The differentiation of epidermis

I. The interrelationship of epidermis and dermis in embryonic chicken skin

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INTRODUCTION

When the whole skin of embryonic chickens is grown in culture, epidermal differentiation quite closely resembles that in vivo (Miszurski, 1937; Fell, 1957; Wessells, 1961). Separated epidermis, however, lacking its connective tissue, develops less well; in hanging drop cultures it may be maintained occasionally as an epithelial sheet which does not differentiate (Champy, 1914; Drew, 1922; McLoughlin, 1961a), while in organ culture the epidermis curls up and either remains undifferentiated (Gomot, 1958; Matoltsy, 1960) or becomes largely necrotic (Wessells, 1962). Some degree of cytodifferentiation was observed by McLoughlin (1961a), however; she found that in isolated limb epidermis of 5-day embryonic chickens, while many cells degenerated, the live cells organized themselves into nodules and underwent squamous changes, but there were very few mitoses. In none of the above work did isolated epidermis survive and differentiate as an organized, keratinizing tissue.

On the other hand, when epidermis was combined in culture with its own mesenchyme, it survived and differentiated as in whole skin grown under the same conditions (McLoughlin, 1961b; Wessells, 1962). Thus, for its histodifferentiation, the epidermis is dependent upon the underlying connective tissue.

In addition to its involvement in epidermal histodifferentiation, the underlying mesenchyme acts on the epidermis in the morphogenesis of integumentary appendages, as in the development of the preen gland (Gomot, 1958), feathers (Sengel, 1958; Rawles, 1963) and scale, beak and spur (Rawles, 1963). A similar tissue interrelationship has been demonstrated in other developing organs—e.g. mouse salivary gland (Grobstein, 1953, 1956) and pancreas (Golosow & Grobstein, 1962), ureter of mouse (Grobstein, 1955) and chicken (Calame, 1961), and lung of chicken (Dameron, 1961) and mouse (Alescio & Cassini, 1962)—which shows that for their continued growth and differentiation, embryonic epithelia usually depend upon their subjacent mesenchyme.

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The nature of the epidermal dependence was indicated by McLoughlin's (1961a) finding that epidermal cells became organized into nodules around a cell-free mucoid material from the dermis; the cells of the nodules appeared to form some keratin and those next to the mucoid survived the longest. Moreover, the pattern of epidermal differentiation was altered when the epithelium was combined with heterotypic mesenchyme (McLoughlin, 1961b); it was thought that the various mesenchymes exerted their characteristic effects by means of the different intercellular material produced by the fibroblasts. Furthermore, Wessells (1962) demonstrated that in metatarsal epidermis of 11-day chicken embryos, mitosis and palisading of the basal cells and a limited differentiation occurred in epidermis separated from dermis by various Millipore filters. Thus the orientation and division of the basal cells in both young (5-day) and older (11-day) embryonic chicken epidermis seemed to occur in response to extracellular material from the dermis.

To understand the relationship between the two tissues, it is necessary to know the nature of the interdependence and what components and characteristics of the one tissue are essential for the continued functioning of the other. It is with this in mind that the present analysis of the relationship between homologous epidermis and dermis has been made. In this investigation the two tissues have been separated, and the epidermis has been recombined with the dermis, after the latter had been subjected to various treatments; the recombined tissues have been grown in organ culture to determine the effect on the epidermis of the treatment of the dermis. In other experiments the epidermis has been explanted on different gels, including acetic acid-extracted collagen. The criteria of epidermal growth and differentiation have been the maintenance of a healthy stratum germinativum, as indicated by the appearance and mitotic activity of the basal cells, the stratified organization of the epithelium, and the formation of keratin, as judged by histological staining, birefringence, and the development of a superficial cornium. The dermo-epidermal junction has been investigated also, since any interactions between the two tissues must take place across or at this boundary. It was hoped that the combination of these techniques would demonstrate the relative importance of different dermal constituents for the survival and differentiation of the epidermis.

A preliminary report of this work has appeared elsewhere (Dodson, 1963).

MATERIALS AND METHODS

Epidermis and dermis were obtained from the scaly skin of the anterior tarsometatarsal region of 12-day embryonic chickens (stages 37$\frac{1}{2}$–38$\frac{1}{2}$ of Hamburger & Hamilton, 1951).

Separation of epidermis and dermis. The skin from each foot was dissected off and cut into 3–6 small pieces, each 1–2 mm square, which were washed in calcium- and magnesium-free Tyrode's solution (CMFT), soaked in 2 changes of
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0.04 % Versene (ethylene diamine tetra-acetic acid, disodium salt) in CMFT at room temperature (18–20 °C) for a total of 20 min, and then transferred to 0.02 % Versene, in which the epidermis was carefully peeled from the dermis. As the epidermis separated, it curled up, with the basal surface on the outside; it was then placed in clot exudate (see below, under 'Cultivation of tissues'), in which the curling slowly relaxed. The total time in Versene solutions was 25–60 min.

Separation of epidermis from dermis occurred at the junction of basal cells and basement membrane (Plate 1, fig. A); histological examination showed that each tissue was free from cells of the other (Plate 1, fig. B) and this was confirmed by subsequent cultivation of the separate tissues.

Cell suspensions. Dermis was trypsinized (Moscona, 1952) in 2 % trypsin (Difco 1:250) in CMFT at pH 8 for 30 min at 37.5 °C; horse serum was added to 25 % and the tissue was dissociated by repeated pipetting; the suspension was filtered through the supporting grid of a Swinny hypodermic adapter, centrifuged, and the cells were washed by resuspension and centrifugation in clot exudate. The cells were dispensed as a thick suspension or they were resuspended in clot exudate to an appropriate dilution. In viability tests, only 5–10 % of the cells were stained by 0.05 % eosin and by phase contrast microscopy at least 60 % appeared viable.

Freezing and heat treatment of dermis. Tubes containing several pieces of dermis in a small volume of Tyrode's solution were frozen at −25 °C for 30 min, then thawed at 37.5 °C; this procedure was repeated 3 times. Tubes containing other fragments in Tyrode's solution were held in boiling water for 4 min, the temperature of the Tyrode's solution being 98 °C. To determine whether the dermal cells were killed, samples of both frozen and heat-treated dermis were examined histologically immediately after treatment and after cultivation for 10 days; no viable cells were seen.

Trypsinization after freezing. Frozen-thawed dermis was incubated at 37.5 °C for 1.5 h in 0.1 % crystalline trypsin (Armour) or in 2 % Difco 1:250 Trypsin in CMFT, and was then soaked in horse serum for 15–30 min to inactivate the trypsin. Controls were incubated in CMFT alone. Residual proteolytic activity in the treated dermis was determined by the digestion of the gelatin emulsion of photographic film (H. B. Hewitt, personal communication) as follows. Pieces of treated dermis or single drops of standard trypsin solutions were placed on 35 mm film (Kodak Microfile) in moist chambers, and were incubated at 37.5 °C for 2 or 19 h, after which the dermis or enzyme solution was washed off with a gentle stream of tap water; where there had been proteolytic activity, the gelatin emulsion had been dissolved, leaving a clear spot in the film. The minimum detectable concentration of trypsin was 10⁻⁵–10⁻⁶ mg/ml. No proteolytic activity was detected in treated dermis inactivated in horse serum.

Preparation of collagen gels. One collagen solution (I) was prepared under non-sterile conditions by extracting rat tail tendon with 0.1 % (v/v) acetic acid,
filtering the extract through sintered glass filters nos. 1-3, and dialysing it against glass-distilled water, all procedures being carried out at 4-6 °C. A second preparation (II) was made aseptically; collagen was extracted as for solution I and was then precipitated with 5% sodium chloride, resuspended in 0.1% acetic acid, precipitated by dialysis against 0.5 M sodium acetate, redissolved in acetic acid, and finally dialysed against glass-distilled water. Solutions of collagen were stored at -25 °C. Preparation I contained 2.01 mg material per ml and preparation II 3.36 mg per ml; the nitrogen contents (method of Kanchukh, 1961) were 18.1% and 17.8% respectively, indicating that the solutions were relatively free of non-collagenous material, a conclusion that was supported by amino acid analyses made with a Technicon autoanalyser.

Collagen solutions were gelled with ammonia vapour (Ehrmann & Gey, 1956), strips of rayon cloth (Schaffer, 1956) being incorporated to render the transparent gels visible. The cloth with attached gel was cut into 2-3 mm squares and was washed with water and Tyrode's solution. Gels made with collagen solution I were sterilized either by ultraviolet light (UV) or by soaking them for 2 days in antibiotic solutions containing 500 i.u. penicillin and 500 μg streptomycin per ml. Gels from collagen solution II, prepared under aseptic conditions, were not sterilized further. Some pieces of UV-sterilized collagen gel were trypsinized by the procedure used on frozen-killed dermis.

Preparation of other gels. Agar gels were made from 3% agar (Agar-agar, New Zealand, Oxoid Co.) and from 0.7% and 0.5% agar (Difco). Gels of alginate were prepared by mixing equal quantities of 1% or 0.67% calcium alginate (CS/LH/F) and sodium alginate (Manucol SS/LH, Alginate Industries). Fibrin clots were obtained by adding 80 units of thrombin (Parke-Davis and Co.) to each ml of 2% fibrinogen solution (Bovine plasma Fraction I, Armour Co.). Agar solutions were sterilized by autoclaving and other solutions by membrane filtration. Rayon cloth was incorporated into the various gels.

Gelfilm and Millipore filter. Pieces of Gelfilm (Upjohn Co.) and of Millipore filter (type HA, pore diameter 0.45 μ, thickness 25 μ) were also used as substrata for the epidermis.

Cultivation of tissues. Before combination with epidermis, connective tissues, gels, and other substrata were soaked in fresh clot exudate (H. B. Fell, personal communication): a clot of 3 ml fowl plasma and 2 ml of embryo extract was incubated at 37.5 °C for 30 min, broken up, incubated further for 1 h, then centrifuged. Epidermis, whose orientation was recognizable from the characteristic curling, was spread over a particular substratum and, except when gels were used, the tissues were placed on rafts of rayon cloth (Schaffer, 1956). The explants of epidermis on its substratum were then cultivated at 37.5 °C by the watch-glass method of Fell & Robison (1929), on a clot composed of 12 drops of plasma and 8 drops of embryo extract. The extract was made by centrifuging a mixture of equal parts of Tyrode's solution and the finely minced pulp of a 13-day chicken embryo.
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Antibiotics (penicillin 50 i.u./ml and streptomycin 50 μg/ml) were added to the culture medium only when gels made from collagen solution II and those from solution I sterilized with antibiotics were used. The explants were transferred to fresh medium on every second day.

**Histology.** Tissues were routinely fixed in acetic-Zenker; other fixatives used included Bouin's fluid, Carnoy's fixative, and Lillie's alcoholic lead nitrate (Lillie, 1954). After dehydration, the rayon cloth was removed from the adherent explants with acetone. Serial paraffin sections were cut at 6 μ and stained with the azan method, the periodic acid-Schiff technique (PAS), alcian blue (Pearse, 1960) or van Gieson's stain; diastase and acetylation controls (Lillie, 1954) were performed with the PAS method.

For electron microscopy, tissues were fixed in 1% osmium tetroxide in Tyrode's solution at pH 8 (Fitton Jackson, 1956) and were embedded in a mixture of 85% methyl and 15% butyl methacrylate; some whole tissues were treated with 1% phosphotungstic acid in absolute alcohol, and some thin sections were stained in 1% uranyl acetate.

Pieces of skin were also incubated at 37.5 °C with the following enzymes before fixation: 2% trypsin (Difco 1:250) in CMFT, pH 7.5, for 30 min; 0.1% crystalline trypsin (Armour Co.) in CMFT, pH 7.5, for 30 or 60 min; 0.01% crystalline papain (Nutritional Biochemicals Corp.) in CMFT, pH 7.5, containing 0.005 M cysteine hydrochloride, for 40 or 60 min; testicular hyaluronidase (Light and Co.) 600 i.u./ml in British Pharmacopoeia diluent (sterilized) pH 7.0, for 1 or 24 h. Controls were treated for similar periods with diluent only.

Living material was examined with the dissecting microscope and by phase-contrast microscopy. A Zeiss polarizing microscope was used to observe birefringence in unstained sections, after removal of wax and mounting in xylene.

**RESULTS**

1. The dermo-epidermal junction of the skin at 12 days

The basement membrane (the term as used in this paper refers to the structure observed in the light microscope) is seen in transverse sections as a continuous line, 0.3–0.5 μ in diameter, whose staining properties indicate that it contains PAS-positive, protein-bound carbohydrate, but very little or no acid polysaccharides, that it may include collagen, and that it can often be distinguished from the basophilic surfaces of the basal cells; it is birefringent in a plane parallel to the surface, indicating that the molecules have a regular orientation. In this investigation, the basement membrane has been identified usually by its affinity for aniline blue and its PAS-reactivity. Electron microscopical examination of the region (Plate 1, fig. C) shows that beneath the lower surfaces of the basal cells, and separated from them by a narrow electron-translucent area, there is an electron-dense line similar to that which many authors have seen at the junction
of epithelia and connective tissues; it is referred to here as the ‘adepidermal membrane’ (Salpeter & Singer, 1959). Randomly scattered below the adepidermal membrane are typical collagen fibrils.

When epidermis and dermis of the anterior metatarsal region were separated at stage 37 without prior treatment, the basement membrane usually adhered to the epidermis, but at stage 38 separation was rarely possible. When the skin of stage 38 embryos was treated with Versene, which binds divalent cations, the dermo-epidermal junction appeared normal by light and electron microscopy, yet the epidermis could be cleanly separated. The basement membrane then remained on the dermis together with occasional wisps of basal cell cytoplasm (Plate 1, fig. A). Trypsinization of the skin removed both basement membrane (Plate 1, fig. D) and adepidermal membrane (Plate 1, fig. E), and the lower surfaces of the basal cells developed numerous projections, between which there remained recognizable collagen fibrils. After treatment, the epidermis could be separated from the dermis.

Papain caused histological changes similar to but less pronounced than those of trypsin, but the epidermis was less readily separated from the dermis. Hyaluronidase neither altered the appearance and staining properties of the basement membrane nor eased separation of epidermis and dermis.

2. The development of whole skin

A study of normal epidermal histodifferentiation from the twelfth day of embryonic development gave results that agreed closely with those of Wessells (1961). Grown in organ culture for up to 10 days, whole skin of stage 38 differentiated in the manner described by Fell (1957) and its histodifferentiation differed from normal in (i) the development of the periderm: some cells were greatly swollen, the granules were fewer than in vivo, and the cells degenerated after 4–5 days. In addition, the periderm at first secreted small amounts of a viscid substance (McLoughlin, 1961b; Fell, 1962); (ii) no subperiderm (Wessells, 1961) appeared in vitro; (iii) the basal cells were often cuboidal rather than columnar; (iv) a s. corneum appeared more rapidly than in vivo, i.e. after 3–4 days instead of after 6 days. No scales were formed.

The appearance of the basement membrane did not alter in culture, but the lower surfaces of the basal cells developed small spiky protrusions similar to those seen in vivo in the hatched chicken. The dermal cells survived and produced intercellular material, but thick bundles of collagen did not develop.

3. Growth in vitro of the isolated skin tissues

(a) Epidermis

Freshly separated epidermis was free of basement membrane (Plate 1, fig. B); the lower surfaces of the basal cells sometimes showed protrusions similar to those in whole skin after trypsinization had removed the basement
membrane. Prolonged Versene treatment damaged the basal cells first, and dividing cells seemed most susceptible to the lack of divalent cations.

Fifty-eight explants of isolated epidermis were grown in culture and histologically examined. Sheets of epidermis placed directly on a clot or on a rayon raft contracted irregularly after 12–30 h in vitro, and by 2 days had formed tightly twisted knots of tissue. Histological examination showed that within the first 3–6 h of cultivation the basal cells lost their columnar orientation and became flattened (Plate 2, fig. F). Damaged cells with pycnotic nuclei were found below the lower surface and appeared to have been squeezed out by healthy tissue; there was no apparent phagocytosis of the damaged cells. Subsequently, as the epidermal sheet contracted in area, its thickness increased and the cells of the central region, between the periderm and the lower flattened layer, were unoriented, dividing, and resembled intermediate cells.

By 16–20 h there was a single layer of s. spinosum below the periderm, while on the lower surface were three or four layers of flattened cells resembling spinous cells. Mitoses still occurred sporadically in the central region at this time. Thus the epidermis, instead of being unipolar and differentiating in one direction only, had acquired a bipolar structure, with flattened, differentiating cells on both sides of a layer of undifferentiated elements.

After about 20 h, some cells of the central region became associated in round groups or whorls, consisting of rounded central cells enclosed by one or two more flattened layers. The cells between these groups had no particular orientation. Mitosis had now ceased in this region. Glycogen appeared first in the periderm at about 16 h and, later, in the upper spinous cells.

After about 40 h in culture the cells began to degenerate (Plate 2, fig. G). They swelled slightly and their outlines became more distinct, the nuclei became pycnotic and disintegrated, basophilia was lost, and the cytoplasm appeared empty. The changes occurred first in the unoriented cells of the central region and in the flattened cells of the s. spinosum and of the lower surface, then in the flattened cells surrounding the whorls, and finally in those in the centre of these groups; the last-named cells retained their basophilia until between the second and third days in culture. The whorls showed signs of keratinization, the oriented, outer flattened cells having thick outlines that were faintly birefringent and stained orange-red with the azan technique.

Basement membrane material was very occasionally associated with the basal cells at the margin of some explants and the nearby cells oriented themselves around this substance, forming a well-defined whorl that was better developed than those in the centre of the explant. Cells next to this material retained their basophilia and normal appearance longer than the rest and the surrounding squamous cells developed a little more keratin, but in no isolated epidermis did living cells survive for more than 3 days in culture.

The periderm of isolated epidermis, like that of whole skin in culture, differentiated abnormally fast. The characteristic peridermal granules had appeared
after 16 h in culture; they were most abundant in the secondary periderm. The periderm often spread on to the flattened cells of the lower surface, thus partly surrounding the explant; this migration occurred at the same time as the thickening of the epidermis, as though the migrating peridermal cells pulled the epidermis together.

Thus isolated epidermis did not merely die, but the cells underwent a characteristic rearrangement and a partial differentiation. It survived for a few days only and did not keratinize in culture.

(b) Dermis

When pieces of dermis, or thick suspensions of dermal cells placed on a rayon raft, were cultivated on clots, the isolated dermis survived and produced intercellular material. In pieces of whole dermis the basement membrane often remained visible after cultivation and the orientation of existing fibres was preserved; fibres formed during cultivation, however, had no particular orientation. The isolated dermis usually became enclosed in a protective layer of closely packed cells which had little intercellular material, a phenomenon that was particularly obvious in reaggregates formed from dermal cell suspensions.

EXPLANATION OF PLATES

The tissues illustrated below are from the scaly, anterior tarso-metatarsal skin of 12-day embryonic chickens. Micrographs from the light microscope are of material fixed in acetic Zenker’s solution. PAS, Periodic acid-Schiff technique; AB/PAS, alcian blue followed by PAS.

PLATE 1

Fig. A. Epidermis partly separated from the dermis in skin treated with Versene solution. The epidermis can be peeled away from the dermis, leaving the basement membrane \( (Bm) \) on the latter tissue. (Azan: \( \times 920 \).)

Fig. B. Separated epidermis in Versene solution. The basal cells are contracted and there are large spaces between them and the intermediate cells (arrow). The lower surfaces of the basal cells are marked by basophilic spots and lines, but there is no attached basement membrane or dermal material. (Azan: \( \times 1690 \).)

Fig. C. The dermo-epidermal junction of untreated skin, showing the adepidermal membrane \( (Am) \). (Cf. fig. E.) \( Cm \), Cell membrane of epidermal basal cell; \( Cf \), collagen fibril. (Osmium tetroxide-fixed material treated with uranyl acetate: \( \times 58,000 \).)

Fig. D. Skin fixed after 30 min trypsin digestion. The basement membrane has disappeared and the smooth junction between epidermis and dermis has been disrupted by numerous protrusions \( (P) \) from the basal cells. (Azan: \( \times 1410 \).)

Fig. E. The dermo-epidermal junction of trypsinized skin. There is no adepidermal membrane at the basal surface of the epidermis. The protrusions of the basal cells contain profiles of cytoplasmic organelles. (Osmium tetroxide-fixed material treated with uranyl acetate: \( \times 49,900 \).)
4. The differentiation of the epidermis after experimental recombination

(a) Epidermis and whole living dermis

When epidermis and dermis were separated, then recombined and grown in organ culture, a basement membrane re-formed in less than 14 h, the earliest time examined, and the tissues differentiated almost exactly like explants of untreated skin (Plate 2, fig. H).

(b) Epidermis and dermal cell suspensions

Suspensions of dermal cells, containing little intercellular material, were placed against the basal surface of sheets of epidermis (thirty-four explants); the tissues adhered to each other, survived, and differentiated to re-form a complex structure that resembled normal 12-day skin grown in vitro for the same time. After 2 h incubation the dermal cells had begun to spread out; those near the epidermis flattened against the basal cells, whose protrusions disappeared; thus both tissues were involved in the initial contact reaction. After 3–6 h in culture at 37.5 °C (or in less than 14 h at 20 °C) a structure appeared that resembled the normal basement membrane seen in the light microscope (Plate 3, fig. I). Subsequently dermal intercellular material was deposited and after 2 days the dermis had a normal amount of fibres. The distribution of the fibres was often similar to that of normal skin in that there was less fibrillar material immediately beneath the epidermis than at a deeper level. The epidermal cells retained their orientation and organization and developed a s. corneum of birefringent keratin; in manner and rate of differentiation the epidermis resembled that of normal intact skin under the same conditions.

When suspensions of dermal cells were placed on the peridermal surface of the epidermis (thirty-two explants), there was no contact reaction, and within 2–3 h a gap appeared between the two tissues; the organization of the epidermis was lost and the sheets behaved like epidermis grown by itself. In areas where dermal cells contacted the basal layer, as at the margins of the sheet, the epidermis was normal; this normal region was continuous with abnormal epithelium which had not made contact with the dermal cells.

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Plate 2

Fig. F. Epidermis cultivated in isolation for 3 h. The epidermis is thicker than at explantation; the basal cells are no longer columnar and already some (Fl) are flattened on the lower surface of the explant. The tissue was placed on a rayon raft on a plasma clot. (Azan: × 920.)

Fig. G. Isolated epidermis after 2 days in culture. The tissue has thickened and the cells on the lower surface are flattened. The secondary periderm (2P) contains characteristic granules, but most other cells have lost their basophilia and have pycnotic nuclei. (Azan: × 240.)

Fig. H. Epidermis and dermis after separation and recombination; in culture for 10 days. The epidermis is healthy with basal cells (B), s. spinosum (Ss), and a thick s. corneum (Sc). The dermis (D) contains living cells and intercellular material, mainly collagen fibres. (Azan: × 920.)
(c) Epidermis and frozen-killed dermis

Epidermis was placed with its basal cells next to either the upper surface of the dermis, bearing the basement membrane, or the lower surface: 52 explants of the former and 8 of the latter had healthy epidermis in contact with killed dermis. In 16 other explants, living dermal cells proved to be present; in these the epidermal growth was intermediate between that on live dermis and that on killed tissue. In the results reported below, only epidermis on wholly dead dermis is considered.

The epidermis spread over the frozen-killed dermis and when it reached the margins, after 4–6 days in culture, the upper surface of the explant became smaller, as though the enclosing epidermis drew together the connective tissue. Frozen-killed dermis explanted without epidermis showed no change in area, demonstrating that the decrease was due to the epidermis. The epidermis quickly became closely associated with the treated connective tissue; the basal cells retained their normal appearance, orientation, and rate of mitosis. The periderm at first produced a little secretion and later granules appeared. The epidermis thickened, became stratified, and a s. spinosum and s. corneum developed as in control explants of epidermis with living dermis (Plate 3, figs. J, K). A basement membrane was formed during the first 6 h after recombination; it was not derived from the original one left on the dermis at separation, since it followed very closely the outlines of the basal cells, and it appeared even when the epidermis was explanted on the undersurface of the dermis (Plate 4, fig. L).

A common and characteristic feature of the recombinations was the presence, after 2–3 days, of intrusive spurs of basal cells within the dead tissue. Some of the cells developed small spiky projections resembling those seen in whole skin in vivo and in vitro. Associated with the spurs and projections was a reorientation of the dermal fibres (Plate 3, fig. K; Plate 4, fig. L): originally mainly parallel to the dermo-epidermal boundary, after 2–3 days fibres appeared that were

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**Plate 3**

Fig. I. Epidermis recombined with a suspension of dermal cells on its basal surface; in culture 3 h. Some dermal cells are close to the basal surface of the epidermis and a definite basement membrane (Bm) has formed already, although some of the dermal cells are still rounded and there is little dermal intercellular material. In the region of the basement membrane the basal cells are columnar (cf. fig. F). The dermis does not make contact with the periderm and there is a gap (G) between them. (Azan: × 300.)

Fig. J. Epidermis on frozen-killed dermis; in culture 2 days. The epithelium is healthy and has a well developed s. spinosum (Ss). An epidermal spur (Sp), bounded by a basement membrane (Bm) penetrates the killed dermis. (Azan: × 240.)

Fig. K. Epidermis on frozen-killed dermis; in culture 10 days. The epidermis has healthy basal cells on a basement membrane (Bm), it is stratified, and has a well developed s. corneum (Sc). There is a small epidermal spur, associated with which are dermal fibres (C) that are reoriented to a position approximately at right angles to the dermo-epidermal boundary. (Azan: × 240.)
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approximately perpendicular to the basement membrane. There was no alteration of the fibres in frozen-killed dermis explanted alone, showing that the rearrangement was caused by the epidermis.

(d) Epidermis and frozen-killed dermis treated with trypsin

After trypsinization, pieces of frozen-killed dermis were looser and less firm than before; histological examination indicated that cellular remains and some interfibrillar material were removed, leaving predominantly collagen fibres.

Epidermis was placed with the basal cells on either the outer or the inner surface of frozen-killed dermis treated with crystalline trypsin (79 explants) or Difco 1:250 trypsin (25 explants); the patterns of development were the same in all groups. In twenty-two control cultures dermis was treated with CMFT only; all developed like epidermis on untreated frozen-killed dermis. Of the experimental groups, the development of 11% resembled that of the controls, but 89% were very dissimilar.

When first explanted on the trypsinized, frozen-killed dermis, the epidermis appeared healthy. A basement membrane was formed and after 2 days in culture the basal cells were still basophilic and dividing; the epidermis was much thicker than in controls and a thin s. corneum had already differentiated (Plate 4, fig. M). The morphological relationship with the treated dermis was abnormal, however, for the epidermis did not spread over the connective tissue and spurs projecting into the dermis, together with numerous isolated epidermal cells, were much more common than in control cultures. After a further 2½–3 days, keratinization extended right to the lower layers; the basal cells were degenerate or partly cornified and were detached from the basement membrane on the dermis (Plate 4, fig. N).

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**Plate 4**

Fig. L. Junction of epidermis with frozen-killed dermis; in culture 10 days. The basal cells have short projections (P) which are outlined by the PAS-reactive basement membrane (Bm). Reoriented dermal fibres (C) are associated with the lower surface of the basal cells. (AB/PAS: ×1410.)

Fig. M. Epidermis on trypsinized frozen-killed dermis; 2 days in vitro. Although the epidermis is alive and its cells are basophilic, it has not spread over the dermis and it is much thicker than on untreated frozen-killed dermis. Many epidermal spurs (Sp) project into the treated dermis and some epidermal cells are isolated (I) from the rest of the epithelium. A little keratin (K) has been formed. (Azan: ×240.)

Fig. N. Epidermis on trypsinized frozen-killed dermis after 5 days in vitro. Most of the epidermis has cornified (Sc), but the basal cells have degenerated and lost contact with the dermis. The positions of former epidermal spurs are marked by gaps in the dermis, which contain necrotic basal cells (B) and are surrounded by reoriented collagen fibres. The remains of the basement membrane (Bmr) can be seen in places. (Azan: ×240.)
(e) Epidermis on dermis killed by heat

After heat treatment, the dermis was contracted and the intercellular material appeared to be denatured. All nineteen explants of epidermis placed with its basal surface next to dermis killed by heat were detached from the dermis and resembled the epidermis grown in isolation. Only the periderm differentiated and after 2 days contained the characteristic granules; some peridermal cells on the lower surface appeared to phagocytose the denatured dermis.

(f) Epidermis grown on gels of collagen

The role of collagen in epidermo-dermal relationships was investigated by replacing the dermis with collagen gels. Examined as squash preparations in polarized light, the gels were anisotropic and appeared to be made up of closely coherent, parallel, birefringent fibrils, which were only just resolvable; the solution was isotropic. Electron-microscopic examination of one gel showed no banded collagen fibrils, but interwoven filamentous material of about 50 Å diameter.

Epidermis was arranged with the basal surface next to the gel and cultivated for periods of up to 14 days: 41 explants of epidermis were grown on gels from collagen solution I sterilized by UV irradiation and 30 on gels sterilized by antibiotics; 17 pieces of epidermis were cultivated on gels from collagen solution II. The growth and differentiation of the epidermis were similar on all the gels and the following description is based on all 88 cultures. The epidermis did not curl up, but spread over the collagen and the diameter of the epithelial sheet usually

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**Plate 5**

Fig. O. Epidermis on collagen gel; 24 h in vitro. The basal cells (B) are still columnar and dividing; a s. spinosum (Ss) has developed already and the periderm (P) has produced much secretion (Ps). Occasional gaps between basal cells and collagen gel (Cg) may be artifacts. (Azan: × 920.)

Fig. P. Epidermis on collagen gel; 2 days in vitro. The epidermis has thickened and there is a prominent s. spinosum (Ss). Some of the basal cells (B) are flattened on the gel (Cg), but they remain healthy. (Haemalum-chromotrope: × 920.)

Fig. Q. Epidermis on collagen gel; 10 days in vitro. The basal cells are flattened but still healthy. There is a s. spinosum and a thick s. corneum. In the area shown, the junction of the epidermis with the collagen gel (Cg) is marked only by a cell membrane (Cm). (See also figs. S, T.) (Azan: × 920.)

Fig. R. Epidermis on collagen gel; 10 days in vitro. The s. corneum and s. spinosum are birefringent. Arrow marks base of epidermis. (Unstained section in polarized light: × 370.)

Fig. S. Epidermis on collagen gel; 10 days in vitro. Basal cells, although flattened, can still undergo mitosis. The junction of epidermis with gel (Cg) is marked only by the cell membrane (Cm). (Azan: × 920.)

Fig. T. Junction of epidermis and collagen gel; 2 days in vitro. In this area, at the boundary of epidermis and gel (Cg) there is a layer of material (Bml) that stains with the PAS technique. (PAS and Mayer’s acid haemalum: × 920.)
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increased 2-3 times. It often covered the whole of the upper surface of the stratum and when this happened, at 6-8 days in vitro, the gel sometimes became reduced in area. The opacity that indicates cornification (Fell, 1957) appeared after 3-4 days in culture.

Histological examination showed that much of the epithelium was attached to the collagen after only 3 h incubation; attachment resulted in retraction of the basal blebs and in cuboidal or columnar orientation of the basal cells, whereas in unattached epidermis some of the lower cells were flattened on the basal surface. After about 12 h in vitro the whole of the epidermis was attached to the gel. Mitoses were at first fewer in attached epidermis than in the free portion, but had increased after 12 h in culture; intermediate cells and those of the secondary periderm also divided, though at a lower rate, and the epidermis thickened rapidly. The periderm, between 12 and 24 h in culture, produced some secretion (Plate 5, fig. O).

During the second day the tissue thickened further and began to differentiate (Plate 5, fig. P). The basal cells became flattened, but still divided; the mitotic count was lower than on the first day, but resembled that of whole skin of the same age in culture (about 4 mitoses per 1000 basal cells). Tonofibrils appeared in the numerous layers of the s. spinosum and granules developed in the healthy periderm, although some peridermal cells were swollen. A true s. corneum appeared at about 3 days and gradually thickened, while the number of layers in the s. spinosum lessened, indicating that cells were cornifying more rapidly than undifferentiated cells were being supplied. After 10 days in culture there was a thick birefringent s. corneum while the s. spinosum was reduced (Plate 5, fig. Q).

The keratin was less birefringent than in vivo but it resembled that of whole skin in culture (Plate 5, fig. R). The basal cells were extremely attenuated, but in the healthiest explants were still dividing (Plate 5, fig. S).

The lower surfaces of the basal cells were smooth during the first few hours in culture and were closely apposed to the gel; later, occasional small cavities appeared between the cell membrane and the substratum: these may have been histological artifacts and/or they might indicate regions of weak attachment.

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**Plate 6**

Fig. U. Epidermis on Millipore filter; in culture 3 days. The epidermis is not attached to the filter (F) and has degenerated exactly as if on a plasma clot. (Azan: x 210.)

Fig. V. Epidermis on Millipore filter; in culture 4 days. In this explant the epidermis is attached to the filter (F) and the lower cells are arranged in layers, but the upper part of the tissue is unoriented and degenerate. (Haematoxylin and eosin: x 360.)

Fig. W. Epidermis on Millipore filter in the presence of extra embryo extract (see text); in culture 4 days. The whole tissue is attached to the filter (F); it has a regular layered arrangement and a s. corneum has developed. The basal cells are very compressed but in some explants are still dividing. (The gap in the filter is a post-fixation artifact). (Haematoxylin and eosin: x 430.)
After 5–10 days in culture, some of the basal cells inserted small spiky processes into the gel, thus resembling epidermis cultivated on live or frozen-killed dermis.

Spurs of epidermis invading the collagen were rarely seen on smooth-surfaced gels, but when the collagen was known to contain cracks, epidermal spurs were common, indicating that the epithelium crawled over the surface and into any defect in it. The epidermis did not appear to break down the collagen gel.

The junction of epidermis and collagen gel varied in structure. The cell membrane could often be discerned and in some places was the only structure present. A region resembling a basement membrane was present in some but not all areas: it was 0.3–0.4 μ wide and stained with aniline blue and with the PAS method after removal of glycogen (Plate 5, fig. T). Amorphous or finely fibrous material, which stained deeply with aniline blue but not with the PAS method, was distributed irregularly along the boundary, extending up to 5 μ below the cell membrane; this may be an artifact arising from compression of the gel during sectioning, or it may have originated in the epidermis. Fine fibres, arranged almost perpendicularly to the epidermis, were sometimes also present, usually associated with the basal spiky processes. The occurrence of these various structures varied greatly in different regions of the same explant and from one culture to another, but each was found in many explants.

The evident flattening and expansion of the basal cells may be related to the spreading of the epithelium over the gel. During the first 5 days in culture, the explant usually increased in diameter by 2–3 times, i.e. increased in area by 4–9 times. While the mitotic count appeared to be too low to account for this extension by cell division, individual basal cells, on the other hand, expanded in area by 16–49 times. It is clear therefore that not all the basal cells spread out and some of them must have moved upwards as other basal cells expanded. An upward movement of this kind may have played a part in the rapid increase in epidermal thickness during the first 24 h of culture and may account for the subsequent thickening during the next 4 days, when the mitotic index was low.

A few explants were grown on trypsinized collagen gels. Epidermis spread over both treated (5 explants) and control gels (4 explants), and after 6 days in vitro the epithelium of both series was attached, healthy, and had formed keratin. Thus the survival and differentiation of the epidermis on collagen gels does not appear to depend upon trypsin-labile material in the gels.

(g) Epidermis on other kinds of gel

Epidermis was also grown on other kinds of gel: agar (8 explants), alginate (12 explants) and fibrin (7 explants). On all these substrata the epidermis retracted and curled up to some degree; sections showed that it failed to become attached and developed as though in isolation on a clot. The surfaces provided by these gels were therefore unsuitable as substrata for the epidermis.
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(h) Epidermis on Millipore filter

Sixty-three explants of epidermis were cultivated for up to 4 days on pieces of Millipore filter placed on a clot. Development was very variable. Although in contact with the filter, the epidermis did not always attach itself to the substratum, and if some regions of an explant were attached, others often remained free. All but one of the explants thickened greatly and 71% (forty-five explants) became bipolar and developed exactly as though isolated on a clot (Plate 6, fig. U); 17 explants (27%), however, contained some regions in which the lower cells were flattened and arranged in layers, although the upper cells of the thickened epidermis were unoriented (Plate 6, fig. V). One explant closely resembled epidermis grown for the same length of time (48 h) on a collagen gel, all the cells being arranged in regular layers. The basal cells, next to the filter, were nearly always flattened, even after only a few hours, but they were occasionally cuboidal (7 explants) or even columnar (2 explants). Mitoses were found in ten of the pieces of epidermis (16%), after 24–42 h of incubation. Keratinization was limited to three or four loose layers of partly cornified cells.

Twenty-three explants were grown on Millipore filter on a clot containing an increased concentration of embryo extract (equal parts of embryo extract and plasma) and were fed each day with an extra drop of the extract. Development was again variable, but the epidermis differentiated further than in the previous series: after 4 days in culture, 44% of the explants consisted of well-arranged layers of cells, with a definite, birefringent, s. corneum (Plate 6, fig. W) and some of the basal cells, although flattened, were still dividing. All the cultures, however, contained some thickened regions of unoriented cells and none were as healthy as epidermis on a gel of collagen, or on living dermis.

These results show that, under conditions similar to those in which other substrata were used, Millipore filter is not a suitable substratum for epidermal differentiation, although the tissue sometimes develops slightly further than when placed directly on a clot. In the presence of an increased concentration of embryo extract, however, the filter may support some epidermal development.

(i) Epidermis on Gelfilm

Since gelatin normally liquefies under conditions of culture, Gelfilm, a stable non-antigenic form prepared from purified, specially treated gelatin, was used. When epidermis was grown on pieces of Gelfilm, some explants (six) became bipolar and degenerated like epidermis explanted on a clot. Four other explants remained unipolar, however, and the cells flattened, partially keratinized, and were arranged in loose layers; after 4 days most of the lowest cells were degenerate or partly differentiated and the epithelium was detached from the film. As a substratum for epidermal differentiation, therefore, Gelfilm is similar to Millipore filter and is much less suitable than a gel of collagen.
DISCUSSION

The differentiation of the epidermis in culture

The present investigation demonstrated that in culture the metatarsal epidermis of 12-day embryonic chickens keratinized when reassociated with its own dermis killed by freezing, with frozen-killed dermis treated with trypsin and also when cultivated on a gel of reconstituted collagen. The process of keratinization in the experimental recombinants histologically resembled that in control explants of whole skin cultivated in vitro under the same conditions; it differed from in vivo keratinization in its rapidity and, perhaps associated with this, the absence in vitro of a subperiderm.

The periderm differentiated on all the substrata used—not only when the epidermis keratinized, but also when the epidermis failed to develop. Wessells (1962) described a similar result in epidermis placed on Millipore filters. Hence these upper two layers of cells appear to be less dependent on the dermis than is the rest of the epidermis, and are able to differentiate in the absence of dermal components. Since the time of explantation is only shortly before the periderm normally forms granules, it may be that differentiation has already begun at 12 days in ovo, and so continues whether or not the underlying cells are healthy.

The dermo-epidermal junction

The results of treating the whole skin with various agents indicate that the dermo-epidermal junction can be divided into three levels, with differing characteristics. (1) The adhesion between dermis and adepidermal membrane; this is not dependent on divalent cations and in anterior metatarsal skin it increases between stages 37 and 38. (2) The adepidermal membrane itself, which includes trypsin-labile peptide bonds of non-collagenous protein and appears to be unaffected by the removal of divalent cations. (3) The attachment of the epidermis to the adepidermal membrane; this is dependent on the presence of divalent cations. Furthermore, acid mucopolysaccharides do not appear to be important structural components in these regions.

Versene may diminish the rigidity of the epidermal cell surface directly, by removing divalent metal ions from the protein framework, and/or by inactivating ATPase, which requires calcium and magnesium; the last would reduce the energy available for maintenance of cell form. It may also affect other enzyme systems through the removal of trace metals, as suggested by Dornfeld & Owczarzak (1957). Thus the Versene treatment may have weakened either the lower plasma membrane of the basal cells, or the adhesion of the cell to the adepidermal membrane. The wisps of cytoplasm left on the basement membrane, however, indicate that the plasma membrane in this area is probably ruptured.

When the skin was incubated in trypsin the basement membrane as seen in
the light microscope had almost disappeared, and electron microscopy showed that, although collagen fibrils were still present, the treatment had removed the adepidermal membrane. This observation supports the view that the electron-dense line shown by electron microscopy contributes largely to the basement membrane seen in the light microscope; the difference in the widths of the two structures (basement membrane: 0.3-0.5 \( \mu \); adepidermal membrane: c. 600 Å) might be explained by overlapping of material in the thick histological sections (e.g. Gersh & Catchpole, 1960).

A basement membrane appeared in culture within 3–6 h of recombination of epidermis with living or treated dermis; the rapidity of its development suggests that this structure is important in the interactions of basal cells and substratum. The basement membrane might be formed by the dermal cells, either directly or in part by condensation of ground substance (e.g. Gersh & Catchpole, 1949, 1960) and/or collagen (e.g. Kallman & Grobstein, 1965), or by the epidermis (e.g. Pierce, Midgely & Sri Nam, 1963). The appearance of a basement membrane between epidermis and trypsinized or untreated frozen-killed dermis demonstrates that neither trypsin-labile components of the ground substance nor living dermal cells are necessary for the production of the membrane. The evidence for basement membrane formation in explants of epidermis grown on collagen gels is ambiguous, but material resembling a basement membrane appeared in some regions though not in others. Thus the evidence from this investigation supports the view that the epidermis is concerned in the formation of the basement membrane and that it may produce at least some components of this structure.

**Morphological relationships between epidermis and dermis**

Although the epidermis survived and differentiated in the absence of living dermal cells, the morphological association between the two tissues was altered. Epidermis spread over frozen-killed dermis or collagen gel until it completely covered the substratum, whereas on living dermis it often became surrounded by the connective tissue and formed a cyst; thus in normal skin, *in vitro*, epidermal spreading appears to be controlled at least in part by the dermal cells.

The dermal fibres were reoriented in frozen-killed dermis when the latter was combined with epidermis, but not when it was explanted alone, indicating that the rearrangement was caused by the epidermis. In explants of epidermis with living dermis, the fibres were not altered, showing that the dermal cells and/or their products either prevented the epidermis from reorienting the collagen fibres, or removed those that had been rearranged, perhaps laying down new ones in the required direction. The mechanism by which the epidermis altered the position of the collagen fibres is not clear; the close attachment of the basal cells to the fibres beneath them, however, and particularly the association of fibres with the cell projections, suggest that the epithelium pulls the fibres into a new
orientation. This may be associated with the contraction in area noted in these explants.

The basal cells of the epidermis are normally columnar and this shape has been used as an indication of the initial stages of epidermal differentiation (Wessells, 1962, 1964); in the present experiments, however, the columnar orientation was not always retained. The rapidity with which the basal cells became flattened when the epidermis was isolated on an unsuitable surface, together with the bipolarity of the isolated tissue and the fact that the flattening and bipolarity were prevented when the epithelium was placed on a suitable substratum, indicate that the primary effects of attachment are not only to keep the basal cells in a columnar orientation, but also to maintain the polarization of the epithelium. When grown on collagen gels for more than 2 days, the basal cells became flattened yet the epithelium remained unipolar and healthy; thus for epidermal survival and differentiation the polarity of the tissue is essential, but the columnar form of the basal cells is not necessary at all stages of epidermal development.

The dermal requirements for epidermal survival and normal differentiation

Although the epidermis keratinized when combined with its own live dermis, it did not survive or differentiate when cultivated in isolation on a plasma clot. The final structure formed in the isolate, with groups of concentrically arranged cells surrounded by necrotic tissue, is similar to that illustrated by Wessells (1962); the arrangement of the cells resembles that noted by McLoughlin (1961a) in the isolated epidermis of 5-day embryonic chicken skin, but the degree of cytodifferentiation was slightly less than that described in the younger epidermis. On Millipore filter also, under similar conditions of culture, the epidermis failed to develop fully. The result supports the view mentioned earlier (see Introduction) that for its normal functioning the epidermis requires certain environmental conditions provided by the dermis.

Epidermis differentiated normally when suspensions of dermal cells were placed on its lower surface, but it did not survive when they were deposited on the periderm; hence the necessary dermal constituents must be associated with the basal surface, a conclusion that agrees with those of Gomot (1958), McLoughlin (1961b) and Wessells (1962).

The survival of epidermis on frozen-killed dermis and on collagen gels (Dodson, 1963), confirmed by Wessells (1964), together with the lack of epidermal differentiation on other gels and surfaces, as discussed in this paper, indicates that the epidermis requires a substratum with certain specific properties. The experimental recombinants reveal something of these requirements.

The keratinization of epidermis on frozen-killed dermis demonstrated that epidermal survival and differentiation are not dependent on the continued activity of dermal cells. Although the treated dermis contained the remains of cells, the growth of epidermis on collagen gels showed that cellular remains are
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not essential for epidermal differentiation; this suggests that it is the extracellular material of the frozen-killed dermis that is important, a conclusion that agrees with those of McLoughlin (1961b) and Wessells (1962). This view is also supported by the fact that heat-killed dermis was not a suitable substratum for the epidermis; the heat treatment would cause many alterations, including shrinkage and possible degradation of the collagen and also changes in the ground substance.

When the frozen-killed dermis was trypsinized, the epidermis soon became detached and degenerated, although it still keratinized; this detachment might be due either to residual tryptic activity, or to the removal or alteration of the cellular debris, the collagen, or the non-collagenous intercellular material, i.e. the ground substance. Since cell remains are not essential for epidermal survival, however, their elimination is not likely to affect the maintenance of the epidermis. Moreover, the continued attachment of epidermis to collagen gels trypsinized in a similar manner indicates that neither residual tryptic activity nor an effect of the trypsin on the collagen contributed to the detachment of epidermis from trypsinized dead dermis; it is possible that some trypsin-labile component of the ground substance is necessary for the continued survival of the epidermis.

This conclusion seems to conflict with the fact that the epidermis can grow on ordinary or trypsinized collagen gels, which contain little or no dermal ground substance; the structure of collagen gel, however, differs from that of the trypsinized frozen-killed dermis. The histology and fine structure of the gels indicated that the collagen was distributed homogeneously as fine filaments and perhaps individual macromolecules would also be present; thus the surface of the gels would be almost continuous and fairly smooth. The trypsinized frozen-killed dermis, on the other hand, formed a loose scaffolding of collagen fibres, from between which the ground substance had apparently been removed; the surface of this tissue therefore would have a coarse, net-like structure. That the two surfaces were very dissimilar was also implied by the behaviour of the explanted epidermis: on collagen gels, the epithelium formed downgrowing spurs only when it encountered cracks in the otherwise smooth surface, while the many small spurs and abundant isolated cells of epidermis on trypsinized frozen-killed dermis indicated the presence of many small breaks.

It is suggested therefore that one of the trypsin-labile components of dermis required by the epidermis is the ground substance which forms a permeable continuum between the collagen fibres. On collagen gels the epidermis reacts to the flat collagenous surface in the same way as to the ground substance plus collagen of normal dermis. The failure of epidermis to grow on other gels such as agar, alginate or fibrin, and its detachment from the collagen of trypsinized frozen-killed dermis, indicate that neither continuity of surface nor a collagenous nature alone renders a substratum adequate, but that both these characteristics may be necessary to enable the epidermis to survive and differentiate.

The behaviour of epidermis when grown on Millipore filter, however, casts
some doubt on whether a substratum must be continuous and collagenous. Wessells (1964) found that when embryo extract (20 %) was present, Millipore filter supported some epidermal development: the basal cells retained their columnar orientation and continued to incorporate thymidine; further differentiation was not described. Cohen (1965) confirmed that the presence of embryo extract (20 %) improved the maintenance of chicken epidermis cultivated on Millipore filter and found that when epidermal growth factor isolated from mouse salivary gland was also added to the medium, the epidermis keratinized. The present investigations, however, showed that when epidermis was placed on Millipore filter in the presence of the same concentration of embryo extract (20 %), columnar cells were rare, mitoses were found in only 16 % of the explants, and the epidermis developed poorly compared with that under similar conditions on a gel of collagen. Only in the presence of an increased concentration of embryo extract did the epidermis survive; it was not as healthy as when on a collagen gel, but it differentiated to form a s. corneum. This supports the finding that embryo extract is important and seems to indicate that a non-collagenous substratum may allow epidermal survival, although a possible contribution by the collagen in the embryo extract has not yet been excluded.

Although the culture experiments showed that living dermal cells were not essential for epidermal differentiation, it must be emphasized that cellular constituents were present in the embryo extract in the natural media used. In the normal relationship between epidermis and connective tissue, dermal cells are necessary to produce the substratum and to modify it in relation to stress; they appear to play some part in the control of epithelial spreading and in the maintenance of correctly oriented dermal fibres. The dermal intercellular material is important for epidermal histodifferentiation, but by itself it is static; for changing relationships between tissues, such as occur in morphogenesis, cells of the connective tissue also are necessary.

SUMMARY

1. Experiments were made on the tarso-metatarsal skin of 12-day chicken embryos to determine the relative importance of different dermal constituents for the continued growth and differentiation of the epidermis. The two tissues were separated after exposure to Versene and the isolated epidermis was either recombined with the dermis which had previously been subjected to various treatments, or explanted on different gels, including acetic acid-extracted collagen. After growth in organ culture on natural medium, the explants were examined histologically.

2. The isolated epidermis did not survive in culture when laid directly on a plasma-embryo extract clot, but it grew and keratinized when placed with the basal surface downwards on dermis killed by freezing and thawing or on a gel of collagen; when frozen-killed dermis was trypsinized, the epidermis differentiated at first, but then degenerated. The epidermis did not survive on heat-
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killed dermis, on agar, alginate, or fibrin gels, nor on Gelfilm. The epidermis survived on Millipore filter only in the presence of an increased concentration of embryo extract, when it differentiated to form a s. corneum. A periodic acid-Schiff-reactive basement membrane was formed in the absence of living dermal cells.

3. It is concluded that, under the conditions of the experiments, the survival of embryonic chicken epidermis as an organized, keratinizing tissue depends on the contact of the basal surface with a suitable substratum, but not on the presence of living dermal cells. In the normal substratum provided by the dermis, both collagen and trypsin-labile ground substance are required by the epidermis, yet a gel of collagen can provide a suitable surface on which the epidermis is capable of normal differentiation in vitro. It has been suggested therefore that the epidermis reacts to the flat surface of collagen gels in the same way as to the extracellular moiety of the dermis.

RÉSUMÉ

La différenciation de l'épiderme. I. Relations mutuelles entre l'épiderme et le derme dans la peau de l'embryon de poulet

1. On a expérimenté sur la peau du métatarsar de 'embryons de poulet de 12 jours, pour déterminer l'importance relative de divers constituants dermiques pour la continuation de la croissance et de la différenciation de l'épiderme ; les deux tissus ont été séparés après exposition au Versene et l'épiderme isolé a été ou bien recombiné avec le derme qui avait été préalablement soumis à divers traitements, ou bien explanté sur différents gels, y compris du collagène extrait à l'acide acétique. Après croissance en culture d'organes sur milieu naturel, les explants ont été examinés histologiquement.

2. L'épiderme isolé n'a pas survécu en culture quand on l'a placé directement sur un coagulum de plasma et d'extrait embryonnaire, mais s'est accru et kératinisé quand on l'a placé, la surface basale vers le bas, sur du derme tué par congélation et dégéllement, ou sur un gel de collagène ; quand le derme tué par le froid a été trypsinisé, l'épiderme s'est d'abord différencié mais a ensuite dégénéré. L'épiderme n'a pas survécu sur du derme tué par la chaleur, sur des gels d'agar, d'alginate ou de fibrine, ni sur du Gelfilm. L'épiderme n'a survécu sur filtre Millipore qu'en présence d'une concentration accrue d'extrait embryonnaire, et s'est alors différencié pour former un stratum corneum. Une membrane basale PAS-positive s'est formée en l'absence de cellules dermiques vivantes.

3. On conclut que, dans les conditions de l'expérience, la survie d'épiderme embryonnaire de poulet, en tant que tissu organisé et kératinisant, dépend du contact de la surface basale avec un substratum adéquat, mais pas de la présence de cellules dermiques vivantes. Sur le substrat normal constitué par le derme, du collagène et une substance fondamentale sensible à la trypsine sont tous deux nécessaires à l'épiderme ; pourtant un gel de collagène peut fournir une surface
convenable sur laquelle l'épiderme est capable de se différencier normalement in vitro. On a suggéré, par conséquent, que l'épiderme réagit à la surface plane des gels de collagène de la même manière qu'à la partie extracellulaire du derme.

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