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Basal Lamina Changes During Tissue Interactions in Hair Follicles—An In Vitro Study of Normal Dermal Papillae and Vitamin A-Induced Glandular Morphogenesis

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Skin pieces from 14-day fetal mice were cultivated for 1–10 days prior to fixation and sectioning. Subsequently, sections were studied by light and transmission electron microscopy. In a standard medium the lateral hair follicle walls showed progressive maturation of the basal lamina, while the hair matrix, at the time of a known tissue interaction, showed the formation of gaps in the basal lamina, with heterotypic cell contacts through the gaps. In a vitamin-A enriched medium similar changes occurred, not only at the hair matrix, but also at lateral follicle walls, at the sites of, and prior to, budding and glandular morphogenesis. This study shows that the induction of hair matrix by dermal papilla may perhaps be added to the list of normal tissue interactions in which heterotypic cell contacts occur. It also suggests that vitamin-A induced glandular morphogenesis might come about through a mechanism resembling a normal tissue interaction.

In the first part of our ultrastructural study of the effects of vitamin A on fetal mouse skin in organ culture [1], we showed that some segments of the basal lamina which lies below the epidermis were lost after 1 day in vitamin A-enriched medium. After 2 days, contacts between epidermal cells and dermal cell processes (heterotypic cell contacts) took place through the gaps in the basal lamina, and after 3 days the differentiation of epidermal cells was seen to be altered. In skin grown in the standard medium without added vitamin A, no gaps in the epidermal basal lamina were found, there were no dermal-epidermal cell contacts, and the differentiation of the epidermis proceeded normally for 10 days. It seemed that there might be a causal relationship between those events that are described above as effects of vitamin A, i.e., basal lamina gaps, heterotypic cell contacts, and altered epithelial differentiation.

The developing vibrissa follicles in fetal mouse skin underwent glandular morphogenesis and mucous metaplasia when exposed to excess vitamin A in organ culture [2]. Because this profound change occurred only when the follicles were treated at defined stages of follicle development, required a relatively short exposure to vitamin A, and was apparently irreversible [3], it was suggested that the mechanism of vitamin A action on follicles might resemble that of an embryonic induction. Earlier studies of the reversible mucous metaplasia in chicken embryonic skin treated with high doses of vitamin A had suggested that the action on the latter tissue was a modulation of expression of differentiation [4,5].

The existence of at least two embryonic inductions, or tissue interactions, during the normal development of mouse vibrissa follicles in vivo has been established by the enzymic separation of tissues and the preparation of tissue recombinants which were grown as grafts. By this method Kollar [6] showed that, in the mouse at about 12 days of gestation, an aggregation of dermal cells at a specific site instructs the epidermis to make an appendage (the "nonspecific dermal message") [7,8]. The induced epidermal cells respond according to the class-specific properties of the epidermis [7] by growing downward into the dermis to form a "hair-bud" (stage 2) [9]. The aggregate of dermal cells, or "prepapilla," then interacts by transmitting a second "specific dermal message" [8] to the adjacent epithelial cells. After the epithelial cells have surrounded the prepapilla cells to form a dermal papilla (stages 3a–c; 13.5–14 days), the cells of the "hair matrix" (the proliferating epithelial cell population surrounding the dermal papilla) differentiate to form the hair and the epithelial root sheaths of the follicle (stages 4–8; 14.5–17.5 days). It may be argued, by analogy with feather formation in chicken skin [10], that this interaction is also instructive in nature.

In this second half of our ultrastructural study of developing skin in vitro, part of which has been the subject of a preliminary report [11], attention will be focussed on the sites of known inductive tissue interaction, or possible interaction, in the follicle. First, in the standard medium, the lateral follicle wall, where the dermis is adjacent to the epithelium but is not thought to induce changes in it, will be compared with the hair matrix area, which is known to be interacting with the dermal papilla. Second, the lateral follicle walls in a vitamin A-enriched medium will be compared with those in standard medium. Areas of induced morphogenesis in the lateral walls will be compared with (a) areas of apparent stability in the lateral walls and (b) the hair matrix regions. These comparisons should enable us to answer three questions: (1) Does the basal lamina area morphology change during a normal tissue interaction? (2) Does the basal lamina area undergo similar changes at the sites of initiation of glandular morphogenesis in the presence of excess vitamin A? (3) Are the morphologic changes described above limited to the areas of (a) normal dermal induction and (b) vitamin A-induced morphogenesis, respectively? Positive answers to these questions would support the hypothesis that the action of vitamin A on the hair follicles resembles that of an embryonic inductor.

Although vitamin A and the retinoids continue to show great potential for the prevention and treatment of cancer [12,13], the mechanisms by which they affect differentiation remain unknown. Meanwhile, it would seem to be useful to explore any viable hypotheses about their mode of action.

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MATERIALS AND METHODS

Fetuses of known age were obtained from an inbred strain of Swiss albino mice (Experiments 1, 2) and the BALB/c strain (Experiments 3, 4). Recorded fetal ages were adjusted to conform to standard descriptions of external features at each age [14].

All observations were made on developing vibrissa follicles in skin dissected from the upper lip of 14-, 15-, and 17-day fetuses and newborn mice. Organ cultures were prepared from the skin of 6 litters of 14-day fetuses, using Maximow slides as described previously [15]. Each explant contained 1 or 2 rows of follicles. Experiments 1 and 2, in which 6 μg/ml of retinol was added to the standard biologic medium, also provided material for the study of epidermal changes produced by vitamin A, which has already been published [1]. In Experiments 3 and 4, retinyl acetate was used at doses of 4 μg/ml and 8 μg/ml, respectively. Control cultures in each experiment received the ethanol solvent only. (Retinol and retinyl acetate were known to give very similar results in this and other differentiating epithelial systems [16] (Hardy, unpublished observations).

Cultures were fixed after 1, 2, 3, 4, 5, 6, or 10 days. Many were fixed in Bouin's or Zenker's fixative for paraffin sections which were examined by light microscopy. The remainder were fixed in 3% glutaraldehyde in isotonic Sorensen's phosphate buffer at pH 7.2 with 0.5% sucrose, followed by OsO_4 in Millonig's phosphate buffer, 0.15 M, and embedded in Epon. In Experiment 1, a second set of tissues was fixed directly in the OsO_4 solution. Semithin sections (1–2 μm) were cut serially right through the blocks, and ultrathin sections from selected regions were stained with uranyl acetate followed by lead citrate. The latter was examined by transmission electron microscopy. All other methodologic details are indicated in earlier papers [1,17] and two theses [18,19].

RESULTS

General Description of Follicle Development

At the light microscopic level, the development of vibrissa follicles in vivo and in standard medium (Fig 1*a,b*) resembled that described previously [9,20]. Likewise, the normal development of skin explanted in a standard medium corresponded to previous descriptions [20]. The accounts of ultrastructure are based on follicles that appeared normal and healthy by light microscopy and would be expected to continue their normal differentiation in vivo or in vitro.

The skin explants in medium containing 6 μg/ml of retinol underwent various degrees of glandular morphogenesis of the follicles [2]. The main features of this morphogenesis at the light microscopic level, described elsewhere [3], were: (1) budding in apparently random locations from the lateral follicle walls after about 3 days of culture (Fig 1*c*), (2) extrusion of dermal papilla cells and rounding of the bases of many follicles by about 6 days, and (3) loss of dermal sheath, and of normal differentiation of follicular epithelial layers above the hair matrix by about 6 days. Growth, branching, and differentiation of the lateral follicle buds continued throughout the remainder of the 10-day period of culture. In many follicles a lumen formed in the original follicle downgrowth and in the branches. The descriptions of ultrastructure are based on these follicles, which show a lateral budding pattern of morphogenesis. Their dermal papillae, although appearing "normal" after 3 days in vitro, would be expected to disappear by 6 days.

At the lower dose of retinyl acetate administered in Experiments 3 and 4 (4 μg/ml) the changes were essentially similar to those indicated above for retinol. At 8 μg/ml of retinyl acetate, the typical follicle morphology was at first different. The dermal papilla cells were apparently extruded before 3 days, leaving a rounded epithelial follicle base. By about 3 days, the epithelial cells near the base had begun to rearrange themselves into parallel solid cords. By 4 days a lumen appeared in some of the cords (Fig 2), and later the cords developed into tubes which formed the lower branches of a gland. The upper part of the original follicle showed lateral budding and later a central lumen. Thus the final morphology of a branching gland was similar to that found in medium containing 6 μg/ml of retinol. The description of the ultrastructure of these follicles, which undergo a more rapid remodelling in 8 μg/ml of retinyl acetate, is limited to the first day in vitro.

The 14-day skin contained rows of vibrissa follicles ranging from stage 2 to stage 3c [9], and in any one explant there was always a range of developmental stages. The following descriptions, except where otherwise indicated, refer to the more advanced follicles of each explant.

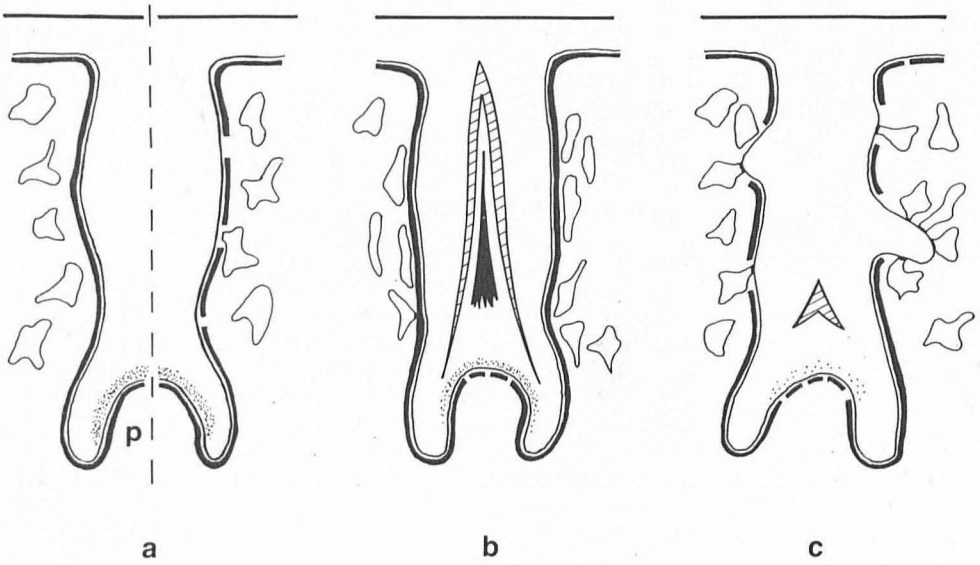


FIG 1. Schematic diagrams of developing vibrissa follicles. *a*, A typical follicle at stage 3c [9,20]. The deep dermal papilla (*p*) is surrounded by the (epithelial) hair matrix (*stippled*). After 1 day in vitro in standard medium the basal lamina (*thick line*) remained continuous around the lateral follicle wall and the hair matrix, as shown on the *left half* of the sketch. After 1 day in vitamin A-enriched medium, gaps appeared in the basal lamina, randomly distributed along the lateral follicle wall, as shown on the *right side* of the sketch. *b*, A follicle similar to that shown in *a*, which has reached stage 6 after 3 days in standard medium. Note the keratinized hair (*black*) and the hardened Henle's layer of the inner root sheath (*hatched*). The dermal cells are no longer randomly oriented, but some have aligned with the lateral walls to form a dermal sheath. The basal lamina is continuous, except at the apex of the dermal papilla. *c*, A follicle similar to that shown in *a*, after 3 days in vitamin A medium. There has been limited differentiation of cells arising from the hair matrix, and only a small hardened Henle's layer of the inner root sheath (i.e., early stage 4). Buds are extending from the lateral follicle walls through gaps in the basal lamina, and the exposed epithelial cells are contacted by the (now unaligned) dermal cells.

Basal Lamina Regions in 14-Day Fetuses (Stage 3c Follicles)

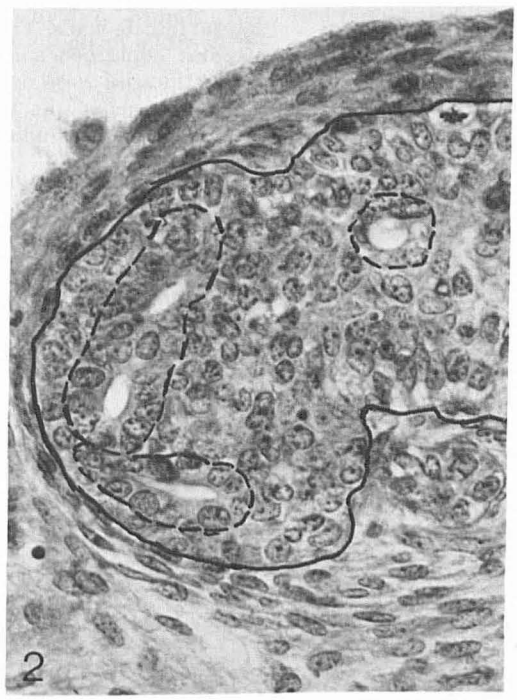
The basal lamina was continuous around the lateral *follicle walls* (compare Fig 1*a*). The beginnings of hemidesmosomes were indicated by attachment plaques (Fig 3). Beyond the basal lamina, a small amount of extracellular material was occasionally found. This was either amorphous or consisted of randomly oriented microfibrils 3–6 nm in diameter.

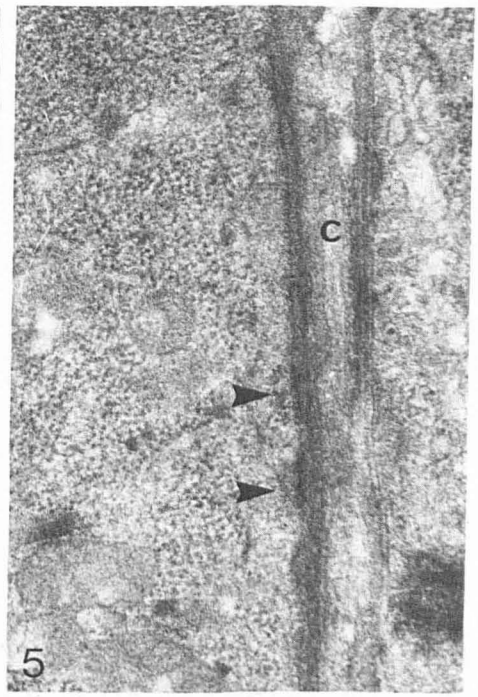
The more advanced follicles (stage 3c) showed a continuous basal lamina below the *hair matrix* (compare Fig 1*a*). It was, however, thin and of more variable density and thickness in comparison with that of the lateral follicle walls (Fig 4). No indications of hemidesmosome formation were seen. Extracellular material was much less conspicuous than in the follicle wall region.

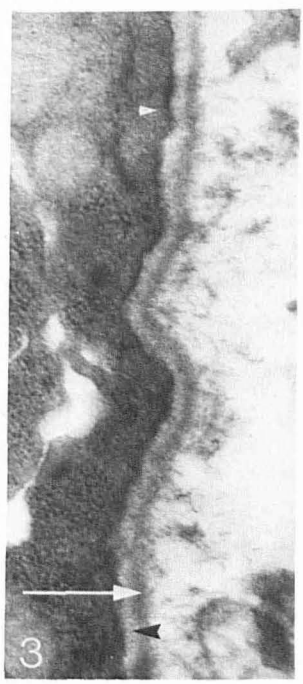
Basal Lamina Regions in 17-Day Fetuses (Stage 6)

The basal lamina of the *follicle walls* was continuous, and more "mature" than that in 14-day fetuses, having many well-developed hemidesmosomes. In the region of the future dermal root sheath, the mesenchymal cells were tightly packed around the follicle (Fig 1*b*), with their processes occasionally touching the lateral margin of the lamina densa. Where there was a space between the basal lamina and the surrounding fibroblasts, it was usually filled with extracellular material including unbanded collagen fibrils of about 20 nm diameter (Fig 5) as well as amorphous material.

Instead of appearing as a smooth line in the light microscope, the line of junction of the dermal papilla with the *hair matrix* was very irregular. This was perhaps due to the constant movement of the hair matrix cells undergoing mitosis. In the electron microscope the basal lamina of many regions, especially in the lower half of the dermal papilla, was continuous, and the lamina densa was more uniformly thick and dense than in the 14-day dermal papillae. However, over the upper one-







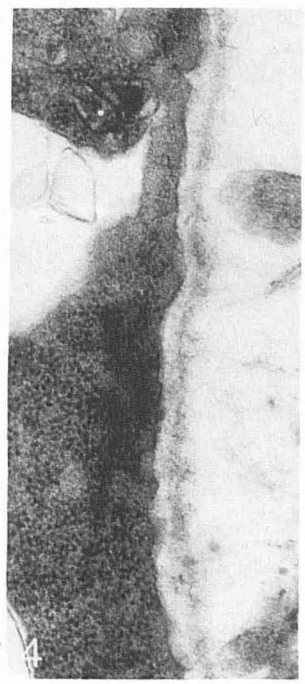


FIG 2. Transverse section near the base of a modified follicle from 14-day skin after 4 days in vitro with 8 μg/ml of retinyl acetate. The former follicle shows a very irregular epithelial boundary (*continuous ink line*). The dermal papilla has disappeared, and parallel cords of cells (*dashed outlines*) within the epithelial base of the follicle have begun to form lumina. Paraffin section, Mallory trichrome stain. × 470.

FIG 3. Right lateral margin of a follicle from 14-day skin, showing continuous lamina densa (*white arrow*) and lamina lucida (*black arrowhead*) with adjacent dermal matrix material. An early hemidesmosome is indicated by an attachment plaque (*white arrowhead*). × 37,000.

FIG 4. Hair matrix cells (*left*) next to the dermal papilla (*right*) from 14-day skin. There is a continuous basal lamina, with a less dense lamina densa than that in Fig 2, and sparse dermal matrix material. × 39,000.

FIG 5. Lateral wall of follicle from 17-day skin. There is a continuous basal lamina with frequent hemidesmosomes (*arrowheads*). Unbanded collagen fibrils (*C*) are tightly packed between the basal lamina and the mesenchymal cells. × 23,000.

FIG 6. Hair matrix cell (*upper right*) from 17-day skin, showing irregularities in the basal lamina. At one point (*arrow*), a process of a dermal papilla cell appears to penetrate the lamina densa and approach the plasma membrane of the hair matrix cell. No collagen fibrils are seen, and the dermal extracellular material is sparse. × 37,000.

quarter to one-half of the dermal papilla, segments of continuous lamina densa were interspersed with segments where the lamina densa was replaced by broad masses of less electron dense material, or much thinner lamina densa, or there was no dense material at all. Careful comparison of the images of plasma membranes of the hair matrix cells and dermal papilla cells as well as other structures in the vicinity convinced the authors that the images described above could not be dismissed as oblique sections of basal lamina. The latter were, of course, observed, along with oblique sections of adjacent plasma membranes of hair matrix cells. Dermal papilla cell processes, which frequently touched the lamina densa where it was continuous, came closer to the hair matrix cell plasma membranes in the areas of defective basal lamina (Fig 6), and occasionally appeared to touch that plasma membrane. Hemidesmosomes were not observed at any part of the hair matrix boundary, which may thus be described as "immature." The extracellular material on the papilla side of the lamina densa was scanty, and either amorphous or in the form of scattered microfibrils.

Basal Lamina Regions in Newborn Mice (20 Days, Stage 8)

The *follicle walls* were similar to those in 17-day mice, but showed an increase in the number and thickness of collagen fibrils. The *hair matrix* region also was similar to that in 17-day embryos, and there was no marked increase in electron-dense extracellular material in that area.

Effects of 1 and 2 Days in Standard Medium (Stages 4, 5)

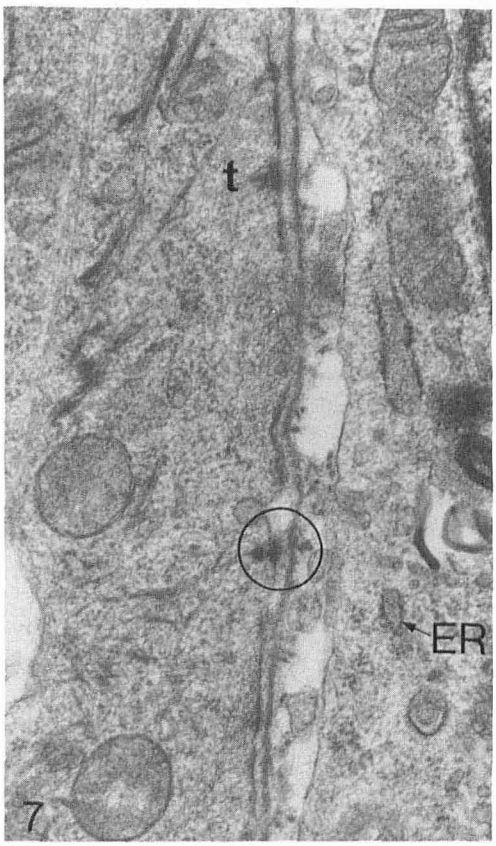
As in the 14-day embryos at the time of explantation, the basal lamina was continuous around the lateral *follicle walls*, but after 1 day in vitro it had a more mature appearance (Fig 7). The lamina densa was of a rather uniform thickness, about 30 nm, while the lamina lucida was of more variable thickness, usually 30–40 nm. Hemidesmosomes were more numerous than at 14 days, and showed tonofilaments extending from the attachment plaques into the cytoplasm of the outer root sheath cells. After 2 days in vitro, there was an increase in the number of hemidesmosomes and there were fine filaments crossing the lamina lucida from the attachment plaques. The associated peripheral densities in the lamina densa were very distinct. After 1 and 2 days in vitro, many fine processes from mesenchymal cells of the dermal root sheath were seen close to the basal lamina, some being only 30 nm from its lateral margin. Occasionally a cell process appeared to touch the lamina densa but did not penetrate it. Extracellular material was not always identified in this region after 1 day, but after 2 days, both amorphous material and unbanded fibrils of about 20 nm diameter were frequently seen (Fig 8).

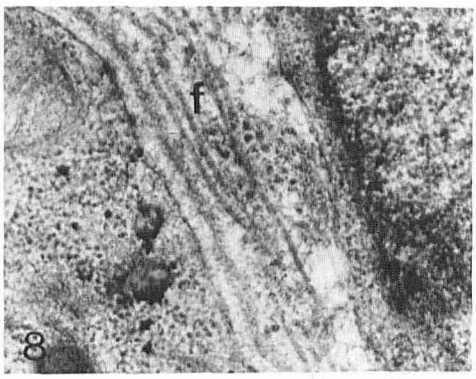
In the light microscope, the dermal papilla outline was irregular. Consequently the lamina densa seen in the electron microscope was not so closely applied to the *hair matrix* cells in all areas as it was to the epithelial cells of the lateral follicle walls. In comparison with those of the lateral walls of the same follicles, the basal laminae surrounding dermal papillae remained "immature," as in Fig 4. Hemidesmosomes were not seen, and the lamina densa was usually thinner than on the lateral follicle walls. The lamina densa surrounding the dermal papillae contained some wider, pale fuzzy areas, but actual discontinuities were not observed. Processes of dermal papilla cells approached the lamina densa but did not penetrate it. Very little extracellular material was observed.

Effects of 3 Days in Standard Medium (Stage 6)

The basal lamina of the lateral *follicle walls* resembled that seen after 2 days in that it was continuous (Fig 1*b*) and relatively mature, with numerous hemidesmosomes. The dermal cells were aligned with the lateral follicle walls (Fig 1*b*). Contacts of mesenchymal cell processes with the basal lamina were frequent, and the amount of extracellular amorphous and fibrillar material was increased in some parts of the follicle shaft (Fig 9).

The lamina densa of the *hair matrix* appeared to be thinner than that of the lateral follicle wall. It was continuous for the most part, but, as at earlier stages, it was frequently widely





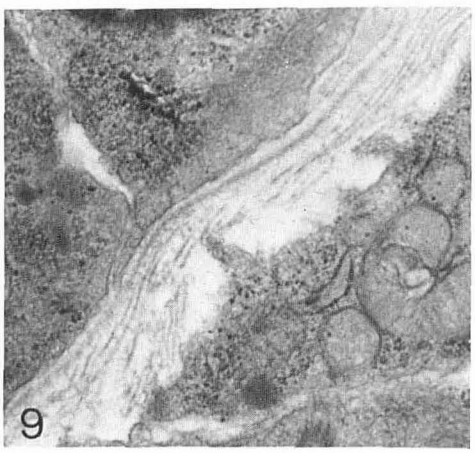


FIG 7. Lateral wall of follicle from 14-day skin after 1 day in standard medium. Several hemidesmosomes are seen. The epidermal cells are distinguished by a high density of free ribosomes and the presence of tonofilaments (*t*), some of which terminate on attachment plaques. Some hemidesmosomes (*circle*) show an increased density of the lamina lucida which is due to anchoring filaments. The fibroblast has some rough endoplasmic reticulum (*ER*) and fewer free ribosomes. × 20,000.

FIG 8. Lateral wall of follicle from 14-day skin after 2 days in standard medium. The basal lamina is continuous, and the lamina densa width is about 30–35 nm. Fibrils (*f*) of 20–25 nm diameter, running mainly in the plane of the image, or normal to that plane, are seen in the adjacent dermal matrix. × 40,000.

FIG 9. Lateral wall of follicle after 3 days in standard medium. The basal lamina is continuous, over 40 nm thick, and surrounded by extracellular matrix fibrils of approximately 20 nm thickness. × 20,000.

separated from the surface of an individual hair matrix cell. In the latter situation, formation of a second basal lamina next to the plasma membrane was sometimes observed. Hemidesmosomes were not apparent. Discontinuities of lamina densa were sometimes found, with contacts between mesenchymal cell processes and hair matrix cells through these gaps (Figs 1*b*, 10). (In this and other cases of epithelial–mesenchymal contacts in this study, the identification of cells and processes as epithelial or mesenchymal was confirmed by differences in density of cytoplasm and ribosomes, in frequency of rough endoplasmic reticulum and Golgi apparatus, and in mitochondrial morphology and lateral cell wall association.) Extracellular material at this peripheral region of the dermal papilla was insignificant or absent.

Effects of 6 Days in Standard Medium (Stages 7, 8)

The lamina densa of the *follicle walls* was always regular and continuous. In the region of the thick dermal root sheath it was surrounded by collagen fibrils (Fig 11) or by 3- to 6-nm microfibrils. Some 40-nm collagen fibrils were found in addition to the 20-nm ones.

The lamina densa was absent from very large areas of the *hair matrix*. Through these gaps there were numerous direct contacts between dermal papilla cells and hair matrix cells (Fig 12). Extracellular material was minimal, and no fibrils were seen.

Effects of 10 Days in Standard Medium (Stage 8)

No further changes were observed at the lateral *follicle walls* after 10 days, except that collagen fibers were predominantly of 60 nm diameter.

The *hair matrix* margin was similar to that seen at 6 days.

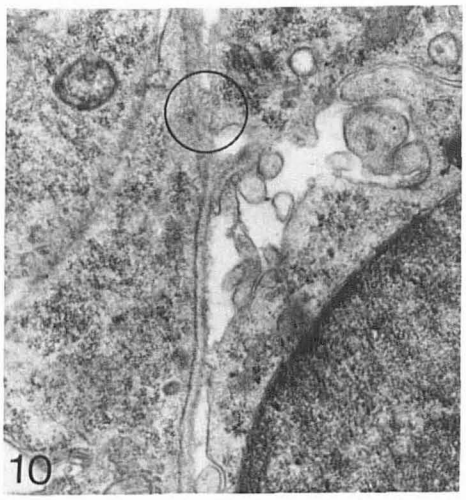
Effects of 1 Day in Vitamin A Medium (Stages 3c, 4)

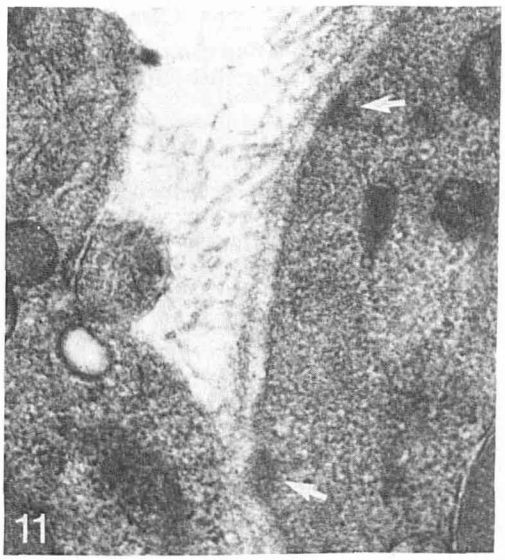
Whereas 1 day in standard medium resulted in some maturation of the basal lamina of *follicle walls*, 1 day in vitamin A medium resulted in no further maturation, but rather, in widespread damage. In many areas the lamina densa was ballooning, folding, or stripping off, and at the lower doses there were occasional gaps (Fig 1*a*). At the 8 μg/ml dose of retinyl acetate, large areas of lamina densa were missing. At all dose levels the hemidesmosomes, if present, were less frequent and less developed than in follicles grown in standard medium. Sometimes a fine feltwork of microfibrillar material was found in the position usually occupied by lamina lucida and lamina densa. The processes of mesenchymal cells of the dermal root sheath approached the basal lamina region, and occasionally a process passed through a gap in the lamina densa to make contact with the plasma membrane of an outer root sheath cell.

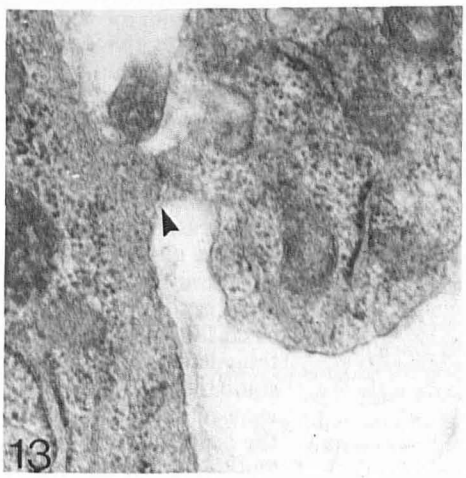
The *hair matrix* region resembled that in explants grown in standard medium, except that in one explant, 2 dermal papillae had large areas where the lamina densa had disappeared.

Effects of 2 Days in Vitamin A Medium (Stages 4, 5, and Modified Follicles)

The basal lamina of *follicle walls*, where present, was still of the immature type, with only a few early hemidesmosomes, and there were fewer attached tonofilaments than in standard medium. The lamina densa was missing from large areas. Many processes of outer root sheath cells projected through gaps in the basal lamina, and some made direct contact with the dermal sheath mesenchymal cells through these gaps (Fig 13). Amorphous material and fibrillar material were sometimes seen lateral to the intact basal lamina, as it was in the explants grown in standard medium.







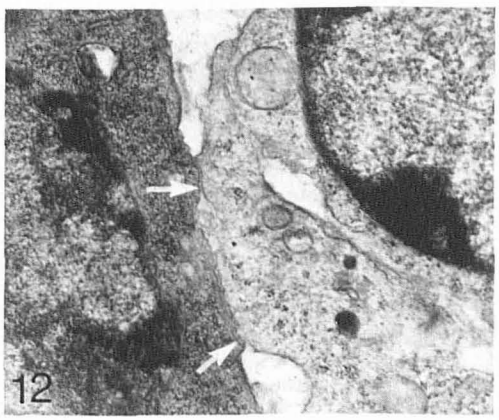


FIG 10. Basement membrane of follicle hair matrix after 3 days in standard medium shows discontinuity, with apparent contact (*circle*) between hair matrix cell and mesenchymal cell process through the gap. Very little extracellular matrix material is seen. There are no hemidesmosomes. × 20,000.

FIG 11. Lateral wall of follicle after 5 days in standard medium, showing a continuous basal lamina and mature hemidesmosomes (*arrows*). The extracellular material consists mainly of collagen fibrils. × 38,000.

FIG 12. Portion of the junction of hair matrix and dermal papilla of a follicle after 6 days in standard medium, with only a small fragment of the thin lamina densa remaining. One of the many areas of close contact between the plasma membranes of a dermal papilla cell (*right*) and a hair matrix cell (*left*) is shown (*between arrows*). The distance between cell membranes appears to be less than 10 nm. × 18,000.

FIG 13. Lateral wall of follicle after 2 days in vitamin A medium (6 μg/ml retinol), showing a lamina densa of reduced density and variable thickness. Through a small gap in the basal lamina there is contact (*arrowhead*) between the epithelial cell (*left*) and a mesenchymal cell. × 39,000.

The *hair matrix* area resembled the corresponding area after 1 day in vitamin A medium, in that some papillae had large areas where the lamina densa had disappeared.

Effects of 3 Days in Vitamin A Medium (Stages 6, 7, and Modified Follicles)

At this stage many lateral buds from the *follicle walls* were recognized. A semithin section of a bud contained 1–20 cells extending from a follicle wall (Fig 14). The basal lamina was absent from the surface of most of each bud, but continuous in the areas between the buds (Figs 1*c*, 14–16). At the surface of the buds were extensive areas of close contact between the epithelial and mesenchymal cell bodies and processes, where the distance between the two plasma membranes was approximately 12–15 nm (Figs 15, 16). Here and there focal close junctions were seen.

Elsewhere the lateral follicle walls usually had a continuous basal lamina surrounded by dermal fibroblasts and their processes. Unlike the follicle walls after 3 days in the standard medium, these areas had very few hemidesmosomes. Collagen fibrils were sometimes present, but were not nearly as numerous as in the control explants after 3 days. The rough endoplasmic reticulum of the fibroblasts in this area was more frequently dilated than in control explants. Occasionally an area that was as yet without lateral budding was found to have a discontinuity in the basal lamina and some contacts between fibroblast processes and outer root sheath epithelial cells.

The basal lamina of the *hair matrix* showed more frequent and more extensive gaps in the vitamin A-treated explants than in the control explants (Fig 1*c*). Direct contacts through these gaps were also frequent. In other respects, the dermal papilla margin resembled that found in the standard medium.

Effects of 6 Days in Vitamin A Medium

Most of the follicles had completely transformed into glands at this time, so that dermal papillae no longer existed. The *lateral walls* of the rapidly growing glands frequently lacked a basal lamina, or else had intermittent segments of basal lamina.

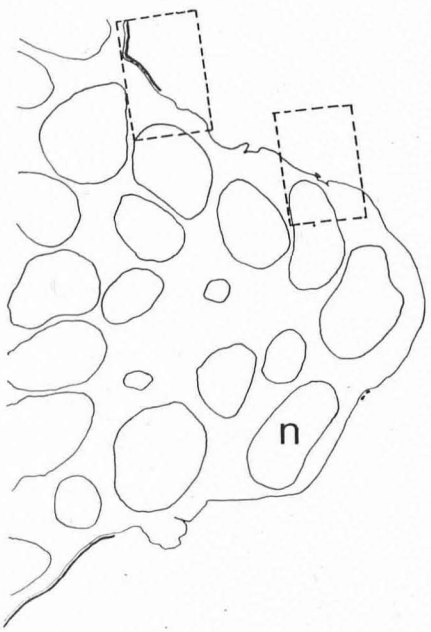


FIG 14. Outline of part of a longitudinal section of a vibrissa follicle from a 14-day embryo after 3 days in vitamin A medium (6 μg/ml retinol), showing a large lateral bud. The outline was traced from a montage of 6 electron micrographs. The *fine line* indicates the plasma membrane of the marginal epithelial cells and the *heavy line* indicates the presence of a basal lamina. The lamina is continuous at the margins of the bud, but only two fragments are seen on the bud itself. The thickness of the lines is exaggerated. (The approximate boundaries of Figs 15 and 16 are indicated by *boxes*, the one *to the left* representing Fig 15).

Effects of 10 Days in Vitamin A Medium

All the follicles were transformed into glands, and dermal papillae were not seen. By this time, a basal lamina had reformed on the surface of most of the glands (Fig 17). This was frequently of a rather immature appearance, being of irregular thickness and variable density, with some early hemidesmosomes. There was some amorphous and microfibrillar extracellular material surrounding the basal lamina in many regions, and collagen fibrils were sometimes recognized (Fig 18).

DISCUSSION

Changes in Ultrastructure of Basal Lamina Regions in Relation to Normal Interactions

In fetal skin at 14 days the basal lamina of lateral follicle walls was intact and beginning to form hemidesmosomes. At 17 and 20 days there was progressive maturation in the area with respect to number and completeness of hemidesmosomes and the orderly deposition of collagen fibrils by the closely packed dermal cells. Breaks in the lamina densa were never seen. The corresponding regions of follicles in vitro were very similar. These findings are consistent with the development of a barrier function usually attributed to basement membranes in such areas [21].

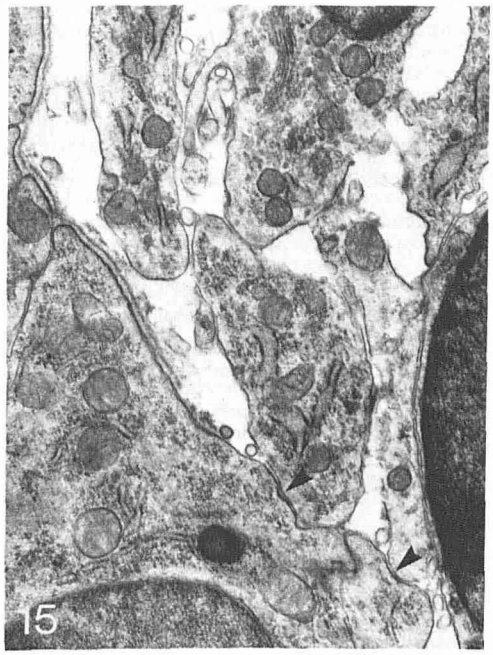
In the hair matrix area at 14 days in vivo, the basal lamina was intact but less mature than that of the lateral follicle wall. The lamina densa of the hair matrix did not mature at 17 or 20 days, but instead began to look irregular and less dense. Mesenchymal cell processes appeared at least to penetrate the lamina densa, and in vitro after 3 and 6 days they progressively invaded the basal lamina region to contact the plasma membranes of the hair matrix cells. Hemidesmosomes were not developed, and extracellular matrix material did not accumulate. Similar situations have been reported in other developing organs such as lung, tooth germ, and salivary gland, at the sites and at the time of known tissue interactions [10,22,23]. Hair follicles may therefore be added to the growing list of organs that may require a temporary heterotypic cell contact for full differentiated expression.

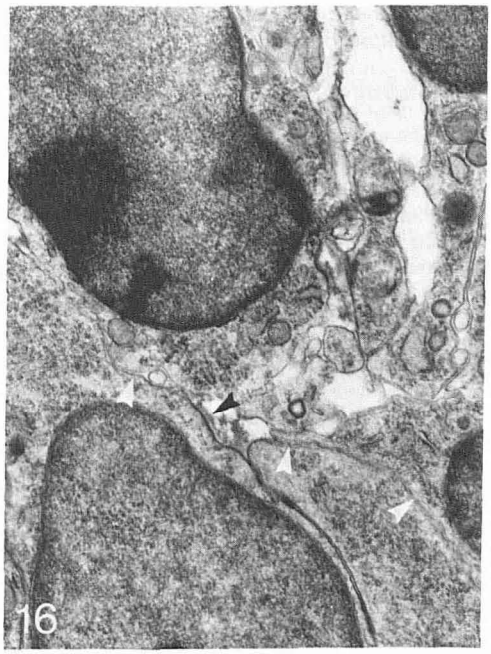
The answer to the first question posed earlier is therefore that the basal lamina area does change its morphology during a known tissue interaction. The change is one that could facilitate opportunities for exchange of material or information between two different cells types.

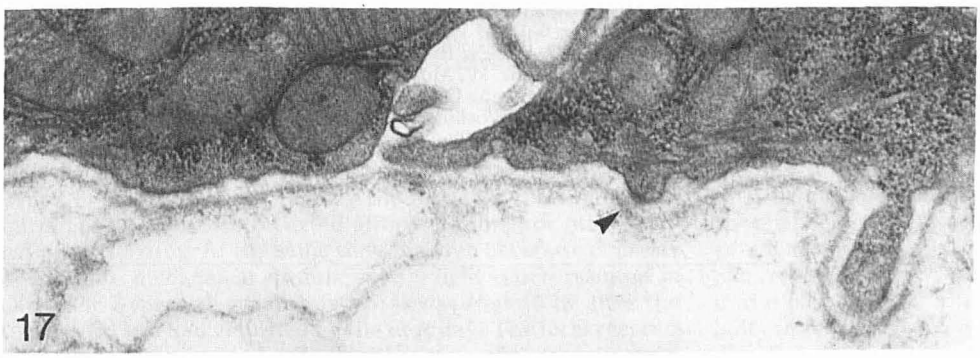
Changes in Ultrastructure of Lateral Follicle Walls in Vitamin A Medium

The sequence of events in lateral follicle walls was very similar to that previously described for the epidermis [1], namely, gaps in the basal lamina, cell contacts, then changes in the behavior of the epithelial cells. In the lateral walls the change was the extension of the epithelial cells through the gaps to form buds which eventually differentiated mucus-secreting cells, rather than an immediate redifferentiation. The sequence of changes was also somewhat similar to the sequence in the dermal papilla area in standard medium, where the final event was normal differentiation of the hair matrix cells. Thus the second question posed earlier is answered by saying that the ultrastructural changes produced by vitamin A were similar to those at the site of a known normal induction by the dermal papilla in a standard medium.

Except at the higher dose of retinyl acetate, much of the basal laminas of lateral walls of follicles remained intact, although these structures did not mature as rapidly as in the standard medium. Individual epithelial cells and groups of cells were not seen to have moved laterally except where a gap in the basal lamina was observed. This focal nature of the lateral budding enables a partial answer to be given to the third







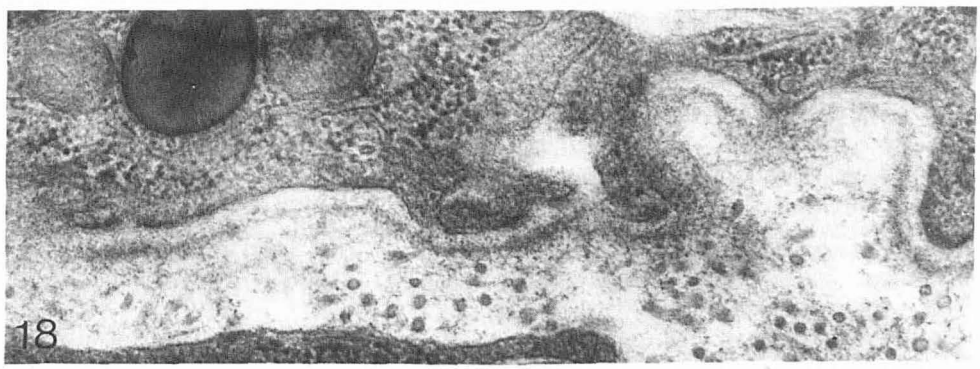


FIG 15. Lateral wall of follicle after 3 days in vitamin A medium (6 μg/ml retinol), at the margin of a large bud growing from the side of the follicle (Fig 14). In the *upper left* of the figure is the continuous basal lamina which surrounds the nonbudding part of the lateral follicle wall (*lower left*). In the *lower right* is the base of the growing bud, where the basal lamina is absent and the epithelial cells make close contacts (12–15 nm, *arrowheads*) with two portions of dermal cells. Scattered focal close junctions were seen in this area at high magnifications. Note the dilated rough endoplasmic reticulum, smaller mitochondria, and looser contacts which distinguish the dermal cells. × 15,000.

FIG 16. Area near the apex of the same bud as in Fig 15 (see Fig 14). *White arrowheads* indicate the junction of epithelium (*below, left*) with dermis (*above, right*). There are many extensive areas of close contact, and no basal lamina except for one fragment (*black arrowhead*). Only the dermal cells show rough endoplasmic reticulum. × 15,000.

FIG 17. Basal lamina at margin of metaplastic gland derived from follicle, after 10 days in vitro. The reconstituted lamina densa is 25–35 nm thick, and an early hemidesmosome (*arrowhead*) is seen. × 35,000.

FIG 18. Basal lamina region of metaplastic gland derived from follicle, after 10 days in vitro. In this region collagen fibrils of varying thickness are found. × 53,000.

question posed earlier. It can be said that, in follicles, either in vivo or in standard medium, gaps in the basal lamina and heterotypic cell contacts occur only at the site of known induction (i.e., the dermal papilla), and that in vitamin A medium, gaps and contacts occur only at sites of either (a) known induction, or (b) presumed interaction leading to glandular morphogenesis and mucous metaplasia.

It is reasonable to think that the larger areas of basal lamina defect in the follicles exposed to 8 μg/ml of retinyl acetate would permit the nonfocal alteration in morphogenesis which was described at the light microscopic level in the third paragraph of Results.

Changes in Ultrastructure of Dermal Papilla Areas in Vitamin A Medium

The changes in dermal papilla areas exposed to excess vitamin A were similar to those in standard medium, except that they were sometimes more marked. This is, however, less relevant to the present discussion because most of the dermal papillae observed after 3 days in vitro would have been everted by 6 days in vitro, and there is no evidence that the normal tissue interaction would have taken place.

Conclusion

The above findings support, but by no means prove, the hypothesis that vitamin A acts on the developing follicles in a manner resembling that of an embryonic inducer. This suggests that the genome of the responding cells may be affected, in a way that so far appears irreversible [3]. Whether the vitamin A is itself an inducer molecule, a releaser of enzymes that attack the basal lamina, or a modifier of dermal tissue, is a question being explored by more direct experimental methods.

The question of the mode of action of vitamin A on the

epidermis remains open, since in that location it has not been possible to establish a point-by-point correspondence between basal lamina breakdown and altered differentiation [1]. The role of the vitamin on the epidermis may yet be shown to be a modulating one.

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Involvement of Sensory Nerve Endings in Cold and Heat Urticaria

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The involvement of sensory nerve endings in acquired cold and heat urticaria was studied in 8 patients. After repeated topical application of capsaicin, the skin was tested for whealing with cold and heat stimuli. Capsaicin pretreatment prevented the urticarial responses for 4–7 days. Since capsaicin is known to induce selective impairment of the chemosensitive nerve endings, the results suggest that these nerve fibers may play an important role in acquired cold and heat urticaria. The possible mechanisms are discussed.

An urticarial response is characterized by slightly erythematous wheals surrounded by a flare. Antidromic electric stimulation of the sensory nerves [1–3] and direct stimulation of the chemosensitive pain receptors by certain chemical agents [4,5] have been shown to induce cutaneous vasodilatation and local edema (neurogenic inflammation), a response closely resembling the urticarial reaction. In spite of this, the involvement of neuronal mechanisms in the development of urtica has not yet been considered. The release of various vasoactive substances, primarily histamine [6], is regarded as the immediate cause of whealing [7,8]. The aim of our experiments was to study the role of the neurogenic factors in this process. Treatment with capsaicin (trans-8-methyl-N-vanillyl-6-noneamide), known to induce selective and reversible functional impairment of the chemosensitive nerve endings [4,9], appeared a promising tool for study of this problem. Therefore, capsaicin was applied topically to patients with acquired cold and heat urticaria, and the treated skin area was challenged with thermal stimuli for whealing.

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METHODS

Eight patients (4 male, 4 female) with acquired cold or heat urticaria or both of 6 months' to 9 years' duration were studied. All of them gave informed consent. Their ages ranged from 23 to 52 years. Medication