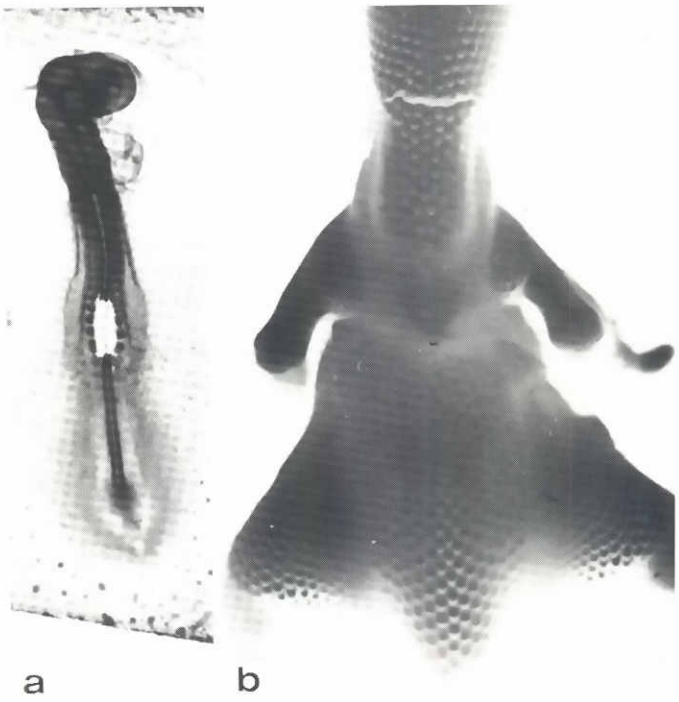


Fig. 2. Production of an experimental featherless area within the spinal pteryla. (a) *2-day chick embryo, just after the surgical removal of a portion of its spinal cord.* **(b)** *Spinalectomized embryo at 10 days of incubation showing the effect of the absence of a portion of the spinal cord on the differentiation of the dermatomal derivatives of the somitic mesoderms lining the neural gap. Beneath the experimental apterium, which occupies the entire width of the pteryla, a portion of the vertebral column is missing (not shown).*

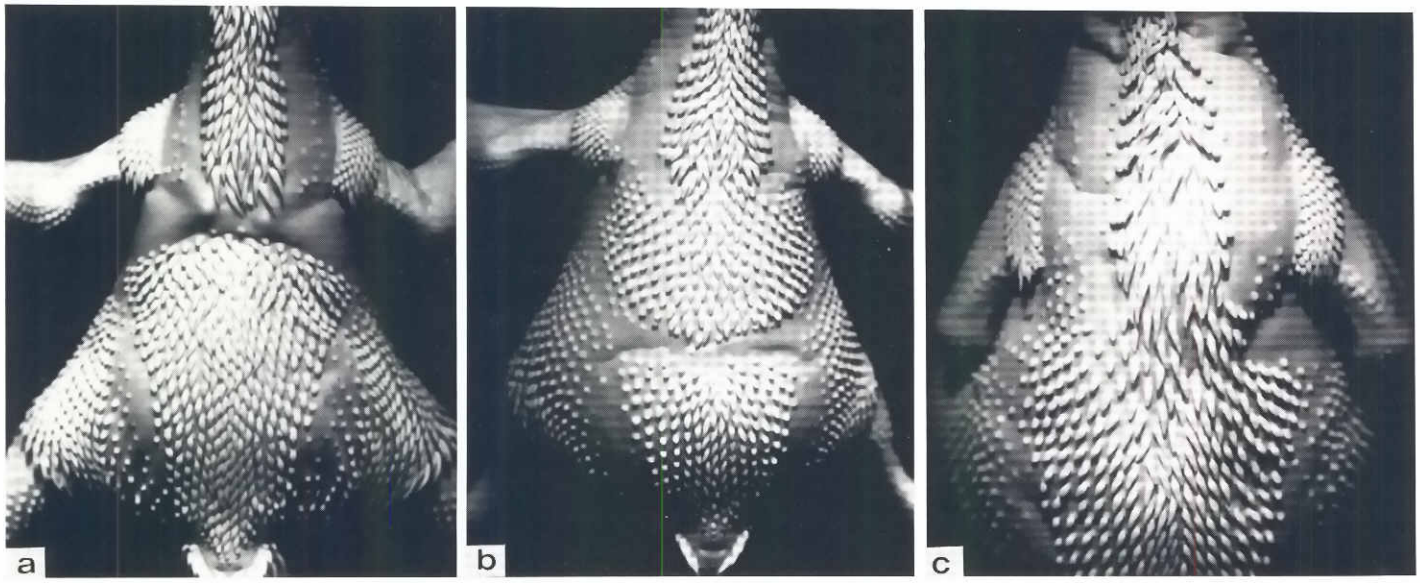


Fig. 3. Experimental apteria produced in the spinal pteryla after localized irradiation of somitic mesoderm, at 2 days of incubation. (a) *and* **(b)** *Transverse apterium in a 12-day embryo after bilateral X-irradiation of spinal cord and adjacent prospective somites 15 to 20* **(a)** *or 22 to 27* **(b)**. *In* **(b)**, *observe the invasion of bare patches by feathers belonging, according to their develomental stages, to the femoral tracts.* **(c)** *Featherless notch produced in the lateral part of the spinal pteryla by a unilateral X-irradiation of right prospective somites 17-22.*

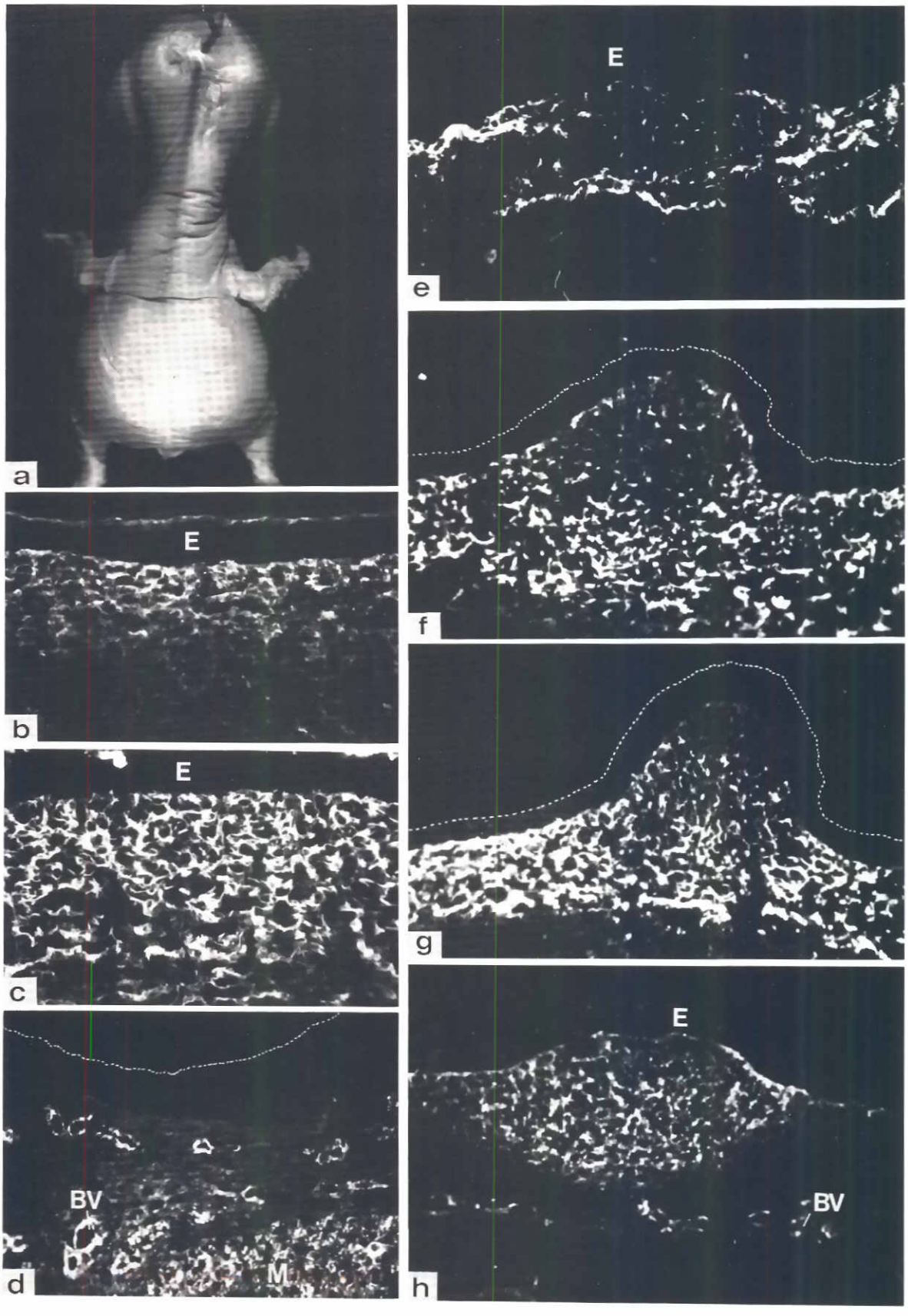
spinal tract always develops normally. This is due to the fact that somitic mesoderm is endowed with a high regulative capacity, the excised somites being rapidly replaced by compensatory proliferation from somitic regions anterior and posterior to the excision site. However, regulation of somitic mesoderm can be inhibited by the implantation of foreign living tissue, such as gut for instance; in this case, a featherless notch appears in the spinal pteryla over the region where the somites were excised. A general feature of these featherlessness-producing experiments is that dense dermis does not form in the integument overlying the operated site.

These experiments show that, when the source of dorsal dermal cells is suppressed by either excision or killed by irradiation, feathers will not form in the region that should have been populated by those dermal cells and their offspring. The result of spinalectomy demonstrates further that even though dermatomal cells are left unscathed by this operation, the presence of the spinal cord at one time in development is necessary for the adjacent somites to undergo further morphogenesis by differentiating into dermatome, myotome and sclerotome, and then to acquire feather-forming properties, i.e. to give rise to feather-forming dense dermis. It must be assumed then that a morphogenetic factor of neural origin is required for the somitic mesoderm to give rise to feather-forming dermis.

Another remarkable feature of all experimentally-produced featherless zones in the spinal pteryla is that they always extend to the very edge of the feather tract. In other words, the experimentally-produced apteria either suppress a complete transverse belt of feathers or affect only lateral rows, leaving the more medially located feathers unaffected. In no case do lateral rows of feathers form where the initial medial ones are missing. The idea that becomes apparent then is that the morphogenetic signal originating from the spinal cord is transmitted in medio-lateral direction conferring feather-forming properties to ever more distally located dermal cells, in such a way that no distal cell can acquire these properties without more medially-located cells having acquired them beforehand. This concept of a morphogenetic wave spreading from the middorsum to the lateral parts of the prospective spinal feather tract (Sengel and Novel, 1970; Novel, 1973) is reinforced by the results of the treatment of embryos by hydrocortisone.

Indeed injection of 0.1 mg hydrocortisone into the yolk sac of 5- to 8-day chick embyos results in extensive deficiencies in the dorsal plumage (Sengel and Züst, 1968; Züst, 1971). Treatment before,

Fig.4. Extracellular matrix in apterous skin and in normally feathered skin. (a) *12-day chick embryo showing nearly total absence of plumage after hydrocortisone treatment at 6 days of incubation. Note the presence of developmentally retarded feathers in thoracic, posterior cervical, and sacral regions of the spinal pteryla (initial rows), in the femoral tract (initial row), in the humeral and cubital tracts (see Fig. 1).* **(b-h)** *Immunofluorescent localization of extracellular matrix components in abnormal featherless skin* **(b-d)** *and in normal feather-forming skin* **(e-h)**. **(b)** *Uniform distribution of type I collagen in the dorsal skin of a hydrocortisone treated embryo, at 7 days of incubation.* **(c)** *Uniform distribution of type I collagen in the dorsal skin of a 9-day scaleless mutant.* **(d)** *Distribution of fibronectin in the dorsal skin of a 12-day scaleless mutant: note the almost total absence of the antigen, except around blood vessels (BV), nerve endings and muscle cells (M).* **(e-g)** *Distribution of type I collagen in normal feather-forming skin, at three stages of feather bud formation: note the disappearance of collagen fibres at the apex of the buds;* **(h)** *preferential accumulation of fibronectin in the dermal condensation of an early feather bud. E, epidermis; in* **(d-f)**, *the outer limit of the epidermis is indicated by white stipple.* **(b-d)** *x110;* **(e-h)** *x80.*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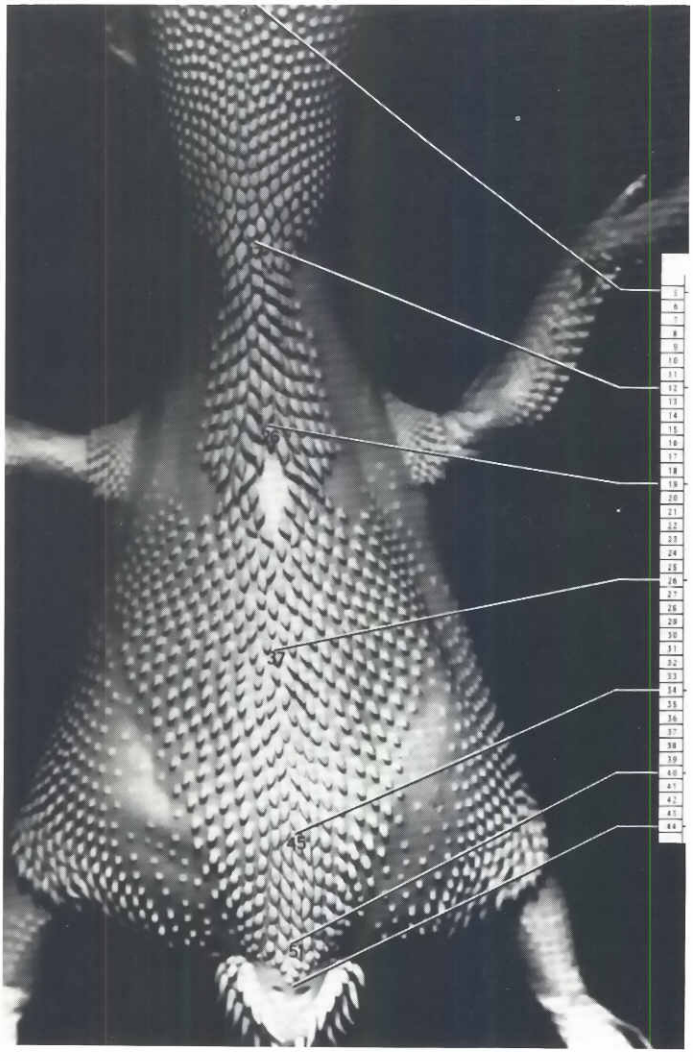


Fig. 6. Dorsal view of a 10.5-day chick embryo showing the extent and dermatomal origin (expressed as somite numbers, on the right) of the spinal pteryla. *Median feathers are numbered in cephalocaudal sequence, feather number 1 (not shown) being located at the level of the anterior edge of the atlas.*

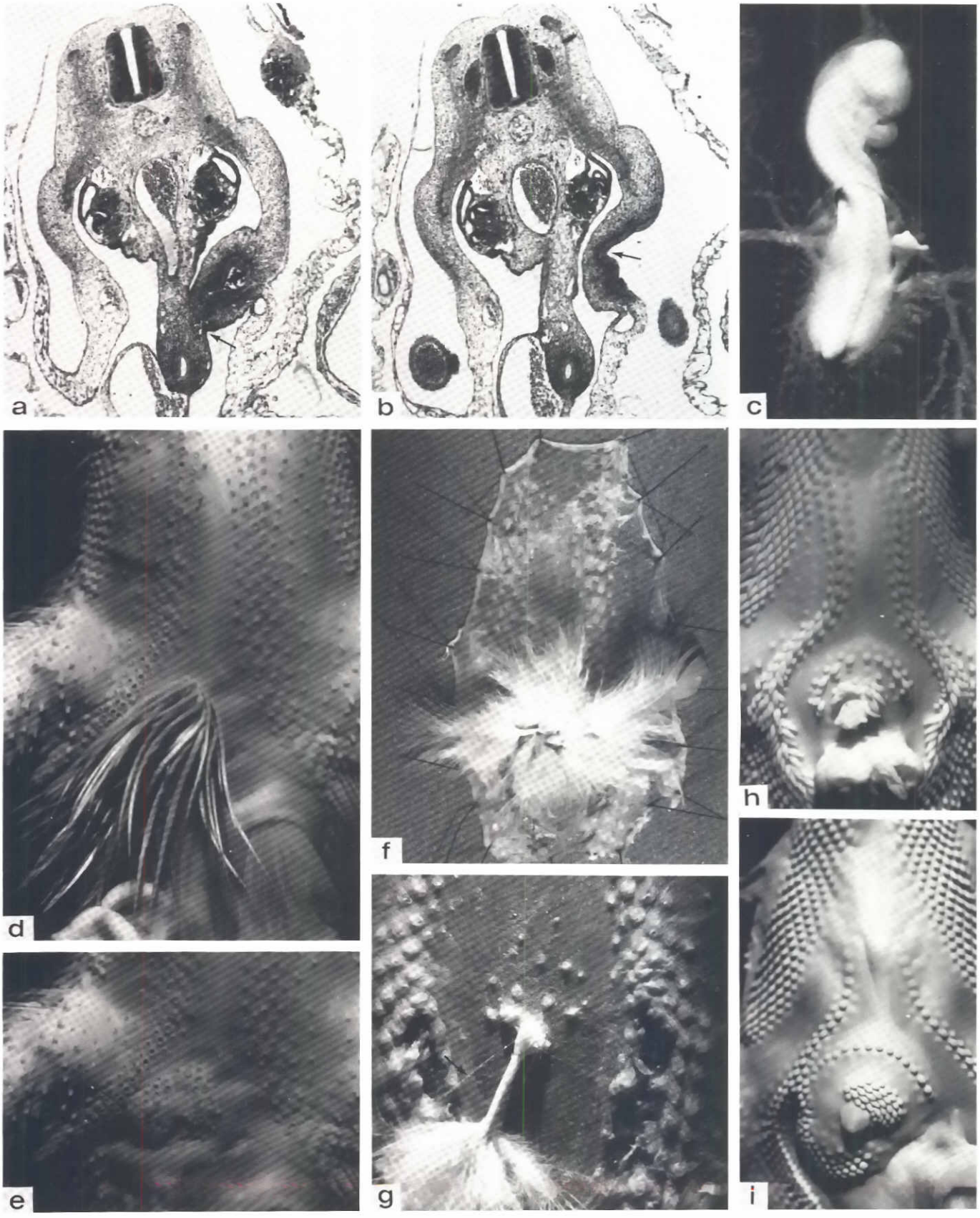
or after, these stages, i.e. at 4, 9, or 10 days, does not produce any deficiencies in the plumage. This means that 48 hours before the onset of feather rudiment formation hydrocortisone has no effect on skin histogenesis. It also indicates that hydrocortisone as such probably does not circulate at high doses for more than 24 hours, since an injection at 5 days produces malformations of the pterylae. Harmlessness of injections at 9 or 10 days demonstrates that once all feather rudiments have been laid down in the skin (at 9 days), they become insensitive to the drug.

When the embryos are treated at ages ranging from 5 to 8 days, extensive deficiencies occur in the plumage. The extent of the featherless zones varies with the age at which the injection is performed. The earlier the injection, the larger the apteria. Treatment at 5 or 6 days may result in the absence of feathers in the spinal tract (Fig. 4a) or in the total absence of feathers in a tranverse band of skin extending over the whole breadth of the spinal pteryla. Treatment at 7 or 8 days affects only lateral rows of feathers leaving the initial medial ones unharmed, with the number of missing lateral rows being larger after treatment at 7 days than after treatment at 8 days. In no case do lateral rows appear when at the same level the medial ones are absent, again suggesting the predominance of the first forming rows over the later ones.

At histology, as was the case after spinalectomy or X-irradiation of somites, it is clear that in hydrocortisone-treated embryos dense feather-forming dermis does not form. It definitively remains in a pre-feather-forming stage of low cell density, as is the case for instance in the normal midventral apterium. Thus hydrocortisone somehow prevents pre-dermis from reaching the density that would allow it to acquire feather-forming properties.

Indeed cell proliferation in the dermis of hydrocortisone-treated embryos is lower than normal (Démarchez *et al.*, 1984), which may in part explain why the dermis does not reach the needed threshold above which feather formation becomes possible. Interestingly, collagen synthesis is increased two- to three-fold as compared to controls, while protein synthesis as a whole is little affected or even downgraded. Immunofluorescent labeling of frozen sections with monospecific anti-collagen antibodies indeed reveals high amounts of uniformly distributed fibrous type I (Fig. 4b) and type III collagen deposits in the dermis of glabrous zones. Contrariwise, staining with anti-fibronectin antibody shows that dermis of featherless zones is completely devoid of this glycoprotein, except around blood vessels and nerve endings. By contrast, in normally developing feather tracts, as described below, interstitial collagens (Fig. 4e-g) and fibronectin (Fig. 4h) become microheterogeneously distributed at the time when feather rudiments begin to form (Mauger *et al.*, 1982a, b).

Fig. 5. Production of supernumerary feather tracts in the midventral apterium of the chick embryo. (a, b) *Transverse section (x40) through a 4-day chick embryo operated in the manner illustrated in* **(c)**, *48 h after implantation of a foreign body in the presumptive area of the midventral apterium. The section passes through the operated region. Note, on the right operated side, an abnormal fusion (***(a)***, arrow) between somatopleure and splanchnopleure, leading, in a neighbouring section (***(b)***, arrow) to an abnormal densification of the subectodermal mesenchyme (compare with contralateral side) in the prospective area of the right ventral abdominal wall, a region destined to give rise to the right half of the midventral apterium.* **(c)** *At 2 days of incubation, a foreign body, either living or inanimate (here an embryonic 7-day cartilaginous tibiotarsus), is implanted in a slit through somatopleure and splanchopleure in the boundary region between right embryonic and extraembryonic areas, at the cephalocaudal level corresponding to the prospective area of the abdominal wall or of the umbilicus (the embryo shown here was fixed and photographed 24 h after the implantation).* **(d-i)** *Resulting ectopic feather tracts in the midventral apterium of 11-day* **(h-i)**, *17-day* **(d-e)** *embryos, and 5-month-old chicken* **(f-g)**. **(d-e)** *Single supernumerary feather tract comprising some 50 feather filaments, arranged in a semi-circular fashion (for clarity, most feathers have been plucked from adjacent abdominal and pectoral tracts* **(d)**, *and also from the supernumerary tract* **(e)**, *leaving the empty feather follicles.* **(f-g)** *Extra single feather tract comprising 18 prepennae (all of which were plucked in (g), 29 filoplumae (most of which were plucked in* **(g)**, *one of which is indicated by an arrow), and 1 penna (which was left in place in its follicle in* **(g)**; *the presence of filoplumae associated with the follicles of pennae is clear evidence that the extra feathers belong to feather tract as such, and are not merely randomly distributed appendages.* **(h-i)** *Two examples of complex extra feathered areas, comprised of the association of at least two distinct feather tracts. Both ectopic formations contain a peripheral semi-circular field and a central field, separated from each other by an apteric zone; in* **(i)**, *the semi-circular field is partially fused with the nearby right ventral tract, a rare occurrence.*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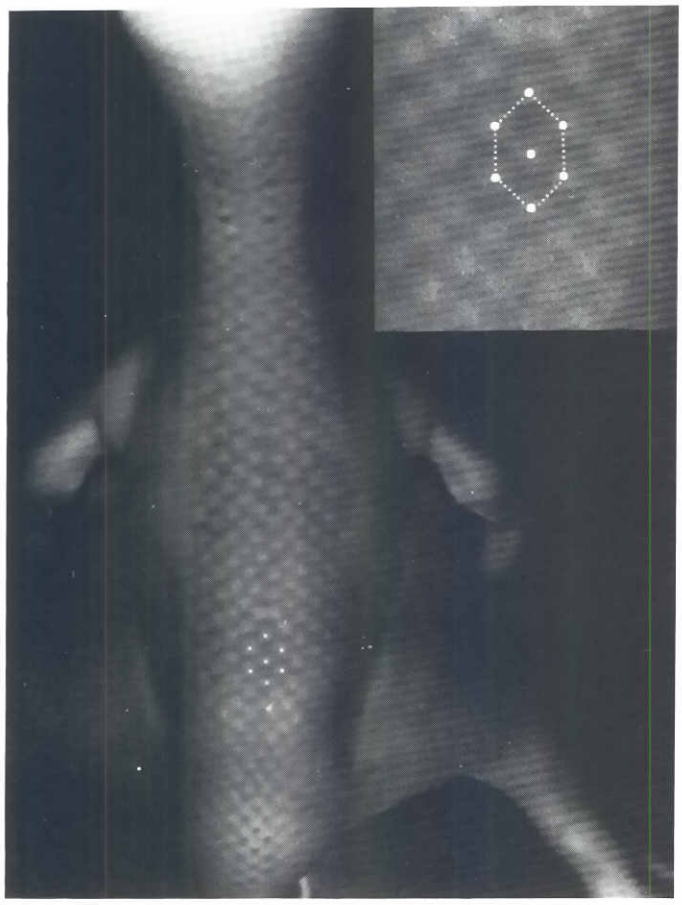


Fig. 7. Dorsal view of an early 8-day chick embryo showing the arrangement of feather rudiments in a hexagonal pattern *(inset, larger magnification, to show that early rudiments are tangent to each other, except that the pattern is already stretched along the antero-posterior axis).*

Finally the examination of a genetically affected chick embryo, such as the scaleless (*sc*) mutant (Sengel and Abbott, 1962, 1963), yields a picture very similar to the one provided by hydrocortisone-treated chicks. The homozygotes *sc / sc* are characterized by a complete absence of scales on the feet and by very deficient plumages: most feathers of the spinal pteryla and other feather tracts are missing. (A few feathers however develop normally, notably in the scapular and caudal tracts. It is noteworthy and somewhat intriguing that the development of feathers in these tracts cannot be prevented by hydrocortisone treatment either. The properties of this «resistant» skin underlying this pecularity are not known, but may be worth investigating). In the glabrous skin of the scaleless embryo, just as in the skin of «naked» hydrocortisone-treated embryos, interstitial collagens are uniformly distributed and heavily accumulated in the dermis (Fig. 4c), at particularly high density along the dermal-epidermal junction, while fibronectin is completely lacking (Fig. 4d) (Mauger *et al.*, 1983a).

Let it be remembered here that the dermis of the normal midventral apterium exhibits strictly the same immunohistochemical features as those of experimentally-produced apteria, namely high density and uniform distribution of types I and III collagen fibers, and scarcity or absence of fibronectin (Mauger *et al.*, 1982b).

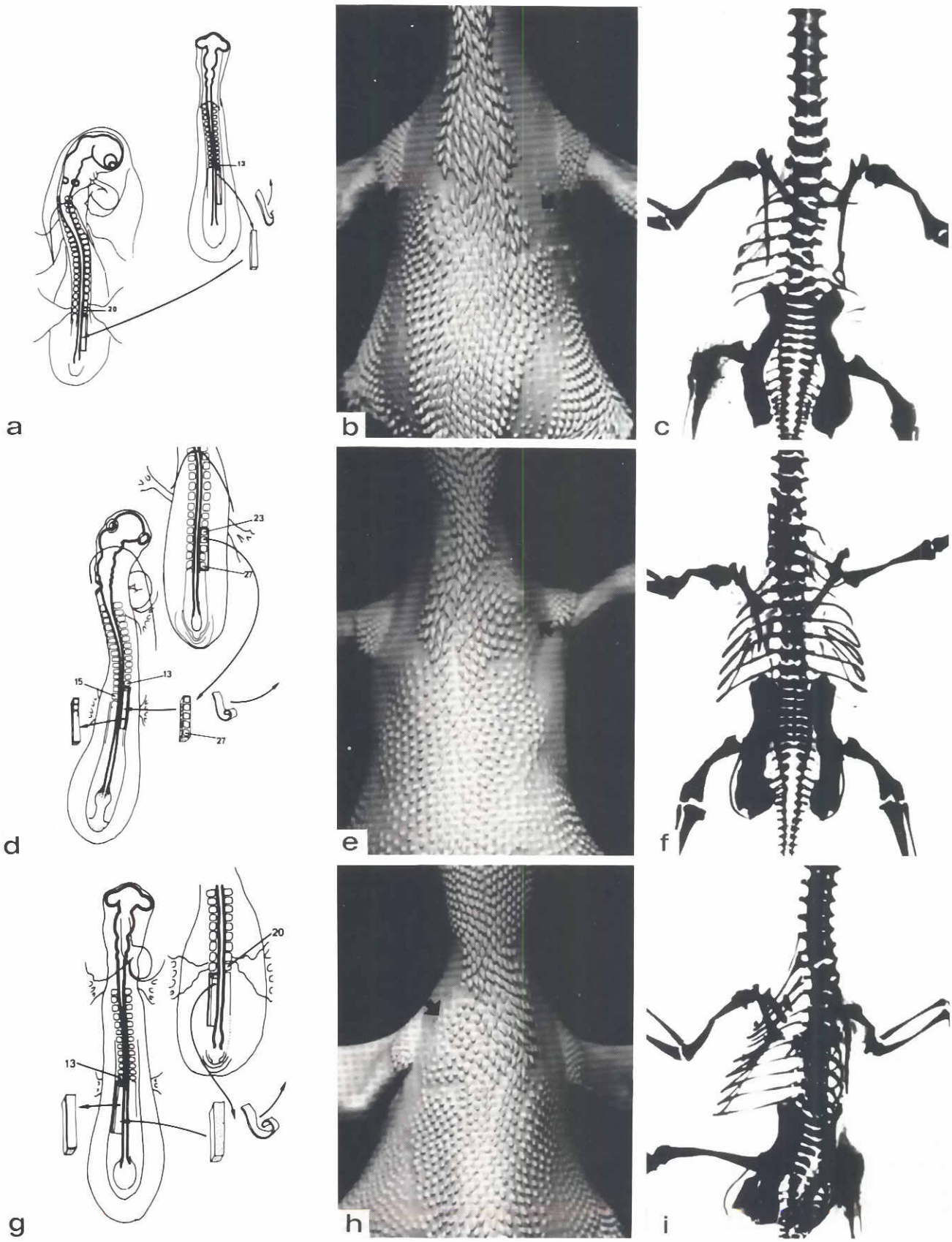
As a conclusion to these experiments or observations on featherless skin, it appears clearly that the absence of feathers is the result of the non-formation of dense dermis at a time when the acquisition of high density is required in feather-forming skin (Mauger, 1970), and is accompanied by an abnormally high production of interstitial collagen fibers and an unusually low content of fibronectin. The latter feature seems to indicate that collagen exerts a stabilizing influence on embryonic organ rudiments, while fibronectin might be necessary for or facilitate morphogenetic movements such as those that are prerequisites for the formation of cutaneous appendages. It also suggests that extracellular matrix components may play a major role in skin morphogenesis, as in the development of other organs. This concept is consolidated by the study of the distribution of extracellular matrix components in normal feather-forming or scale-forming skin, as described below.

The production of ectopic feathers

Much can be learned about feather pattern formation by trying to experimentally bring about the formation of feathers or feather tracts in sites where they normally do not arise. Early manipulation of predermal mesenchyme may result in the formation of abnormally located feathers, either within an apterium or within a pteryla.

The midventral apterium, a bare area of skin located in front of the umbilicus between the two bilaterally symmetrical ventral feather tracts, can thus be induced to produce ectopic feathers (Kieny and Sengel, 1964; Sengel and Kieny, 1967a and b). This is achieved by implanting a living (cf. Fig. 5c) or inert foreign body, such as a piece of neural tube, of cartilage, agar or paraffin, into the right presumptive territory of the ventral body wall of 2-day chick embryos. The implant, provided cells do not readily adhere to it, causes an extraordinary fusion between somatopleural and splanchnopleural lateral plates, resulting in the formation of abnormally dense mesenchyme in the ventral parietal mesoderm (Fig. 5a, b). (Once this fusion has occurred, the implant need not be left in place, indicating that it is the fusion of tissues that is important, not the implant itself). The experimentally produced unusually high density

Fig. 8. Heterotopic transplantations of somitic mesoderm resulting in origin-specific development of feather tracts and axial skeleton. (a-c) *Implantation of right unsegmented somitic mesoderm of the posterior cervical level in place of right unsegmented somitic mesoderm of the thoracic level:* **(b)** *development of part of the posterior cervical tract on the right side (arrow) within the thoracic spinal pteryla.* **(c)** *The corresponding defect in the vertebral column, where half ribless cervical vertebrae have replaced the thoracic ones.* **(d-f)** *Implantation of right thoracic somites in place of right cervical somitic mesoderm:* **(e)** *development of part of the right thoracic spinal pteryla at the level of the right posterior cervical tract (arrow).* **(f)** *The corresponding formation of ectopic ribs and right half of thoracic vertebrae at the level of cervical vertebrae.* **(g-i)** *Implantation of left unsegmented somitic mesoderm from the thoracic level in place of left unsegmented cervical somitic mesoderm.* **(h)** *Development of part of the left thoracic spinal tract at the level of the left posterior cervical tract (arrow).* **(i)** *The corresponding formation of supernumerary ribs and left half of thoracic vertebrae on the left side at the level of the cervical vertebrae.*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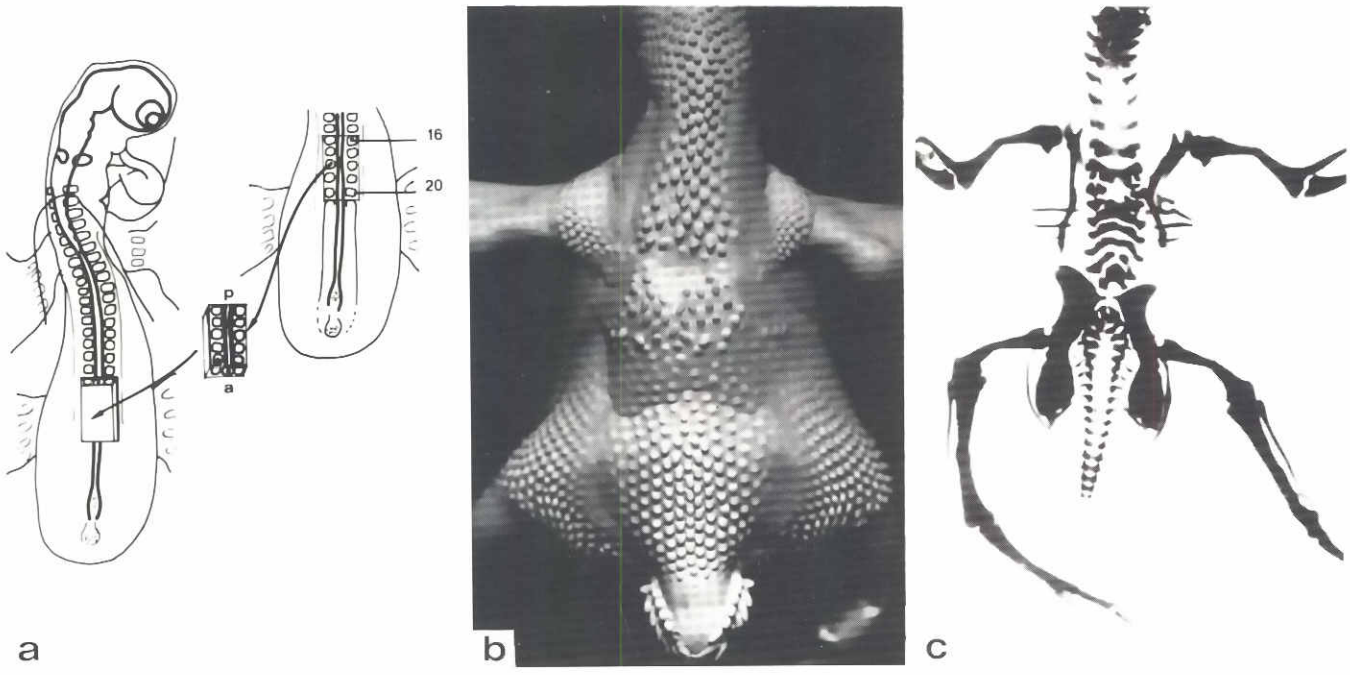

Fig. 9. Production of ectopic dorsal feathers within the spinal pteryla. (a) *Implantation of cephalo-caudally reversed spinal cord with bilaterally adjacent somites into a 17-somite host embryo. The operation leads to the development of feathers growing in reverse polarity, with their apex pointing toward the head of the host* **(b)**, *and to the inversion of a corresponding portion of the vertebral column* **(c)**. Note in **(b)** *the formation of a transverse apterium between the host's cervical tract and the ectopic feather tract.*

of predermal cells in this area apparently confers feather-forming properties to the midventral dermis. The supernumerary feather tract which then arises frequently exhibits bilateral symmetry, although it is borne exclusively by the right (operated) side of the ventral skin (Fig. 5d-i). It is often composed of two parts (Fig. 5d, e, h, i), a central field surrounded in front by a semicircular field; the two fields, when they exist, are always separated from each other by an apterous band. Also, the extra feather tract or tracts are usually separated from the adjacent ventral pterylae by an apterium. The number of supernumerary feathers thus produced varies greatly from one embryo to the other: it may be as low as 4, but may exceed 100; a typical ectopic feather tract in the midventral apterium comprises about 40 feathers. Whenever their number is large enough, extra feathers are clearly arranged in a typical hexagonal pattern.

It is clear that the morphogenetic messages leading to the production of these ectopic feathers do not originate from the implanted foreign bodies, but are contained in the cutaneous tissues of the apterium. Dermal-epidermal recombination experiments between dorsal feather-forming skin and apterous midventral skin of adequate ages have shown that it is the dermis of the midventral apterium that is deficient and unable to trigger off the formation of feather in the overlying epidermis (Sengel *et al.*, 1969). It is therefore probable that the dermis of supernumerary feather tracts acquires feather-forming properties simply because it is formed from abnormally dense dermis produced early (between 2 and 4 days) in this area as a result of the presence of the foreign implant.

Ectopic feather tracts can also develop within a particular pteryla, such as the spinal pteryla. In this case the abnormally located and/or oriented tract interferes with the arrangement of feathers in the host pteryla, in a way which is informative about the mechanism of feather pattern formation.

Before the experiments leading to the production of ectopic feathers within the spinal pteryla are reported however, the main developmental and morphological features of that pteryla should be recalled (Fig. 1 and 6). Because of its well-defined lateral contours, the spinal pteryla subdivides naturally into three regions: an anterior cervical region, a posterior cervical (interscapular) region, and a thoraco-lumbo-sacro-caudal (in brief truncal) region (Mauger and Sengel, 1970). The entire dermis of the spinal pteryla originates from the somitic dermatomes. Shortly before the end of the 7th day of incubation, the first four feather rudiments appear in the lumbar region in a middorsal longitudinal row. After that, more feather rudiments are added in front and behind the first four, while additional rows develop successively on either side of the initial row. In the cervical and thoracic regions, initial feather rudiments are arranged in two parasagittal rows. When they first form, feather rudiments appear as circular spots in the skin, which are initially tangent to one another. As soon as lateral rows have developed alongside the initial one(s), feather rudiments are arranged in a highly regular hexagonal pattern (Fig. 7) where each rudiment is at first tangent to its neighbours, and, except for those sitting in the last formed lateral longitudinal row, surrounded by six equidistant feather rudiments. Soon however the regular hexagonal pattern becomes stretched in cephalo-caudal and also lateral and oblique directions, differently in the three parts of the pteryla. Finally, feathers appear to be arranged in oblique chevron-like rows (Fig. 1

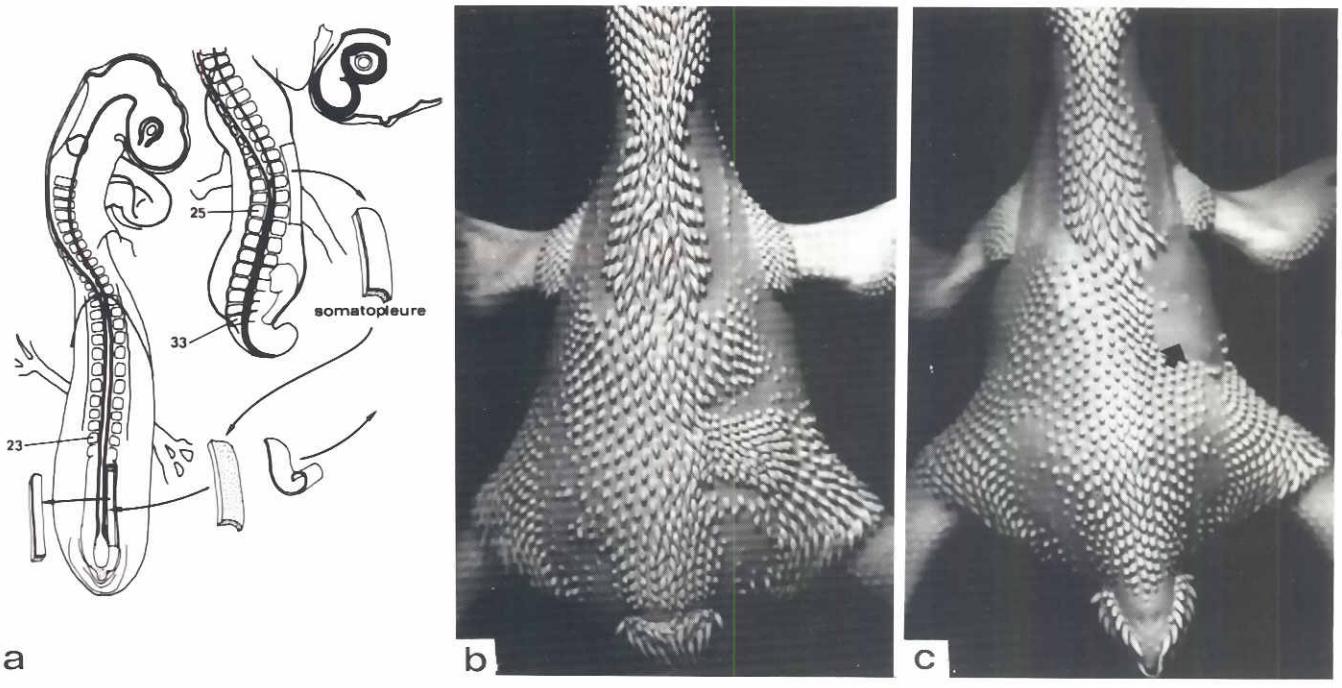

Fig. 10. Origin-specific development of heterotopically-transplanted somatopleural mesoderm. (a, b) *Replacement of thoraco-lumbar somitic mesoderm (on the right side) by somatic mesoderm from the prospective pectoral pteryla: formation of a portion of breast tract, partially fused medially with the indented spinal pteryla, but separated from it anteriorly and posteriorly by narrow apteria.* **(c)** *Formation of a patch of glabrous skin (arrow) within the thoracic spinal pteryla, after replacement of thoracic somitic mesoderm (on the right side) by somatic mesoderm from the prospective midventral apterium.*

and 6), each chevron being centered on one of the initial feathers of the middorsal or parasagittal rows. In the anterior cervical region, feathers are very closely packed and the chevrons appear to open toward the head. The transition from anterior to posterior cervical region corresponds to the narrowest part of the pteryla. In the posterior cervical and in the truncal regions the chevrons appear to open toward the tail, and feathers are less densely packed than in the anterior cervical region.

A fate-map correspondence has been established between somites and initial feathers of the spinal pteryla (Table 1 and Fig. 6) (Mauger and Sengel, 1970; Mauger 1972a).

TABLE 1

CORRESPONDENCE BETWEEN SOMITES AND INITIAL FEATHERS IN SPINAL PTERYLA

Regions	Originating from somites	Number of initial feathers	Number of initial feathers per somitic segment
anterior cervical	5-12	16.0	2.3
posterior cervical	12-19	9.8	1.4
thoracic	19-26	10.8	1.5
lumbar	26-34	8.0	1.0
sacral	34-40	6.0	1.0
caudal	40-44	4.0	1.0

The experiments whereby ectopic feathers can be produced in the spinal pteryla consist of heterotopic transplantations between two prospective regions. A given length of somitic mesoderm (say three to six somites or prospective somites) is excised on one side of a 2-day chick embryo and replaced by an equally long piece of somitic mesoderm (deprived of its overlying ectoderm) obtained from another level of the cephalocaudal axis. Implants are inserted with their three axes (antero-posterior, dorso-ventral, medio-lateral) in conformity with those of the host. Orthotopic control transplantations lead to an undisturbed development of the spinal pteryla. The heterotopic transplantations however result in malformations, where easily recognizable pieces of spinal feather tract develop in ectopic position (Fig. 8) (Mauger, 1972c). For instance, when somitic mesoderm from the posterior cervical region (where the tract is narrow) is transplanted into the thoracic region (where it is wide), a portion of narrow posterior cervical tract develops on the operated side at the thoracic level (Fig. 8a-c). Conversely, somitic mesoderm from the thoraco-lumbar region implanted into the posterior cervical region gives rise to a portion of wide thoraco-lumbar tract at the cervical level (Fig. 8d-i). In all experiments of this kind, width, density of feathers, number and orientation of chevrons is in conformity with the regional origin of the grafted somitic mesoderm. When a short portion of spinal cord and bilaterally adjacent somites is turned by 180° with respect to the cephalocaudal axis of the host embryo, the resultant spinal pteryla is interrupted by an abnormal feather tract in which the feather buds point their apex toward the head of the host, and are thus oriented in conformity with the cephalocaudal polarity of the graft (Fig. 9).

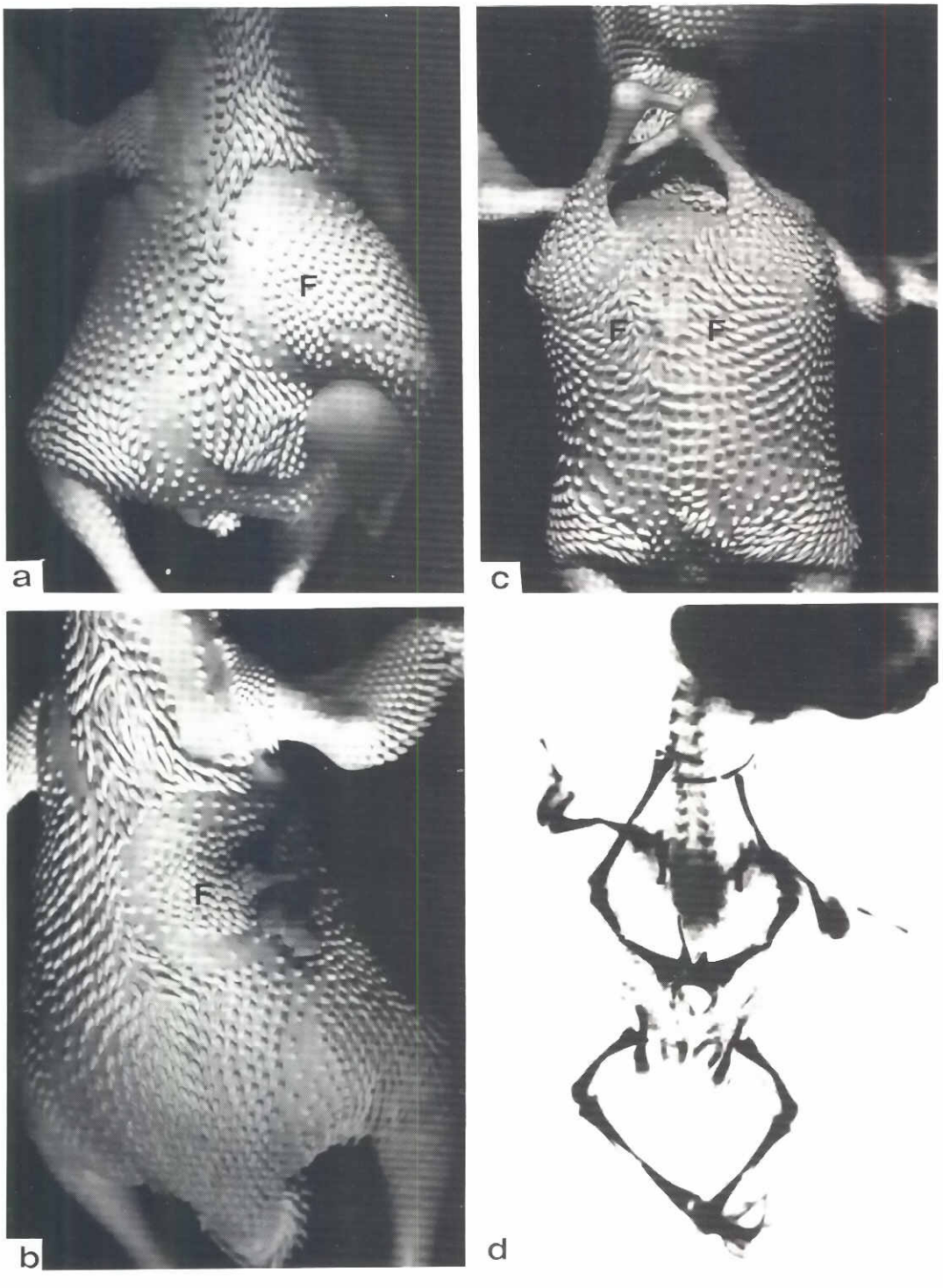

Fig. 11. Production of ectopic feathers within the territory of the spinal pteryla, *after implantation, at 2 days of incubation, of prospective leg-level somatopleural mesoderm in place of the right thoracic somitic mesoderm* **(a, b)** *or of the spinal cord* **(c)**. *The graft induces the formation of a supernumerary leg* **(a, b)** *or of two supernumerary legs* **(c, d)**, *the femoral feather tract (F) of which either deeply indents the spinal pteryla* **(a, b)** *from which it is separated by a featherless zone, or completely replaces it at the level of the operation* **(c)**. **(d)** *The corresponding malformation of the axial skeleton: at the level of the supernumerary pelvic girdle, the vertebral column is absent.*

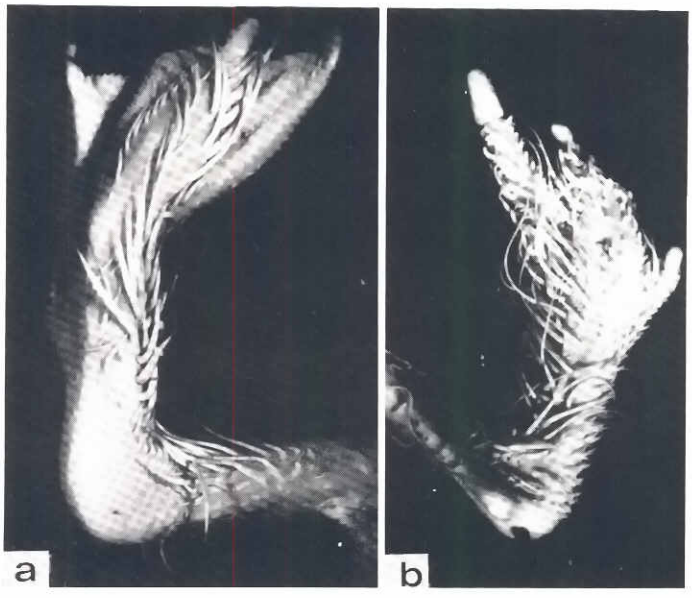

Fig. 12. Predominance of feather over scale morphogenesis revealed by heterotopic recombinations of duck wing bud ectoderm with duck (a) or chick (b) leg bud mesoderm. *The resulting legs develop in conformity with the regional and specific origin of the mesoderm. The feet of these legs are covered by cutaneous appendages, the large majority of which are feathers instead of scales. The latter, when present, carry the ectopic feathers at their distal edge.*

These results demonstrate that the segmented, and also the still unsegmented, somitic mesoderm of the 2-day chick embryo are already regionally determined, not only in their prospective dermatomal component, but also in their prospective sclerotomal component from which the vertebral column and ribs originate (Kieny *et al.*, 1972; Sengel, 1972, 1973). Incidentally, this is not the case of the myotomal component of the somites (Kieny, 1980).

Other heterotopic transplantation experiments have shown that this early regionalization affects not only the somitic dermatomes, but also, and at the same time, the somatopleural lateral plates (indifferently also called somatic mesoderm). When, for instance, a piece of somatic mesoderm from the prospective region of the midventral apterium is implanted in place of thoracic somitic mesoderm (Fig. 10c), a patch of glabrous skin develops within the territory of the thoracic spinal pteryla. Similarly, when a piece of somatic mesoderm taken from the prospective region of the pectoral feather tract is implanted in place of thoracic somitic mesoderm, a piece of breast tract develops within the territory of the spinal pteryla (Fig. 10a, b). Interestingly, this patch of "foreign" breast feather tract in part remains distinct from the spinal tract, in the sense that a narrow apterium forms anteriorly and posteriorly between dorsal and ventral feathers. Another example of this kind of "conflict" between two feather tracts is given by experiments where a piece of leg-forming somatic mesoderm is implanted in place of thoraco-lumbar somitic mesoderm (Fig. 11a, b) or of thoraco-lumbar spinal cord (Fig. 11c, d) (Kieny and Brugal, 1977). This type of operation results in the formation of one – or, in the case of middorsal grafting, two – supernumerary leg(s) sticking out of the back. In this situation the femoral feather tract grows within the territory of the spinal pteryla, by, as it were, "eating itself" into the spinal tract, which consequently becomes deeply indented (Fig. 11a, b). The two conflicting tracts are then usually separated by an apterium. This result again shows that the somatic mesoderm - just like the somitic one - is already determined at 2 days of incubation and, when given the opportunity to develop in a foreign location, expresses its morphogenetic properties in conformity with its regional origin. It further demonstrates that two feather tracts of distinct origin and different directions of growth (the femoral tract adds successive rows in disto-proximal direction, while the spinal pteryla grows in medio-lateral direction) cannot harmoniously melt into each other by coordinating their respective hexagonal patterns. And indeed, in normal development, truncal spinal and femoral pterylae are separated from one another by a distinct partial apterium (Figs. 1 and 6). Thus it may be tentatively concluded that certain distinct portions of the outline of a feather tract reflect the resulting balance between opposing morphogenetic properties.

Naturally, many questions remain to be answered in the puzzling field of pattern formation. Not the least among them are of course those of pattern inception and regularity. If we admit the morphogenetic role of the initial row(s) of feather rudiments, capable of inducing new rows lateral to it in repeating succession, we have to ask what causes the formation of the initial row, or the initial rudiment for that matter, in the first place. Where does the initial information come from, and by what mechanism is the ordered architecture achieved? The ability of dermal cells, once they have attained a certain threshold density, to conglomerate into lens-shaped dermal condensations of feather rudiments is probably an intrinsic property of that type of embryonic connective tissue. And this property in turn resides in the way individual dermal cells are able to secrete and manipulate extracellular matrix and to interact with it and their neighbours in an orderly fashion.

Another important notion regarding the ability of dense dermis to induce feather placodes in the overlying epidermis is that this morphogenetic function is exerted prior to the formation of dermal feather condensations themselves. Indeed, when 7- to 8-day skin is enzymatically split into dermis and epidermis, and when each tissue is then observed microscopically, it is clearly seen that epidermal feather placodes can be detected in advance of dermal condensations, so that incipient epidermal placodes are not straightaway associated with a dermal condensation (Sengel and Rusaouën, 1968). Also, when the outer face of the periderm of 7.5-day chick embryos is examined by scanning electron microscopy, peridermal cells are seen to coordinately change their shape and polarity at sites of future placodes well in advance of the appearance of histologically detectable feather rudiments (Sengel and Mauger, 1976).

Pattern formation in the arrangement of cutaneous appendages

Not only are cutaneous appendages localized, as we have seen, in specialized areas, but they are also ordered individually in regular arrays with respect to their neighbours. The hexagonal feather pattern is a typical example (Fig. 7). Likewise, scales in reptiles, birds and mammals, or hairs are usually arranged according to more or less regular geometrical patterns. The question may therefore be raised as to the origin and ontogenesis of these patterns, and to the

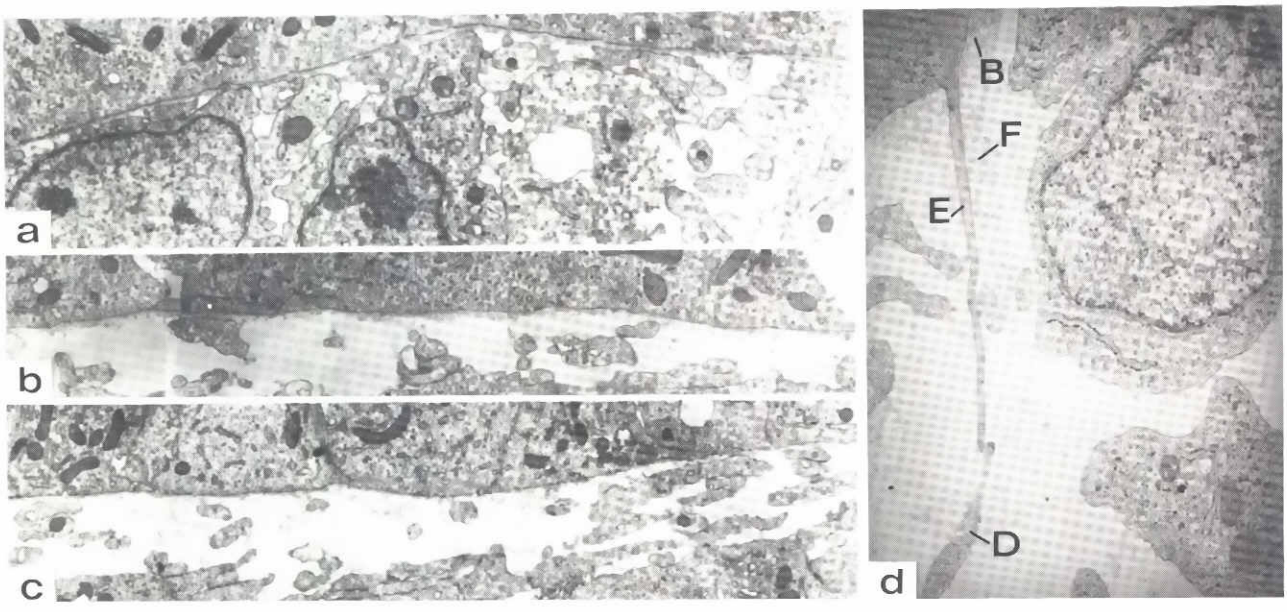

Fig. 13. Ultrastructure of the dermal-epidermal junction in feather-forming skin of 7-day (d) and 8-day (a-c) chick embryos. (a-c) *Differences in the micro-architecture of the dermal-epidermal junction at the peripheral base of a feather bud* **(a)**, *in interplumar skin* **(b)**, *and at the apex of a feather bud* **(c)**. *Note high density and closeness to the basement membrane of dermal cell processes in* **(a)**, *as compared to* **(b)** *and* **(c)**. **(d)** *Direct contact between a dermal cell process (D) and an epidermal cell process (E) protruding through the basement membrane (B) at the site of origin of an anchor filament (F).* **(a-c)** *x11,000;* **(d)** *x20,000.*

respective role of dermis and epidermis in their establishment (Sengel, 1975a).

We have shown before that, in birds, dermis arises from somitic and somatic mesoderm. Since then, as demonstrated by heterotopic transplantations of these precursor mesodermal tissues (see above), presumptive dermal mesenchyme is already determined at 2 days of incubation to give rise to regionally specified portions of feather tracts with their specific density and arrangement of feathers, it appears obvious that dermis is the determinant tissue, responsible for the expression of the hexagonal feather pattern.

That this is so has indeed been demonstrated by a great variety of heterotypic dermal-epidermal recombinations (Sengel, 1958, 1964, 1969, 1971). In these experiments, pieces of skin are obtained from various skin regions prior to the onset of appendage formation, split into dermis and epidermis by enzymatic treatment and mechanical separation, then reconstructed by associating dermis and epidermis from different ages, orientation, regions, or species, and finally cultured *in vitro* or as chorio-allantoic grafts, where they can express their morphogenetic potential. Similar experiments with mammalian or reptilian skin have also been performed and have extended the notion that dermis is determinant in the expression of quite a number of morphological and physiological features of appendage formation. Furthermore inter-class heterospecific dermal-epidermal recombinations between lizard, chick and mouse have shown that the dermal morphogenetic messages can be transmitted to a foreign epidermis and correctly understood and interpreted by it with regard to the arrangement of cutaneous appendages.

A few examples will illustrate the extent of the dermal control over appendage morphogenesis. Recombination of dorsal feather-forming dermis with foot scale-forming epidermis or with epidermis from the midventral apterium yields feathers, while the reverse combination of scale-forming dermis (provided it is obtained from a chick embryo older than 12 days of incubation) with feather-forming epidermis leads to the formation of scales. Contrariwise association of apterous dermis with appendage-forming epidermis results in the formation of glabrous skin. Thus region-specificity resides in the dermis. Epidermis of whatever origin responds to the contents of the dermal message by complying to them. This clear-cut picture, however, appears to be oversimplified and is true only if certain conditions of developmental age, such as those indicated above, are fulfilled. Indeed feathers may arise instead of scales from recombinations of foot scale-forming dermis and dorsal or alar feather-forming epidermis. This is the case, for example, when the two prospective cutaneous tissues are associated very early in development, at 3 or 4 days of incubation, in heterotopically constructed limb buds (wing bud ectoderm/leg bud mesoderm). These heterotopic buds give rise to legs in accordance with the origin of the mesoderm. However, the feet of these legs bear cutaneous appendages, most of which are feathers rather than scales (Fig. 12). When a few scales do form, they often carry one or several feathers at their distal brim (Sengel and Pautou, 1969). A similar result is obtained in recombinants of dorsal feather-forming epidermis and early (younger than 12 days of incubation) foot scale-forming dermis (Rawles, 1963). Thus foot scale-forming dermis, prior to a certain stage, is not able to impose its region-specificity on feather-forming epidermis, but is nonetheless able to do so on non-feather-forming midventral epidermis (Dhouailly and Sengel, 1973, 1975; Sengel and Dhouailly, 1977). This leads to the concept that somehow feather morphogenesis predominates over scale morphogenesis, formation of feathers being, as it were, a more basic function of avian skin than the formation of scales

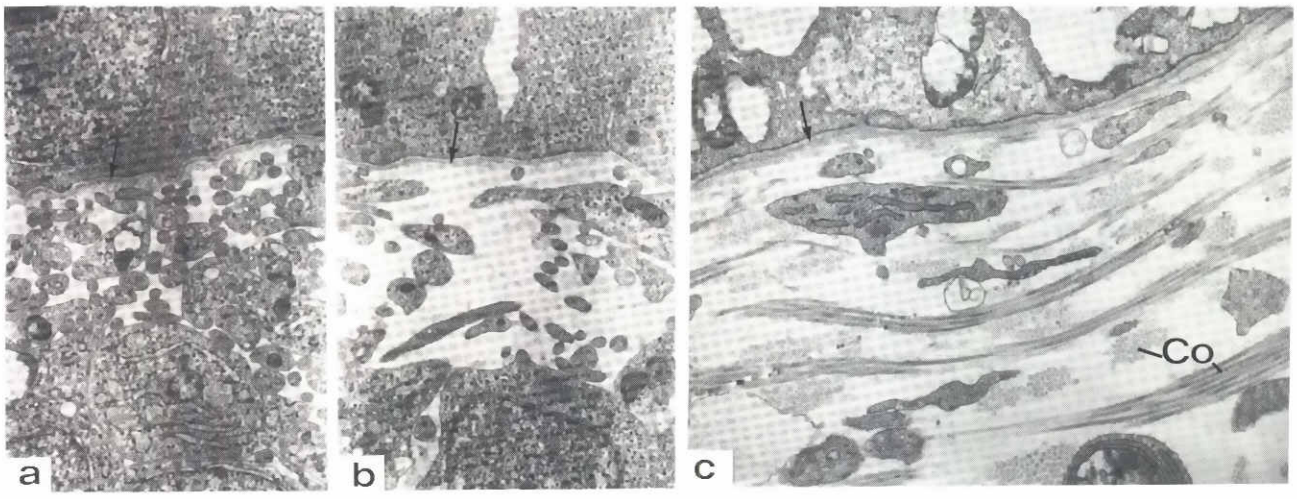

Fig. 14. Ultrastructure of the dermal-epidermal junction in 11-day scale-forming foot skin (a, b) and in 14-day non-feather-forming skin of the midventral apterium (c). *In scale-forming skin* **(a, b)**, *dermal cell processes are oriented preferentially with their long axis at right angle to the basal-apical axis of the scale (in these sections, which are parallel to this axis, most of them appear as small circular bodies); their density is higher at the base* **(a)** *than inside the scale* **(b)**. **(c)** *In the midventral apterium, dermal cell processes are scarce, never close or in direct contact with the basement membrane (arrow), and separated from it by at least one array of collagen fibres (Co). x12,000.*

(Sengel, 1958, 1980; Sengel and Pautou, 1969, Dhouailly and Sengel, 1983).

Results of heteropolar recombinations where the cephalo-caudal axis of the epidermis is turned by 90° or 180° with respect to that of the dermis show that the cephalo-caudal polarity of the hexagonal feather pattern resides in the dermis. However the individual basal-apical orientation of feather buds is dictated by the epidermis.

Heterospecific dermal-epidermal recombinations between chick and duck, two species whose plumages differ by a great number of morphological characters, result in the formation of a species-specific organization of the pterylose which is in accordance with the origin of the dermis. Whatever the specific origin of the epidermis, in the case of chick dermis, chick pterylae or scale tracts are formed; with duck dermis, duck pterylae or duck scale tracts develop. A remarkable exception to the overall dominance of dermis, however, is the shape, size and number of feather barbule cells, which are strictly dependent on the specific origin of the epidermis (Sengel and Dhouailly, 1966; Dhouailly and Sengel, 1972).

Inter-class heterospecific exchanges between lizard and chick, lizard and mouse, or chick and mouse are very informative insofar as they allow a clear distinction between the morphology of individual appendages and their arrangement (Dhouailly and Sengel, 1973). Briefly, to speak only of chick/mouse recombinations, the association of chick feather-forming dermis with mouse hair-forming (or, for that matter, non-hair-forming foot sole) epidermis forms (malformed arrested) hairs, which however are arranged in a typical hexagonal feather pattern. Conversely, the association of mouse hair-forming dermis with chick feather-forming (or non-feather-forming midventral) epidermis gives rise to (malformed arrested) feathers, the arrangement of which however is typical of a triade-type hair pattern.

To summarize then, together with other data not mentioned above but derived from other kinds of dermal-epidermal recombination experiments, the following developmental events of skin morphogenesis are under dermal control:

1. Transformation of ectoderm into epidermis.
2. Basal-apical polarization and subsequent stratification of epidermal cell layers (Sengel, 1958).
3. Maintenance of regulated proliferation of basal epidermal cells and ordered stratification of keratinizing suprabasal layers.
4. Initiation of epidermal appendage placodes.
5. Choice of epidermal placode site.
6. Definition of epidermal placode size, on which the size of the future appendage depends.
7. Specification of epidermal placode distribution pattern.
8. Species-specific determination of the architectural arrangement of epidermal cells in appendages.
9. Regional specification of keratin polypeptides in cutaneous appendages (Dhouailly *et al.*, 1978; Rogers *et al.*, 1978).

Let it be recalled here that appendage-forming properties are acquired by predermal cells only if they are in connection with a "normal" ectoderm. This knowledge stems from heterogenetic dermal-epidermal recombinations between mutant bird or mammal embryos and their wild-type partner. Whenever epidermis is obtained from a mutant embryo, such as the scaleless chick, for example, neither feathers nor scales will form, even though the dermis originates from a normal embryo. On the other hand, when epidermis is taken from a normal embryo and dermis from a scaleless embryo, appendages will form normally. Thus, predermal somitic or somatic mesenchyme is unable to express its morphogenetic properties unless it is (or was, at least for part of its developmental history) in contact with non-defective epidermis. It must therefore be concluded that one important morphogenetic role

of ectoderm (or epidermis) is to systemically induce the underlying dermis to start organogenesis of cutaneous appendages.

The mechanisms of dermal-epidermal interactions

Now that heterotypic dermal-epidermal recombinations have revealed the contents of the morphogenetic messages that the dermis transmits to the epidermis (Sengel, 1975b, 1983), further investigations have attempted to understand by what mechanism the two skin tissues communicate. Since dermis and epidermis are linked to each other by the dermal-epidermal junction and since dermal cells are separated from each other by extracellular matrix, part of the answer to the question of tissue communication might be found in the constitution of these structures in various types of developing skin.

The ultrastructure of the dermal-epidermal junction differs greatly in feather-forming, scale-forming and glabrous chick skin (Démarchez *et al.*, 1981; Sengel, 1986). The distribution, density, orientation and shape of dermal cell processes and of collagen fibers underneath the epidermal basement membrane are characteristic of each skin region (Figs. 13 and 14), as are the existence and density of direct dermal-epidermal cell contacts through perforations of the epidermal basement membrane.

Regarding extracellular matrix, immunolabeling of frozen sections of embryonic chick (Sengel *et al.*, 1962; Jahoda *et al.*, 1987; Mauger *et al.*, 1982, 1983a, b, 1984) or mouse (Mauger *et al.*, 1987) skin with monospecific antibodies reveals that matrix components subdivide into two categories. In a first category are those whose distribution is uniform and remains unchanged during all stages of appendage formation, such as laminin and type IV collagen; these probably do not play a major role in the transmission of morphogenetic messages, since their distribution is the same in appendage-forming as in non-appendage-forming skin. The other category comprises interstitial collagen types I, III, and V, fibronectin and several glycosaminoglycans whose deposition and density vary from site to site and with proceeding developmental stages (Fig. 4e-h). Before the onset of appendage morphogenesis, the extracellular matrix components of the latter category exhibit a fairly uniform distribution and moderate density throughout the dermis. By the time the epidermal feather, scale or hair placodes appear, distribution of these components becomes heterogeneous. Collagen types I, III and V tend to disappear from the apex of growing buds, while the density of their fibers continues to increase at the base of appendage rudiments and in inter-appendage skin. Contrariwise, fibronectin accumulates more heavily in the dermal core of appendage rudiments and buds, whereas its density decreases in inter-appendage skin. Also, interstitial collagens, fibronectin and glycosaminoglycans (notably sulfated GAGs and hyaluronic acid) acquire an asymmetrical distribution pattern along the cephalocaudal axis of reclining feather or hair buds. In glabrous skin (midventral apterium, dorsal skin of hydrocortisone-treated chick embryos, dorsal skin of the scaleless mutant) (Fig. 4b-d), the distribution of interstitial collagens remains homogeneous and their density increases steadily with age, whereas that of fibronectin likewise remains uniform but declines to the point that it almost completely disappears from the dermis, and from the dermal-epidermal junction.

These observations are an indication that the micro-architecture of the dermal-epidermal junction, interstitial collagens, fibronectin, and glycosaminoglycans might play a morphogenetic role in the development of cutaneous appendages (Mauger *et al.*, 1984; Sengel, 1985, 1986). It appears that interstitial collagens are removed and remain sparse or absent in zones of high morphogenetic activity where movements of cells and tissues take place. On the other hand, interstitial collagens accumulate predominantly and form a dense matrix in histogenetically-stabilized zones. By contrast, fibronectin and glycosaminoglycans appear to abound precisely where collagens become sparse. The density of fibronectin and glycosaminoglycans increases in zones of high morphogenetic activity, while it decreases in stabilized regions.

Conclusion

It is clearly apparent that cutaneous pattern formation relies predominantly on the mesenchymal component of skin. Not only does it provide a suitable substrate and nutrient supply for the maintenance, controlled proliferation and stratification of the overlying epidermis, but it is also the depository of decisive information for the production and ordered arrangement of cutaneous appendages. This morphogenetic information is, at least in birds, acquired by presumptive dermal mesenchyme at a fairly early stage of development, i.e. at the time para-axial mesoderm becomes individualized, shortly after gastrulation, as somitic and somatic mesoderm.

The mechanisms of dermal-epidermal tissue interactions are still largely hypothetical. Electron and immunohistological microscopy indicates (1) that direct epidermal-dermal cell's contact across the basement membrane, and close apposition of dermal cell processes against the epidermal lamina densa, and (2) that interstitial collagens (types I, III, and V), fibronectin, sulfated and non-sulfated glycosaminoglycans might play an important role in the transmission of morphogenetic messages. The current view is that the epidermis is able to sense the microheterogeneous texture of its dermal substrate and respond to it accordingly. In brief, large amounts of homogeneously deposited interstitial collagen and sparse fibronectin would cause the rigidification of dermis, and therefore the inability of epidermis to undergo morphogenetic movements, whereas high density of fibronectin and glycosaminoglycans, together with rarefied collagen, would offer a soft substrate allowing and possibly stimulating morphogenetic movements and histogenetic changes in the epidermis. Verification of this hypothesis awaits further investigations, currently under way, where morphogenetic performances of dermal and epidermal cells are tested in artificially simplified *in vitro* systems (Sengel and Kieny, 1984, 1986; Robert *et al.*, 1989a, b). Of particular value is the possibility of constructing three-dimensional dermal equivalents by culturing embryonic dermal cells in hydrated collagen lattices (Sengel *et al.*, 1985; Lachgar *et al.*, 1989).

Summary

The development of skin and cutaneous appendages in amniote embryos has been submitted to a large number of experimental investigations the results of which have led to a better understanding of the mechanisms whereby this multiform organ arises during embryonic development. In birds, the main appendages are the feathers and the foot scales. Their formation results from a series of inductive events between ectoderm (later epidermis) and

subectodermal mesoderm (later individualized dermis). Morphogenetically, the mesodermal (mesenchymal) component of skin is the predominant tissue, insofar as it controls most morphological and physiological features of developing skin and appendages, notably transformation of ectoderm into epidermis, polarization, proliferation and stratification of epidermal cells, initiation, site, size and distribution pattern of epidermal placodes, species-specific architecture of appendages, regional specification of keratin synthesis. The ectodermal (epithelial) component is able to respond to the mesodermal inductive instructions by building feathers and scales in conformity with the specific origin of the dermis. In these epithelial-mesenchymal interactions, extracellular matrix and the microarchitecture of the dermal-epidermal junction appear to play an important role. Indeed extracellular matrix components (primarily collagens, proteoglycans and adhesive glycoproteins) and dermal cell processes close to the epidermal basement mebrane become distributed in a microheterogeneous fashion, thus providing a changing substratum for the overlying epidermis. It is assumed that the latter is able to somehow sense the texture and composition of its substratum, and by doing so to appropriately engage in the formation of glabrous, feathered or scaly skin.

KEY WORDS: *chick embryo, dermis, epidermis, cutaneous appendages, feathers, scales, morphogenesis, epithelial-mesenchymal interactions, extracellular matrix*

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