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Dermal cells form strong adhesions to the basement membrane during the development of feather primordia in chick skin

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SUMMARY

Experiments are described which provide a direct measure of the adhesion between dermis and epidermis during the development of feather primordia in chick dorsal skin in culture. The epidermis was peeled from the dermis and the surfaces so exposed were examined under the scanning electron microscope: regions of strong adhesion between the tissues were revealed as areas where their separation was incomplete. The results show that soon after primorida become morphologically distinct, cells from the surface of dermal condensations form adhesions to the basement membrane which are stronger than those between dermis and epidermis interplumar skin. These adhesions may help to hold the epidermis and dermis together during the outgrowth of the primordium.

INTRODUCTION

The feather primordium comprises both an epidermal placode and a dermal condensation (Wessells, 1965). As the primordium develops, its shape is transformed from a shallow, biconvex disc into a bulbous bud rising above the skin surface (Sengel, 1976). Throughout this process, the dermal and epidermal components remain closely apposed. The question arises, what holds the two tissues together? Prior to the development of feather primordia, there is an obvious candidate for a structure with this function: spur-like projections of the basal epidermal surface are connected to the base of the dermis by 'anchor' filament bundles (Kallman, Evans & Wessells, 1967; Kischer & Keeter, 1971). But in the developing primordium, and later elsewhere, these bundles disappear and the tissue interface becomes smooth (Kischer, 1968; Ede, Hinchliffe & Mees, 1971). The observations described in this report suggest a different mechanism which operates during the elevation and outgrowth of primordia: they show that cells at the surface of dermal condensations form strong adhesions to the epidermal basement membrane.

MATERIALS AND METHODS

In order to investigate the adhesion between dermis and epidermis, the two tissues were gently peeled apart and the dermal and epidermal surfaces so

exposed were examined under the scanning electron microscope. Regions of strong adhesion between the tissues were revealed as areas where their separation was incomplete. These observations were made on cultured skin one day after explanation. Preliminary experiments showed that, in freshly excised skin dissected without using enzymes, any local adhesions that might be present are obscured by a general, strong adhesion between the tissues. The use of cultured, rather than freshly excised, skin simplified dissection and carried an additional advantage. Anchor filament bundles are much less frequently observed in histological sections of cultured skin than in sections of skin fixed *in situ*, or of freshly excised skin (Davidson, 1983a), probably because they degenerate after the base of the dermis is disrupted when the skin is excised. We would therefore expect interplumar dermal–epidermal adhesions to be weakened in cultured skin and easily broken, providing a clean background against which to detect any adhesions localised in developing primordia.

Skin culture and dissection

White Leghorn or Brown Leghorn, eggs were incubated at 38·5 °C in a humidified atmosphere. At stages 29 to 31 (Hamburger & Hamilton, 1951), dorsal, lumbosacral skin was excised and laid flat on the surface of a hydrated lattice of reconstituted rat tail collagen which was surrounded by liquid culture medium in a Petri dish (Davidson, 1983a). The culture was incubated at 38·5 °C for one day during which between two and four new rows of primordia formed. (The collagen substratum incorporated Eagles medium (Flow), 10% newborn calf serum, and 50μg/ml ascorbic acid. The culture medium comprised F10 (Flow), 10% newborn calf serum, 50μg/ml ascorbic acid, 100i.u./ml penicillin, and 100μg/ml streptomycin, buffered to pH 7·2 with MOPS buffer. Cultures were washed in Dulbecco A phosphate-buffered saline (Oxoid) where appropriate.)

In one series of experiments, the skin was treated with cold trypsin before dissection, making it possible to reliably separate the tissues. The culture was rinsed with saline and incubated with trypsin (a 0·02% solution of Difco 1·250 trypsin in saline) for 1–2·5 h at 6 °C, then rinsed once with culture medium, to arrest the action of the enzyme, and twice with saline. Under these conditions, the epidermis did not float free: forceps were used to gently peel it from the dermis under saline, leaving the epidermal sheet attached at one end.

In another, control, series of experiments skin was washed twice with saline and dissected in culture medium without trypsin treatment.

Scanning electron microscopy (SEM)

Each specimen (comprising skin with its partly removed epidermis) was lifted from the collagen, laid dermis side down on filter paper and withdrawn from the saline so that the epidermis folded back. The skin was immediately

drained by touching the filter paper against a paper tissue (taking care not to allow the skin to dry), fixed overnight in 2·5% glutaraldehyde buffered with cacodylate (0·1M, 100mOsM) to pH 7·4, and postfixed in osmium tetroxide. The specimens were dried from acetone by the critical-point method, coated with gold and viewed under a Cambridge Stereoscan S180 microscope.

Transmission electron microscopy (TEM) and light microscopy

Dissected specimens were mounted on millipore filters, fixed in 2·5% glutaraldehyde overnight, postfixed in osmium tetroxide and embedded in Araldite. Sections, 1μm thick, were stained with alcian blue and examined under a Zeiss Universal microscope. Thin sections were stained with lead citrate and uranyl acetate and examined under a Phillips 300 electron microscope.

RESULTS

In lumbosacral skin, feather primordia form first along the dorsal midline then in successive anteroposterior rows on either side. Rows form at intervals of 8–15h in cultured skin, depending on the stage at explanation (Davidson, 1983b). A series of developmental stages can thus be examined in each piece of skin. Epidermal placodes form simultaneously with, or one row before, detectable dermal condensations. Since definitive primordia comprise both components, their number is defined by the number of condensations. In ten specimens, the number of rows of primordia visible immediately before dissection was the same (± one row) as the number of rows of condensations seen in the SEM. The number of rows visible in living specimens corresponds to the number detectable histologically (Davidson, 1983a). Thus, the most lateral row of condensations seen in the SEM generally corresponds to the earliest histologically distinguishable primordia.

Scanning electron microscopy showed that the results of dissection of trypsinized skin varied with the age of the skin and the duration of enzyme treatment. Preliminary results showed that, in advanced skin (with more than seven rows of primordia), the medial epidermis split between its apical and basal surfaces, while in younger specimens after prolonged trypsinization (2·25 or 2·5h) the epidermis with the basement membrane separated cleanly from the entire dermis. Between these extremes, dissection yielded an interesting result: precisely in the positions of developing primordia, cells had been lifted from condensations and remained attached to the epidermal surface. This result, observed in ten specimens with seven or fewer rows dissected after 1·25 to 1·5h trypsinization, is described below in lateral to medial sequence, following the course of development as it is displayed in successively more advanced rows of primordia. (Fig. 1A illustrates a whole specimen and Figs. 1B, C are general views of the dermal and epidermal surfaces in the region of the three most recently formed rows of primordia.)

The entire basal epidermal surface was coated with basement membrane (Fig. 1D). Lateral to feather sites, and between them, the membrane was bare but for a few cells and sparsely distributed, short cell processes, or matrix fibres. In some cases, shallow depressions in the epidermal surface were visible laterally, at the expected positions of incipient placodes. Along the length of the most lateral row of condensations, the dermal surface had an open texture, many cells were spindle-shaped, rather than flat as elsewhere; condensations appeared as groups of loosely-packed, spindle-shaped, or rounded cells. Here, dissection had produced variable results. Generally, there were few cells attached to the epidermal surface. However, in three specimens, individual cells were attached to this surface in a sparsely populated band, about ten cells

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Fig. 1. Scanning electron micrographs of the dermis and epidermis separated by dissection of trypsinized skin.

(A) A general view of dissected skin. The upper right part of the skin remains intact while, on the left, the epidermis is peeled back to expose the originally apposed epidermal and dermal surfaces. Dark depressions in the epidermal surface are feather placode sites which correspond precisely to condensations in the dermis. The region where the two tissues were separating when the skin was fixed can be seen in the middle of the picture. Magnification × 17; scale bar = 1mm.

(B) A general view of the dermal surface illustrating the appearance of the three most lateral rows of condensations. Successive rows lie top to bottom of the field (anterior is upwards). The most recently formed row is on the right (arrow); mesially (to the left) lie condensations from two more mature rows. Note the increase in packing of the superficial cells of progressively more mature condensations. Magnification × 115; scale bar = 100μm.

(C) A general view of the epidermal surface illustrating a typical distribution of attached dermal cells. The most recently formed row of primordia is represented by the bare depressions on the right (arrow). Depressions representing the preceding, more mature rows in the centre and left have large, deep groups of dermal cells attached to them. Magnification × 75; scale bar = 100μm.

(D) Basement membrane at the epidermal surface exposed by dissection. This region is free from attached dermal cells. A tear in the membrane reveals the basal surfaces of epidermal cells. Magnification × 1000; scale bar = 10μm.

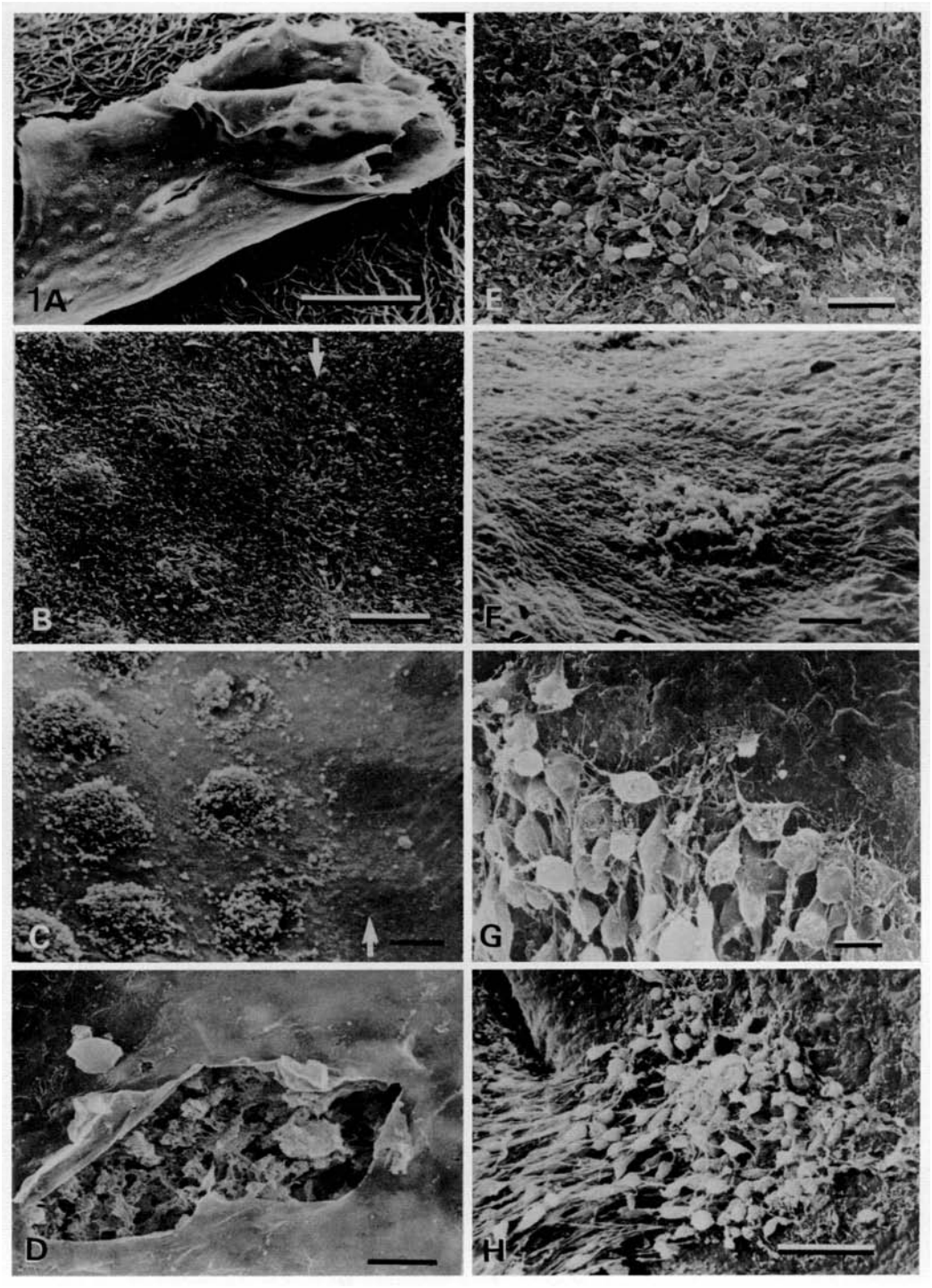
(E) The dermal surface showing a condensation from the row of primordia immediately mesial to the most recently formed row. Note the rounded and closely packed superficial cells in the condensation. Magnification × 330; scale bar = 30μm.

(F) A small group of dermal cells attached to the epidermal surface at the centre of a depression formed by the undersurface of an epidermal placode. Magnification × 300; scale bar = 30μm.

(G) The edge of a group of dermal cells attached to the basement membrane. The marginal cells are attached individually to the membrane by fine processes or fibres. Magnification × 800; scale bar = 10μm.

(H) A region where the epidermal and dermal surfaces were separating at the time of fixation. A group of superficial cells from a dermal condensation lies on the basement membrane. At the left of the picture, cells lie stretched between individual attachments to the basement membrane and the condensation. Magnification × 470; scale bar = 30μm.

A, D, F and H are of the same specimen; B, C, E and G are each of different specimens.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wide, corresponding in position to the most lateral row of condensations. In the next, more advanced, row superficial cells near the centres of the slightly elevated dermal condensations were clearly more rounded and closely packed than those in the most lateral row (Fig. 1E). Cells were attached to the epidermal surface in discrete groups (Fig. 1F), at locations corresponding to condensations. In more mesial rows, the well-developed condensations were markedly elevated and comprised tightly packed cells. Groups of packed cells were attached to the epidermal surface at feather sites. These groups varied in size, but in general, their depth and diameter increased in successively more advanced rows (Fig. 1C); in several cases, the cells appeared to be piled one on top of the other. (Dissection of primordia that had formed before explanation gave the same results.)

Details of the mode of attachment of dermal cells to the basement membrane were clearly visible at the margins of groups of adhering cells (Fig. 1G): the rounded dermal cells were individually attached to the membrane via thin processes or fibres. In the region where the tissues were being peeled apart when the skin was fixed, cells at the surface of each condensation (except in the most lateral row) were stretched between attachments to the basement membrane and the dermis (Fig. 1H): their appearance suggested that they were being pulled individually from the condensation against strong, localized attachments to sites in the dermis. Very few dermal cells were stretched between the two surfaces in interplumar regions.

Light microscopy of sections through undissected skin suggested that dermal cells were closely apposed to a much greater proportion of the basement membrane in primordia than in interplumar skin (Figs. 2A, B). Transmission electron microscopy of dissected skin confirmed that the basement membrane

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Fig. 2. (A–E) Skin after trypsinisation. (F) Skin dissected without enzyme treatment.

(A and B) Light micrographs of sections through intact, trypsinized skin: (A) between feather primordia and (B) within a primordium. These figures illustrate that a greater proportion of the basement membrane is closely apposed to dermal cells in primordia than interplumar skin. Magnification × 690; scale bar = 10μm.

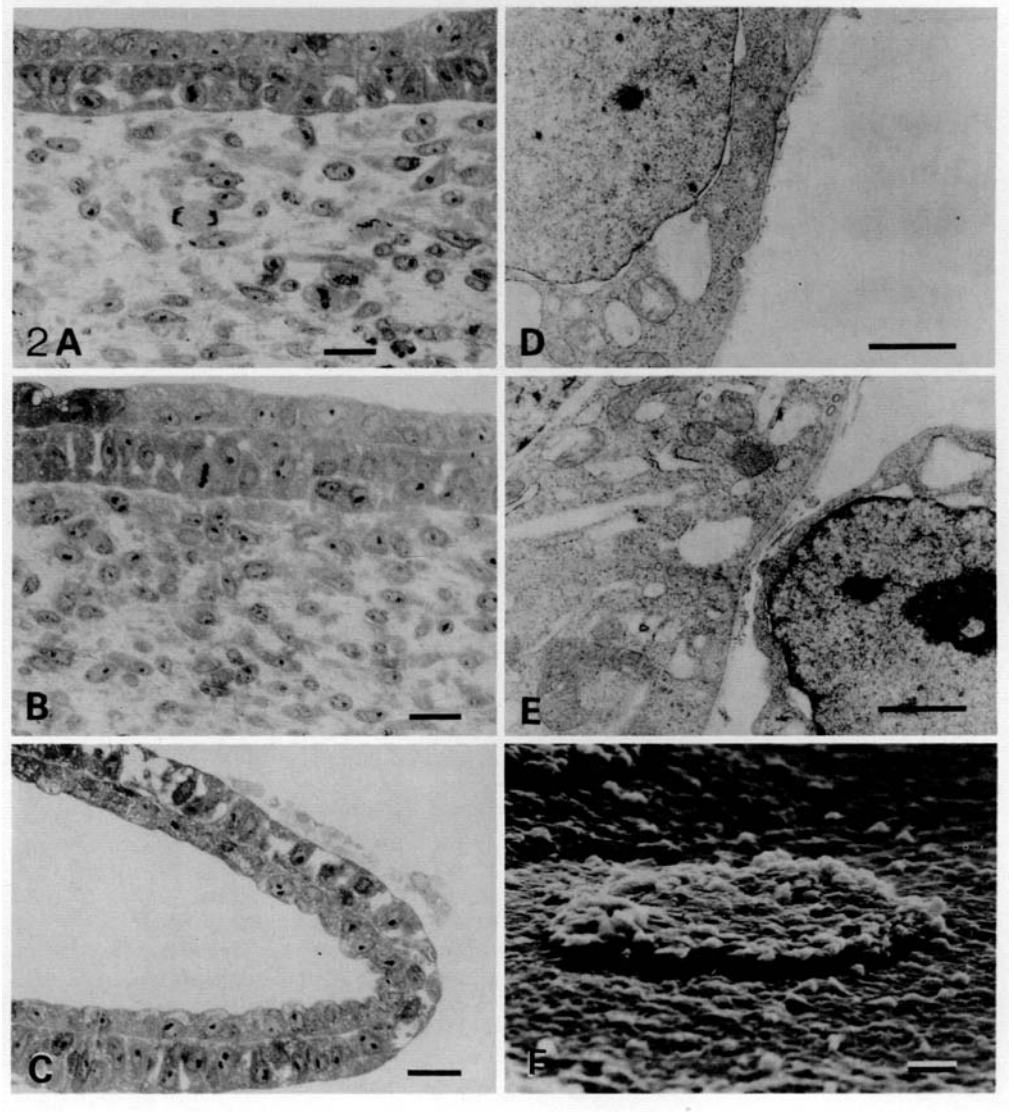
(C) Light micrograph of a section through the epidermis peeled from the dermis, showing part of a group of dermal cells attached to the otherwise bare basal surface of the epidermis. Magnification × 690; scale bar = 10μm.

(D and E) Transmission electron micrographs of the basal surface of the epidermis in dissected skin showing, (D) the basement membrane on the epidermal surface without attached dermal cells, and (E) part of a dermal cell (right) attached to the epidermal surface. Scattered collagen fibrils, identified by their characteristic periodic banding, were abundant at the site of attachment and were found in other locations on the membrane where there were no attached dermal cells. Magnification × 11300; scale bar = 1μm.

(F) Scanning electron micrograph showing a cap of epidermal debris lying over the site of a dermal condensation. Magnification × 600; scale bar = 10μm.

separated with the epidermis and showed that dermal cells were attached to extracellular material near the basement membrane (Figs. 2C–E).

In skin dissected without trypsin treatment (11 cases), the tissues were difficult to separate: in several cases large areas of epidermis fractured, even in skin with only a few rows of primordia. Where the tissues did separate, however, strong attachment of dermal cells to the basement membrane in primordia was indicated by the presence of groups of dermal cells on the epidermal surface. In addition, in parts of some specimens, where the epidermis had split and areas of the dermis were covered with epidermal debris, discrete patches of debris were confined to sites of mature primordia (Fig. 2F).



DISCUSSION

These results show that cells at the surface of dermal condensations form strong adhesions to the basement membrane as feather primordia develop beyond the initial, disc-shaped stage. This conclusion derives from the observation that cells from condensations remain attached to the basement membrane when the epidermis is peeled from the dermis. The phenomenon is not a result of any weakness of adhesion between condensation cells and the surrounding dermis. Intradermal adhesions appear to be at least as strong as those between dermal cells and the basement membrane: during dissection, dermal cells were apparently torn free from strong attachments to other cells or matrix in the condensation. Neither is the result attributable to any local differences in the penetration of, or sensitivity to, trypsin: the same phenomenon was observed after dissection of untreated skin. There is no reason to suppose that these adhesions are peculiar to cultured skin (Davidson, 1983a). However, successive rows form more slowly in culture than *in vivo* (Davidson, 1983b): for example, the three rows illustrated in Fig. 1C represent a domain over which five rows might form *in vivo*.

The present methods provide a rather crude means to demonstrate adhesion. The forces applied during dissection are large compared with the adhesion of individual cells and vary across the tissue. Moreover, a series of static pictures must be interpreted to represent a process. It is impossible to determine precisely when adhesions begin to form and, in particular, whether they develop before or after the tissue interactions which initiate primordium morphogenesis (Sengel, 1976). It is equally difficult to draw conclusions about regions where the tissues do not separate at all. Thus, it is not clear if fracture of the medial epidermis in older skin reflects a general adhesion between the two tissues or derives from the increasing proportion of the tissue interface that lies in the maturing, bulbous primordia. The mechanically resistant adhesions detected by the present assay do not necessarily correlate with the kind of cellular adhesiveness that dictates the behaviour of cells in initial encounters with their surroundings (Grinnell, 1978). The adhesions demonstrated here are more relevant to the stabilisation of structures than, for example, to the guidance of cell movements. The mechanism underlying these adhesions is not known. Kischer (1968) and Sengel & Rusaoën (1969) have noted that dermal cell processes lie close to the basement membrane during primordium elevation and Dèmarchez, Mauger & Sengel (1981) have shown, by quantitative ultrastructural analsis, that dermal cell membranes are closely apposed to a greater proportion of the dermal–epidermal junction at the margin of the feather bud than within it or in interplumar skin. Recent molecular studies suggest possible mechanisms, including those involving cell-surface lectins (Kitamura, 1980) and fibronectin (Mauger, Dèmarchez, and Sengel, 1984).

We can expect that a major mechanical effect of the adhesions demonstrated

here will be to lend structural stability to the feather primordium during the striking morphogenetic change from a disc to a bulb shape. Adhesion can be demonstrated at the stage when anchor filaments disappear from primordia (Kischer, 1968; Ede *et al*, 1971). It is possible that anchor filaments, which appear to tie together the bases of the two tissues, would prevent elevation of the primordium; this difficulty would probably not arise from the adhesion of cells to the tissue interface. Whether this is the primary role of these adhesions remains to be investigated. The observation that deep groups of cells were pulled from the dermal condensations of mature primordia raises the possibility that a system of adhesion extends throughout the condensation. Such a system might help to shape the elevating condensation and give it the stable integrity which Wessells (1965) has demonstrated autoradiographically.

Local intertissue adhesion may play a similar role during the development of other organ primordia. Locally strong attachment of mesenchymal cells to the epithelial sheet has been noted after trypsin–pancreatin-aided dissection of the evaginating primordium of the mouse pancreas (Wessells & Cohen, 1968). However, at least one primordium, that of the mouse lung, shows no evidence of intertissue adhesion during evagination (Wessells & Cohen, 1968).

The present results have immediate relevance to the interpretation of epidermal–dermal combination experiments which use cold trypsin to isolate the tissues (Rawles, 1963). They suggest, for example, a new interpretation of an anomolous result reported by Linsenmayer (1972). Composite skin, comprising thigh epidermis from an 8·5-day-old embryo with several rows of placodes and dermis from a younger embryo, formed primordia at least some of which were apparently induced by the epidermis. This result was unexpected because only the dermis appears to induce primordia in other experimental situations (Sengel, 1976). It can be understood in terms of the activity of dermis from the older donor if we suppose that cells from dermal condensations were carried over with the epidermis. It will be important to check the effectiveness of methods used to isolate the tissues in future dermal–epidermal combination experiments.

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