

FINE STRUCTURE OF DESMOSOMES, HEMIDESMOSOMES, AND AN ADEPIDERMAL GLOBULAR LAYER IN DEVELOPING NEWT EPIDERMIS

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ABSTRACT

The skin of late embryonic, larval, and young postmetamorphic newts, *Taricha torosa*, has been examined with particular reference to areas of cellular attachment. Stereo electron microscopic techniques and special staining methods for extracellular materials were utilized in addition to conventional avenues of ultrastructural study to investigate the fine architecture of desmosomes, hemidesmosomes, their associated filament systems, and extracellular materials. No evidence has been found that continuity of tonofilaments between adjacent cells exists at desmosomes. Rather, most of the tonofilaments which approach desmosomes (and perhaps also hemidesmosomes) course toward the "attachment plaque" and then loop, either outside the plaque or within it, and return into the main filament tracts of the cell. These facts suggest that the filamentous framework provides intracellular tensile support while adhesion is a product of extracellular materials which accumulate at attachment sites. Evidence is presented that the extracellular material is arranged as pillars or partitions which are continuous with or layered upon the outer unit cell membrane leaflets and adjoined in a discontinuous dense midline of the desmosome. A similar analysis has been made of extracellular materials associated with hemidesmosomes along the basal surface of epidermal cells. An adepidermal globular zone, separating the basal cell boundary from the underlying basal lamina and collagenous lamellae during larval stages, has been interpreted from enzyme and solvent extraction study as a lipid-mucopolysaccharide complex, the function of which remains obscure. These observations are discussed in relation to prevailing theories of cellular adhesion and epidermal differentiation. They appear consistent with the concept that a wide range of adhesive specializations exists in nature, and that the more highly organized of these, such as large desmosomes and hemidesmosomes, serve as strong, highly supported attachment sites, supplemental in function to a more generalized aggregating mechanism.

INTRODUCTION

Mechanisms by which cells are ordered into cohesive tissue systems have been the objects of

This report is dedicated to Professor Victor C. Twitty on the occasion of his approaching retirement from The Department of Biological Sciences, Stanford University. The author is deeply grateful for the opportunity of association with Professor Twitty,

long historical interest. Embryologists have employed such terms as contact guidance (Weiss, 1958), contact inhibition (Abercrombie, 1961), and tissue affinity and disaffinity (Holtfreter, 1939) to denote the role of adhesion, contact, or

and the benefit of his insight and warm encouragement.

lack of these, in the behavior of developing cell systems and the ultimate organization of the embryo. Recent experiments demonstrating specific histotypic reaggregate capabilities for dissociated cells (Moscona, 1960) serve to emphasize the recognitive adhesive potentials which many cells possess. After an analysis of the patterns of such selective adhesions in various differentiating systems, Steinberg (1964) has suggested that the requisite selectivity is best explained on the basis of a general adhesive principle common to all or most cell surfaces. According to this view, the relative abundance or lack of equivalent, weakly adhesive sites available in a given cell population at a given time can account for the subtle differentials in cell aggregative behavior.

Identification of such general adhesive properties with discrete morphological units in or on cell membranes is, however, still problematical. In routine electron micrographs, for example, one is unable to detect structure within the electron-lucent zone separating two cells of an epithelium, or in the equally lucent zone separating the same cells from a dense underlying basal lamina (basement membrane).¹ Yet, from other evidence, it is apparent that a trypsin-sensitive, calcium-dependent, and occasionally stainable (see Luft, 1964) cementing material probably occupies such spaces.

Other apparent adhesive components are readily visible at the ultrastructural level, and some of these display complex morphology. They include tight junctions and intermediate junctions (components of terminal bars [Farquhar and Palade, 1963, 1964, 1965]), desmosomes, hemidesmosomes (originally termed "bobbins" [Weiss and Ferris, 1954]), extended areas of membrane fusion termed "nexuses" (Dewey and

¹In this discussion the term basal (or basement) lamina will be applied to denote the finely filamentous layer seen, in electron micrographs, separating epithelia, endothelia, nerve, muscle, and other tissues from connective tissue compartments (Fawcett, 1963). The term basement membrane, commonly used by others in this context, is primarily of light microscopic origin and, in the epidermal system under study, was originally used to denote the basal lamina plus underlying layers of collagen fibers as a unit. Salpeter and Singer (1960) utilized the term "adeipidermal membrane" to denote basal lamina in epidermal systems, but this term shares with "basement membrane" the possible confusion with the unit membrane concept.

Barr, 1964), the "septate desmosomes" in epithelia of invertebrates (Wood, 1959; Locke, 1965), and synaptic contacts between neuronal, sensory, and muscular components. Such junctions have been considered as sites of heightened cell-to-cell permeability or impulse transmission, but the adhesive tenacity of many such sites, as deduced from their persistent attachment during microdissection (Chambers and de Rényi, 1925 [see discussion by Odland, 1958]) or centrifugal disruption (see e.g., the experiments on synapses by De Robertis et al., 1962), implies strong adhesive-supportive properties as well. Steinberg (1964, p. 362) has considered desmosomes and terminal bar components as structural bonding mechanisms, perhaps supplementary to the less visible and weaker, general adhesive mechanism which allegedly confers reaggregate potential. Overton (1962) described the re-establishment of desmosomes as subsequent to the reaggregation of dissociated chick blastoderm cells and noted a correlation in increasing cell-to-cell adhesivity with that re-establishment. Whether or not the stronger attachment sites play any role in selective, aggregative adhesion, it appears probable that the quality, number, and distribution of firm attachment mechanisms at the boundaries of a differentiated cell are related to the proper maintenance of position, morphology, and function of that cell.

The present study represents a re-examination of the ultrastructural morphology of attachment areas within a developing system which displays a rather wide variety of possible adhesive mechanisms. Several technical innovations allow a more refined visualization of fine structure within the attachment sites and closely related extracellular compartments.

MATERIALS AND METHODS

Developing skin utilized in this study was obtained from embryonic, larval, and newly metamorphosed west coast newts (*Taricha torosa*, originating from ponds near Stanford, California). In most cases the skin of the dorsal tailfin was studied, but samples of skin from trunk, head, and gill were utilized as well. Animals were anesthetized with MS-222 (Tricaine Methanesulfonate [Sandoz Pharmaceuticals]) prior to removal of body regions for fixation. Some tissue blocks were fixed for 1 to 2 hrs, with ice-cold 3.75% osmium tetroxide in 0.05 M *s*-collidine buffer (Bennett and Luft, 1959) before dehydration in an ethanol series and embedding in Epon 812 (Luft, 1961). Other blocks were fixed in a solution of

0.5% glutaraldehyde or 0.25% acrolein plus 0.25% glutaraldehyde in a half-strength Niu-Twitty solution (Niu and Twitty, 1953), followed by postfixation in osmium tetroxide utilizing the same solvent system. These aldehyde fixation media had a pH of 7.3–7.5 and total milliosmolarity of 112–132. Permanganate fixation was also used in a few cases for purposes of comparison. For one series of fixations the dye, ruthenium red, was incorporated into the collidine-osmium tetroxide mixture. This method serves to demonstrate acid mucopolysaccharides (particularly extracellular deposits) in thin sections (Luft, 1964, and unpublished data).

To facilitate ultrastructural analysis of extracellular components lying immediately beneath larval tailfin epidermis, a method was devised for stripping off the epidermis, exposing these components without apparent damage. This method consists of the application of small strips of lens paper to one side of tailfins which have been exposed to air just long enough for water to recede from their surfaces. At this time a moist surface mucous coat adheres to the paper. After further drying, removal of the paper with fine forceps produces a denuded area which may be treated with various agents before fixation.

Agents applied to such denuded areas in the present study include: a 0.5% mixture of alpha- and beta-amylase for 10 min; 0.5% Pronase for 10 min; 0.13% collagenase (grade B) for 5 min; pure chloroform, which volatilizes within a few sec.; pancreatic and wheat-germ-derived lipases applied in 0.1 and 2.0% concentrations for 5 and 10 min, respectively; 0.1 and 2.0% hyaluronidase also applied for 5 and 10 min, respectively; and 20% sodium *N*-acetyl cysteine (a nonenzymatic mucolytic agent [Mead, Johnson and Co.]) for 5 min. The above enzyme solutions were made with pond or distilled water and applied at or near neutral pH. Fixation followed these treatments immediately with no intervening rinses.

Tissue blocks were sectioned on a Porter-Blum MT2 ultramicrotome using diamond knives. Thin sections were mounted on carbon-coated grids and stained with half-saturated aqueous uranyl acetate, 8 to 10 min at 55°C, followed by 8 to 10 min treatment with the alkaline lead citrate procedure of Reynolds (1963). Thick (1 to 2 μ) sections were stained with methylene blue-azure II (Richardson et al., 1960) and examined and photographed by light microscopy. Electron micrographs of thin sections were taken on a Siemens Elmiskop I operating at 60 kv with voltage alignment and compensated lateral and rotational chromatic aberrations (Luft, 1963). Stereo electron micrographs were taken using a 10° specimen tilt difference. The resulting stereo pairs were analyzed by: (1) a direct "cross-eyed"

viewing technique (*see* caption under Figs. 8 and 9) which forms a three-dimensional image for some investigators; (2) a first-surface double mirror Wheatstone-type stereoviewer; or (3) a polarized light projection system for stereo lantern slide pairs.

OBSERVATIONS

General Epidermal Organization

At least five basic cell types can be distinguished over much of the epidermis in larvae of this newt species. Apical and basal filamentous epidermal cells provide the major structural framework for the system (*A* and *B*, Fig. 1; Fig. 2). They are characterized by the ubiquitous large (100 to 120 A diameter) tonofilaments which pervade their peripheral cytoplasm. The basal cells overlie basal lamina and collagenous lamellae which comprise the light microscopist's basement membrane (Weiss and Ferris, 1954; Porter, 1954; Salpeter and Singer, 1960). Apical filamentous cells constitute the outer cellular surface of larval epidermis. They display a vesicular mucinogenic free border and are occasionally ciliated. The cytoplasm of these cells contains, in addition to the filamentous complement, mucus-filled vesicles and an extensive rough endoplasmic reticulum and Golgi network (Fig. 3).

Scattered between the filamentous cells in a position which impinges upon neither the free surface of the epidermis nor its basal epidermal junction are found the large, presumably fluid-secreting cells of Leydig (*L*, Fig. 1; Fig. 2). These unusual cells become numerous during midlarval stages and subsequently disappear with the onset of metamorphosis. They are identifiable by their large size, clear cytoplasm, widely scattered vesicles, and the network of tonofilament bundles (Langerhans network) along their peripheral margins (*see* Hay, 1961, for discussion). Like both filamentous cell types, the Leydig cells are mitotically active. A more complete ultrastructural and functional consideration of the Leydig cells will be the subject of a separate report.

Mucous goblet cells seem confined to the anterior head epidermis. These cells have an exposed free apical surface, and they are characterized by many, closely packed mucus granules (Fig. 4).

Finally, a second, though far less common, type of apical epidermal cell has been observed frequently in 1- to 2- μ sections (*G*, Fig. 1) and occasionally by electron microscopy (Fig. 5). This

cell is populated by fewer tonofilaments, but it contains numerous cisternae of rough endoplasmic reticulum and a well defined Golgi apparatus. Its most distinctive feature is a smooth free border underlain by a layer of densely staining, membrane-bounded granules. Electron and light micrographs of these cells, to date, reveal no definite answer as to the origin or fate of these inclusions or the functional significance of this cell type.

In all but permanganate-fixed specimens, regular, wide intercellular compartments appear to be maintained between the various cell types noted above (compare Figs. 2 and 4), a fact which corresponds to the findings of previous authors (e.g., Chapman and Dawson, 1961; Dewey and Barr, 1964; Farquhar and Palade, 1963, 1964, 1965). The compartments are bridged by stubby processes extending from the cells toward attachment points. Such processes are less pronounced along the surfaces of the Leydig cells.

The various cells within this epidermal system are attached to each other and to their adepidermal substrate by numerous and varied specialized adhesion structures. These range from tight junctions and intermediate junctions (which, together with small desmosomes, constitute terminal bars surrounding the apices of surface cells) to large desmosomes along lateral cell boundaries, and hemidesmosomes bordering the adepidermal components and underlying connective tissue. Since the terminal bar components

closely correspond to a typical junctional complex as described in detail by Farquhar and Palade (1963, 1965)² (Fig. 6), the present discussion will emphasize the substructure of large desmosomes, hemidesmosomes, and several adepidermal constituents.

Desmosome Fine Structure

A desmosome is formed in an area of approximation (at a distance of about 300 to 500 Å) of plasma membranes of adjacent cells. This nearly circular region of approximation is underlain on each side by a dense intracellular plaque, the diameter of which is variable (often exceeding 5,000 Å), and toward which numerous tonofilaments converge. In very thin sections cut perpendicular to the plaques and apposed unit membranes, each plaque is seen to be a relatively thin (150 to 200 Å) mat separated from the unit membrane by a less dense zone of slightly less width. Often such sections reveal beaded profiles (100 to 150 Å) within the plaque (Fig. 7). Deviation of section plane from perpendicular, particularly in thicker sections, produces a much broader, dense image of the plaque (lower right, Fig. 8).

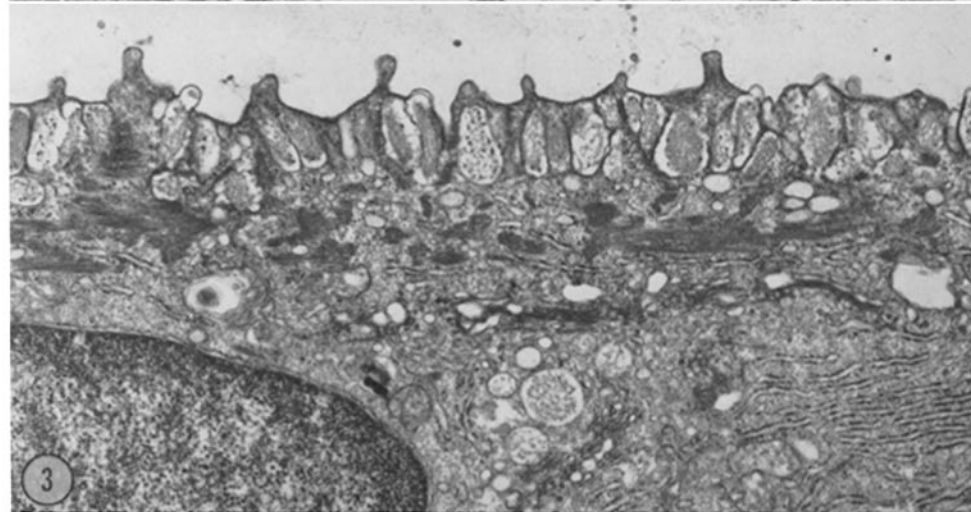
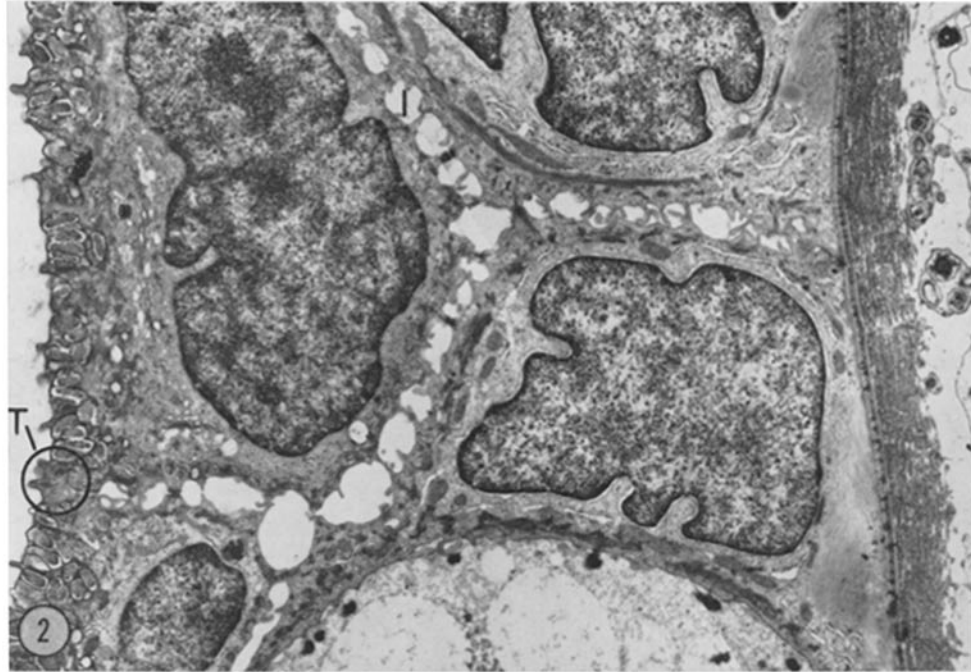
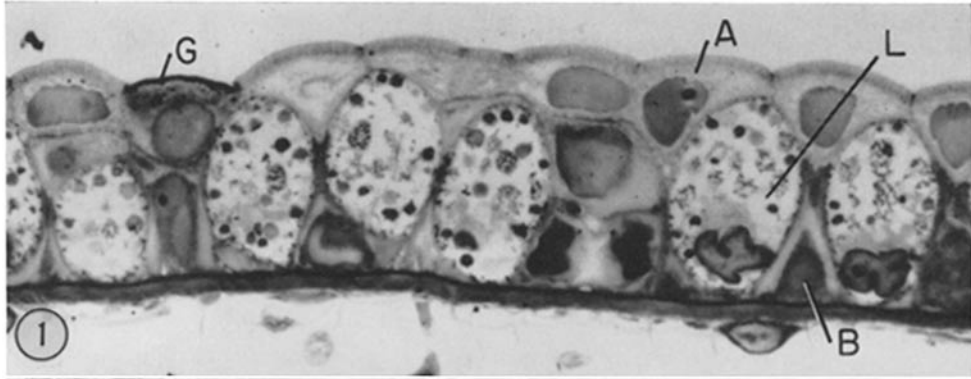
The arrangement of tonofilaments which converge toward the cytoplasm-facing surface of each plaque is most accurately discernible in stereo

²These authors have used the terms *zonula occludens*, *zonula adhaerens*, and *macula adhaerens* to designate, respectively, the tight junction, the intermediate junction, and the desmosome.

FIGURE 1 Light micrograph of a section of Epon-embedded epidermis from the dorsal tailfin of a midlarval newt. Principal cell types include apical (*A*) and basal (*B*) epidermal cells, numerous Leydig cells (*L*), and an infrequent apical granular cell (*G*). Note mitotic basal epidermal cell (right center), and thick basement membrane complex separating the epidermis from underlying mesenchymal components (bottom). $\times 1,000$.

FIGURE 2 Low magnification electron micrograph showing a portion of a field similar to Fig. 1, but rotated 90°. The mucinogenic, epidermal free border is visible (left) as well as an apical terminal bar region (*T*). Collagenous lamellae of the basement membrane complex are also visible (right). A portion of a Leydig cell (bottom) displays typical light cytoplasm, vesicles, and dense filamentous bundles at the periphery of the cell. Note wide extracellular compartments (*I*) bridged by desmosome-bearing cellular processes. Glutaraldehyde-acrolein fixation, lead citrate/uranyl acetate stain. $\times 5,000$.

FIGURE 3 A portion of the free border of an apical epidermal cell showing numerous aligned mucus vesicles (top). An extensive rough endoplasmic reticulum (lower right) and a well developed Golgi complex (center) studded with smaller mucus-containing vesicles are presumably active in mucus synthesis. Tonofilaments are also abundant in the apical cytoplasm. Glutaraldehyde fixation, lead citrate/uranyl acetate stain. $\times 11,000$.



electron micrograph pairs taken of moderately thick sections. Under these conditions, and to a less striking degree in two-dimensional micrographs, it is apparent that the majority of tonofilaments approach the plaque, then loop in a wide arc and course back into the main tonofilament bundles of the cell (Fig. 8). The nature of such filament excursions first toward, and then away from, each desmosome can be visualized as lateral detours from the "mainstream" of peripheral filament tracts within each epidermal cell. While it has not been possible to trace a single filament throughout the entirety of a given excursion, the paths of many filament segments viewed collectively in a given section lead inevitably to the above conclusion.

The majority of filament loops occur at a regular distance from the plaque (usually 400 to 700 Å). Collectively the loops form a recognizable linear density parallel to the plaque (Fig. 8). Current observations do not reveal with certainty what connects the loops directly to the plaque, and this aspect of organization remains conjectural. Some micrographs suggest the presence of filaments coursing parallel to the plaque in the area between it and the outer, main line of loops.

These are believed to represent a small filament population which approaches the plaque near its periphery, coursing through the region in a much more shallow arc (Fig. 18).

Not all filaments loop at the two levels mentioned above. A few course directly into the plaque, where they become obscure. In several cases it has been possible to analyze stereographically the plaque region in sections cut parallel or nearly parallel to the plane of the plaque itself (Fig. 9). Such sections disclose the presence of many oblique, tightly looping profiles of filaments within the substance of the plaque. These may be the tonofilaments which are seen, in perpendicular sections, coursing through the outer looping regions to reach the plaque directly, or they may be the termination of some connection between the outer loops and the plaque. At any rate, the substance of the plaque seems to be an aggregate of filamentous loops and a dense ground substance. Cross-sections of the filamentous components probably account for the beaded (100 to 150 Å diameter) profiles occasionally seen in the plaque in perpendicular sections (Figs. 7 and 8).

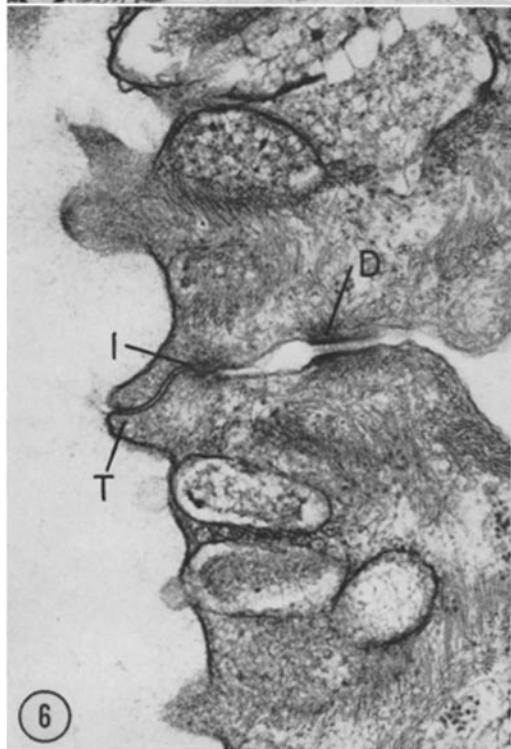
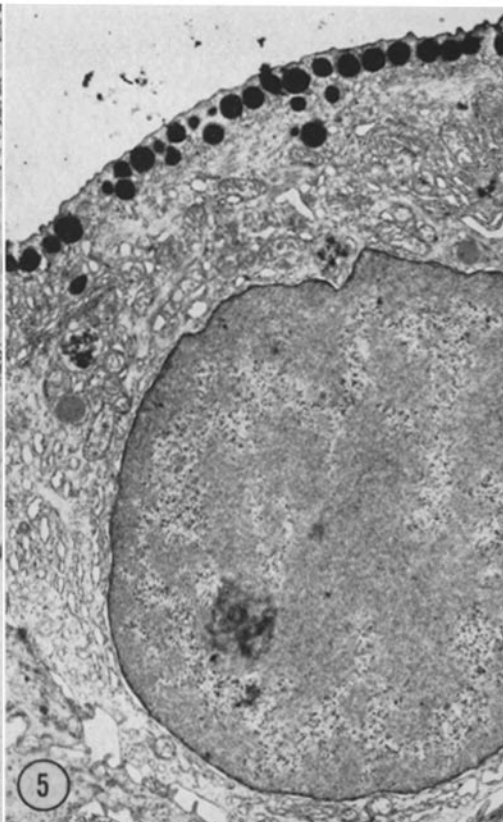
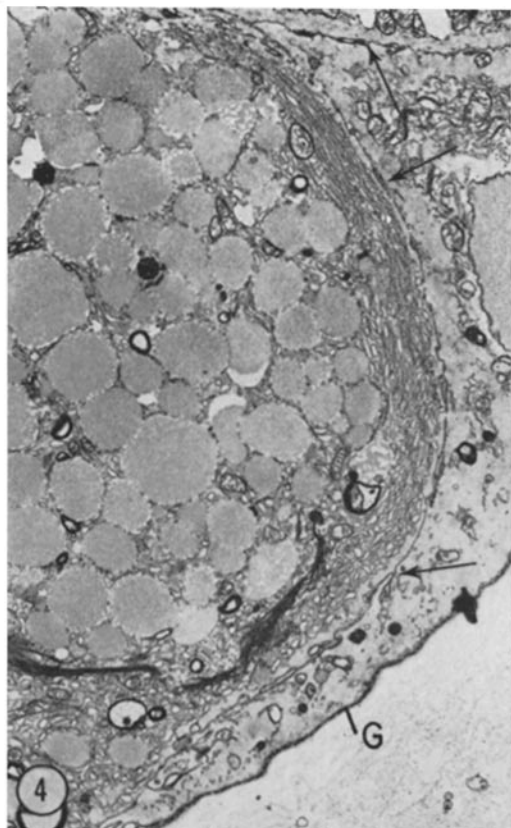
Material linking the plaque with the nearby plasma membrane is not revealed in any of the

FIGURE 4 Low magnification electron micrograph of permanganate-fixed, lead-stained young larval epidermis taken from the head region. This section shows the basal portion of a mucus goblet cell (left), an adjacent basal epidermal cell (right), and the adepidermal components and collagenous lamellae (lower right). The adepidermal globular layer appears as a line of black granules (*G*). Note the lack of open compartments between cells, by comparison to Fig. 2. After this fixation adjacent cell membranes are closely apposed and interdigitated (arrows). $\times 3,000$.

FIGURE 5 The apical portion of one of the infrequent granulated cells found along the surface of larval skin (see Fig. 1). A row of dense granules lines the free margin of the cell. Note the extensive rough endoplasmic reticulum and numerous mitochondria in the cytoplasm. OsO_4 fixation, lead citrate/uranyl acetate stain. $\times 3,000$.

FIGURE 6 Terminal bar region separating the apices of adjacent apical epidermal cells. Adhesive components include a tight junction (*T*), an intermediate junction (*I*) (obliquely sectioned), and a small desmosome (*D*). Glutaraldehyde-acrolein fixation, lead citrate/uranyl acetate stain. $\times 35,000$.

FIGURE 7 A large desmosome between adjacent epidermal cells seen in a section perpendicular to the plane of the desmosome plaques. The cell membranes can be distinguished as single dense lines, separated from the plaques by thin lucent areas. A beaded appearance is apparent along portions of the lower plaque, and looping of tonofilaments approaching the lower plaque is also evident. The intercellular gap of the desmosome is occupied by moderately dense material which displays a discontinuous midplane density (arrow). OsO_4 fixation, lead citrate/uranyl acetate stain. $\times 93,000$.



specimens studied, with the exception of those fixed in osmium tetroxide solutions to which ruthenium red has been added. After this procedure, and particularly in very thin (200 to 400 Å) sections cut perpendicular to the plane of apposition, it is obvious that the lucent zone usually seen between plaque and plasma membrane is lacking (compare Figs. 7 and 10). This finding may indicate the presence of a material, likely acid mucopolysaccharide in nature, which bridges this interval and which binds the dye. However, no structure other than general increase in density can be discerned for this apparent material.

Use of ruthenium red also provides information on materials which occupy the intercellular gap between the apposed plasma membranes of a desmosome. Lacking this dye, collidine-buffered osmium tetroxide fixation (lead citrate and uranyl acetate-stained) makes visible only the inner (cytoplasmic) leaflet of each unit membrane plus a moderately dense, apparently filamentous or stringy extracellular material in the intercellular gap. A dense and usually discontinuous line is often observed within the extracellular material in a plane midway between the two cell membranes and parallel to them (Fig. 7). Glutaraldehyde- and acrolein-glutaraldehyde-fixed specimens display the same features plus a faint image of unit membrane outer leaflet which appears continuous through the desmosome. Ruthenium red imparts clarity to the picture

owing to its binding with the outer unit membrane leaflet or mucopolysaccharide materials residing on the external surface of the leaflet (Fig. 10). These materials then become visible as pillars, partitions, or coatings which extend along the outer leaflet and across the interval to establish contact with similar coatings on the leaflet of the opposite side. In a few instances (inset, Fig. 10) the image of stained material at the junction of the outer leaflet with a pillar is suggestive that the lucent middle leaflet of the unit membrane may be continuous with the interior of the pillar. The discontinuous dense line midway in the interval appears possibly due to (1) a thickened fusion of pillars from the two sides, (2) arches of material from opposite sides which are superimposed at the midplane, (3) wrinkles in the midpoint of pillars or partitions extending the whole width, or (4) a combination of such configurations (see Fig. 18). In no case are any regular elements such as filaments or partitions seen between the dense leaflets of either unit membrane; the lucent middle leaflet appears unbroken over long distances.

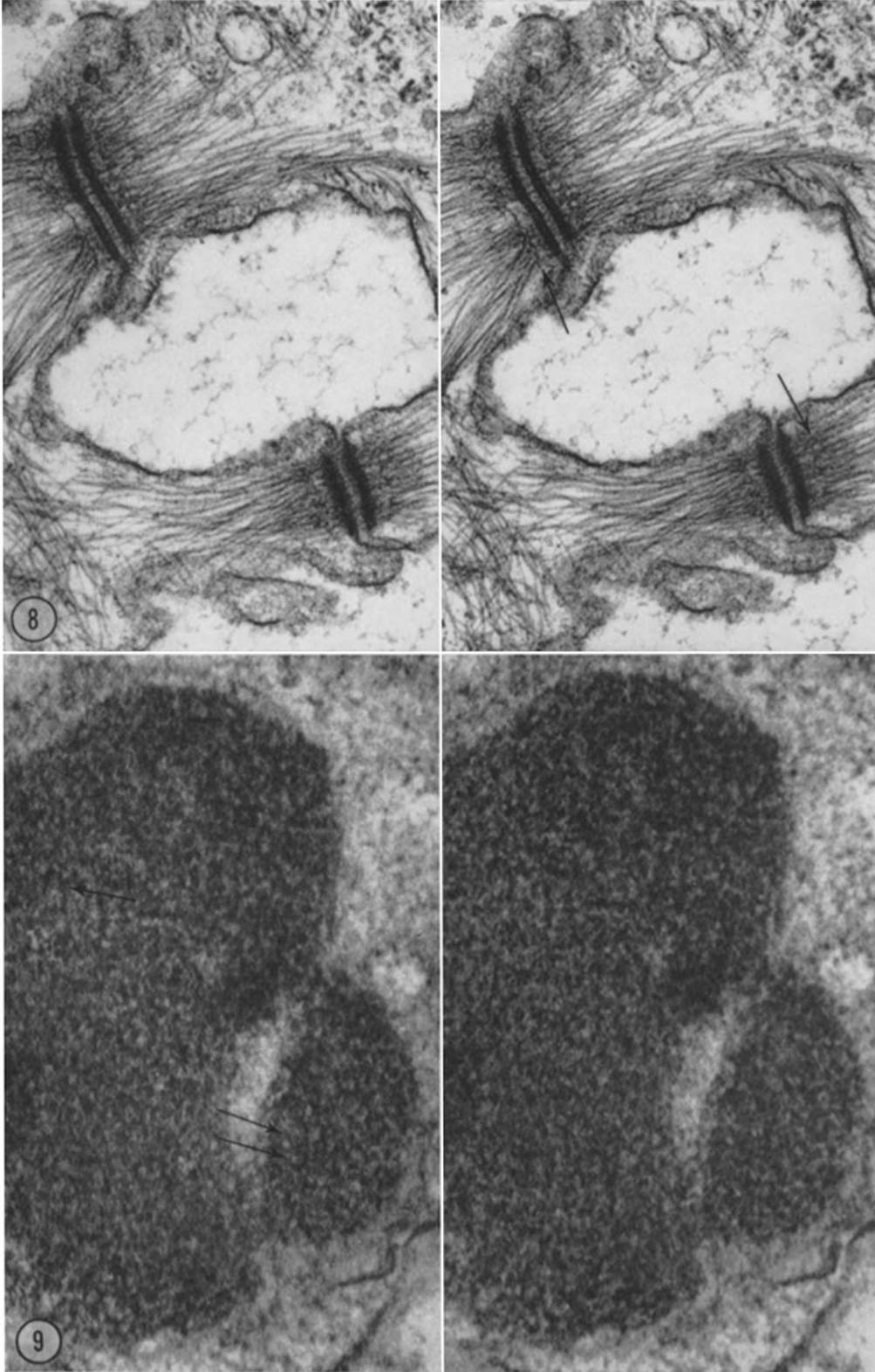
Hemidesmosome Fine Structure

A related organization is found in the hemidesmosomes occupying the basal cytoplasm of epidermal cells bordering the adepidermal components and underlying connective tissue complex (Fig. 11). Because of a basic similarity to one side of a desmosome, the designation "hemi-

FIGURES 8 and 9 are stereo electron micrograph pairs. They may be viewed as fused stereoscopic images by holding the plate at a viewing distance of 15 to 20 in. Crossing of the viewer's eyes will produce double, out-of-focus images of each pair. Gradual relaxation of eye muscles toward normal will then fuse the two central images into a single stereoscopic image. Slight lateral tilting of the head may aid the final maneuver. A cartographer's stereoscope may also be used to provide a three-dimensional image.

FIGURE 8 Two desmosomes in a relatively thick section cut slightly oblique to perpendicular. Looping of tonofilaments as they approach desmosome plaques is obvious, as is the linear density produced by the aggregate of loops (arrows). Looping is wider and more distinct when seen in a plane parallel to the section plane (upper left, lower right). OsO₄ fixation, lead citrate/uranyl acetate stain. × 50,000.

FIGURE 9 Desmosome plaques seen in a plane parallel to the section plane. No filaments are seen entering the periphery of the plaques, but numerous tightly looping profiles are seen in the substance of the plaque (arrows). When viewed stereoscopically, the filaments which form these loops seem to be oriented obliquely to the plane of the plaque (arrows, lower right). OsO₄ fixation, lead citrate/uranyl acetate stain. × 149,000.



desmosome"³ appears more descriptive than the term "bobbin" (Weiss and Ferris, 1954). However, basic differences between amphibian desmosomes and hemidesmosomes also exist. The dense skeins of converging filaments, visible with the light microscope and often referred to as "bodies of Eberth" (see Chapman and Dawson, 1961; Singer and Salpeter, 1961), form a much more tightly packed bundle as they approach the plaque. On the basis of a few observed looping profiles within the crowded bundles, a provisional interpretation is drawn that most of the converging filaments loop as is the case for desmosomes, but in the hemidesmosomes the angle between the two limbs of the loop is much smaller. Hemidesmosomes seen in sections cut parallel to the longitudinal axis of the tailfin (Fig. 11) appear about twice as long as those observed in cross-sections. The plaque is, therefore, ellipsoidal in shape, rather than nearly round like desmosomal plaques, and oriented with its long axis parallel to the long axis of the tailfin. The density of the filament bundle extending from 300 to 1800 Å out from the plaque (Fig. 11) may be accentuated by the presence of a cementing substance in addition to the tightly packed filaments.

Thinly scattered tonofilaments course parallel to the plasma membrane in the most basal epi-

³This designation is essentially equivalent to the term "half-desmosome" used by a number of other authors.

dermal cell cytoplasm. These seem to intermingle at right angles with hemidesmosome bundles. Similar to the case with desmosomes, a linear density which exists in a plane midway between the plaque and the base of the presumably looping filaments may be due to a few filaments which course parallel to the plaque at that level (Figs. 11 and 19). Numerous vesicles and caveolae of the plasma membrane populate the basal cell cytoplasm (Fig. 11), regularly interspersed between the hemidesmosomes. When stained with ruthenium red (Fig. 16), the unit membrane of the basal cell surface is made distinct, and it can be traced as an uninterrupted trilaminar profile through regions of hemidesmosomes.

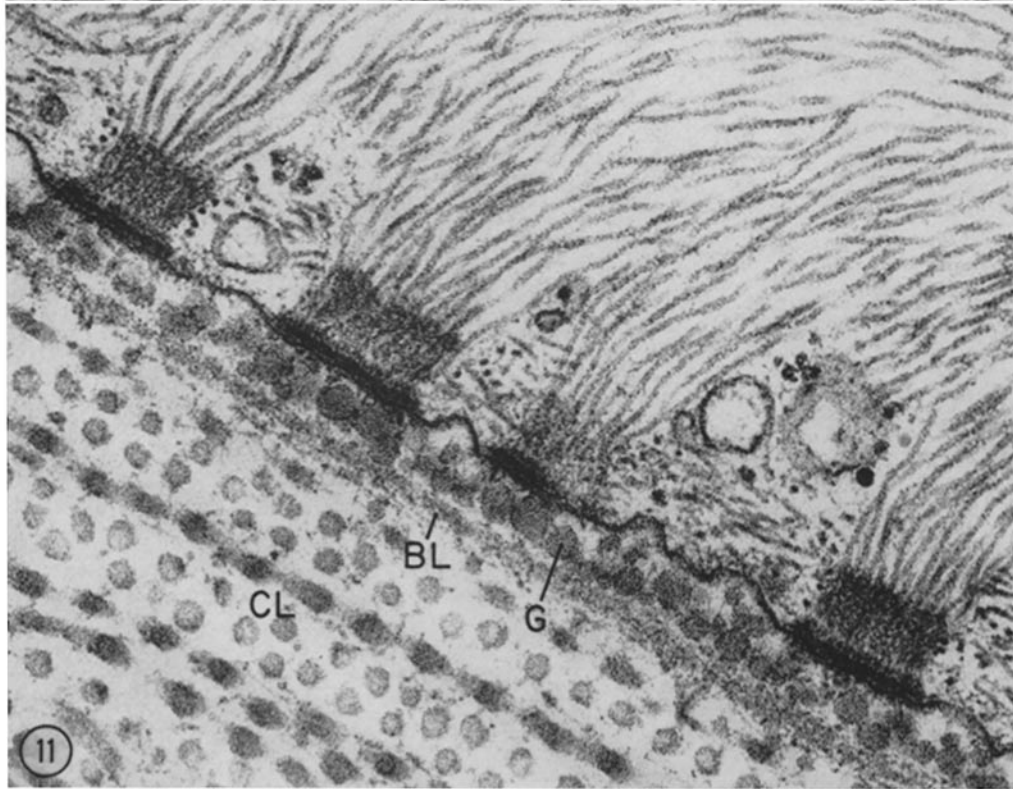
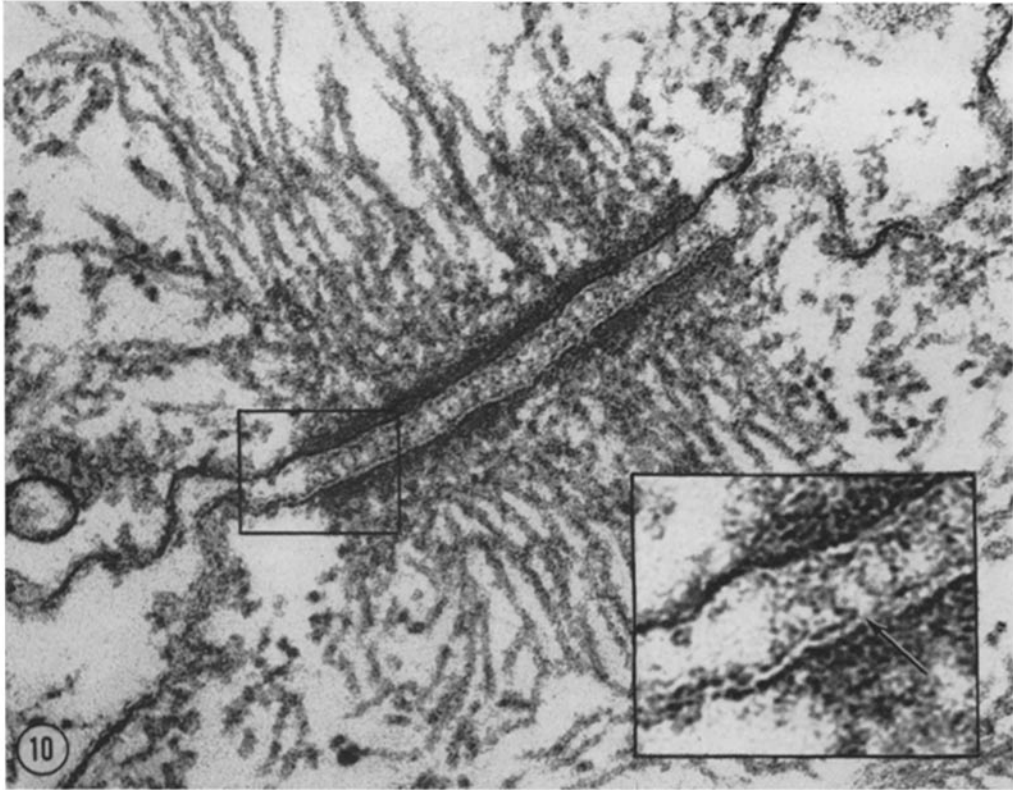
Adepidermal Components and the Collagenous Lamellae

Figs. 1 and 2 disclose, at low magnifications, the highly ordered collagenous foundation upon which the epidermis and adepidermal structures rest. Weiss and Ferris (1954) (see also Porter, 1954), who provided the initial electron microscope analysis, placed special emphasis on the nearly orthogonal arrangement of collagen fibrils which form some twenty or more regular layers.⁴ Some

⁴Weiss and Ferris (1954, and subsequent reports) referred to these layers as the "basement lamella," a term used with varying connotations by subsequent authors (see Hay, 1964, p. 100-101).

FIGURE 10 A very thin (200 to 300 Å) section of a large desmosome from epidermis fixed in OsO₄ to which the dye, ruthenium red, has been added. The usually lucent area separating the plaques from their respective cell membranes (Fig. 7) is dense in this preparation. The unit character of cell membranes is made obvious by binding of the dye to extracellular materials in or on the outer unit membrane leaflet. Dense extracellular material is also visible as pillars or partitions bridging the intercellular gap. A midplane density in the intercellular material is not apparent. The inset shows an area (arrow) which is suggestive, but not conclusive, evidence that the lucent unit membrane middle leaflet may be continuous with the interior of a bridging pillar. Lead citrate/uranyl acetate stain added after sectioning. × 134,000. Inset, × 342,000.

FIGURE 11 A very thin section of the basal portion of a larval epidermal cell. Longitudinally sectioned hemidesmosomes line the cell unit membrane; Plaques and compact perpendicular filament bundles can be seen as intracellular components of each hemidesmosome. Parallel filaments are sparse and course among the hemidesmosomes. Adepidermal constituents overlying the collagenous lamellae (CL) include the basal lamina (BL), and the globular layer (G) which is interposed between the basal lamina and the overlying cell membrane. Thin strands extend from the cell membrane toward the globular layer and basal lamina, particularly in the vicinity of hemidesmosomes. OsO₄ fixation, lead citrate/uranyl acetate stain. × 80,000.



of these lamellae are seen at higher magnification in Fig. 11. The possible developmental significance of the collagenous lamellae and the question of their origin has been considered by numerous authors (*see, e.g.,* Weiss and Ferris, 1956; Weiss, 1958; Porter, 1954; Kemp, 1959; Edds and Sweeny, 1960, 1962; Singer and Salpeter, 1961; Anderson and Kollros, 1962; Hay and Revel, 1963; Hay, 1964; Usuku and Gross, 1965).

The collagenous lamellae are overlain by a 250- to 400-A thick, finely granular or filamentous, basal lamina. In the newt epidermal system a wide (850- to 1000-A) lucent zone ("adepermal space" of Salpeter and Singer, 1960) separates the basal lamina from basal epidermal cell plasma membrane. Weiss and Ferris (1954, 1956) (*see also* Porter, 1954) noted that during larval stages in amphibians this lucent zone is occupied by objects which they described as regularly dispersed "granules" of probable lipid nature. Singer and Salpeter (1961) (*see also* Usuku and Gross, 1965) also noted the presence of this adepermal substance, and the further fact that it is lacking along the basal lamina in adult newts. However, other reports are equivocal as to the reality of the material, and whether it exists as globules or as filaments (*see* Hama, 1960; Chapman and Dawson, 1961; Singer and Salpeter, 1961).

The adepermal substance in question is visible in Fig. 11 as a series of globular bodies, 200- to 600-A in diameter, arranged as a virtually uninterrupted layer on the basal lamina. The

presence of irregular transverse strandlike profiles coursing among the globules to connect plasma membrane with basal lamina is also apparent. The globular material is readily visible after all methods of preparation, is circular in profile in all planes of section and when viewed stereoscopically, and is found in head, trunk, and tail regions. Perhaps also notable is the fact that a globular layer is lacking in gill epidermis, and judging from the micrographs of Anderson and Kollros (1962), it may well be absent in the balancer.

Weiss and Ferris (1954) apparently based their conclusion as to the lipid nature of the globules on the osmiophilia of the material in methacrylate sections. A similar suggestion may be deduced in the present study from the very high density which the globules display by comparison to surrounding structures in sections of osmium-tetroxide-fixed, but otherwise unstained, Epon-embedded tissues (Fig. 12). Lead citrate and uranyl acetate staining appears to reverse this contrast (Fig. 11). Such staining behavior is compatible with, but not conclusive evidence for, the presence of lipid (Phelps, Rubin, and Luft, 1964).

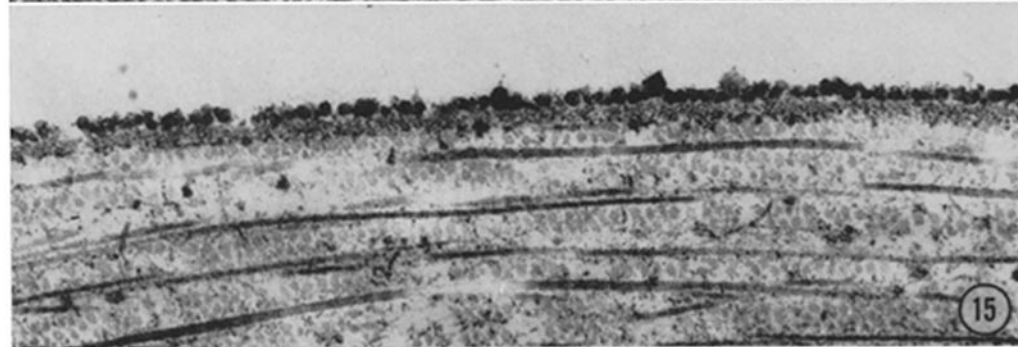
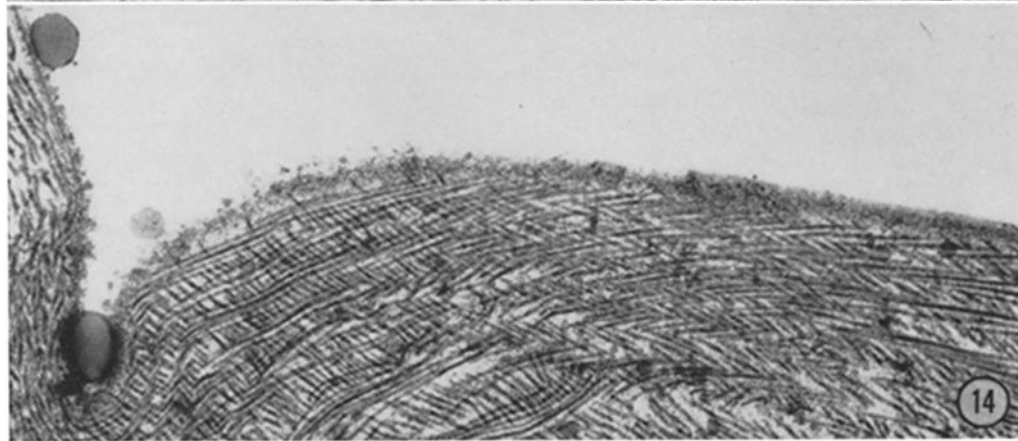
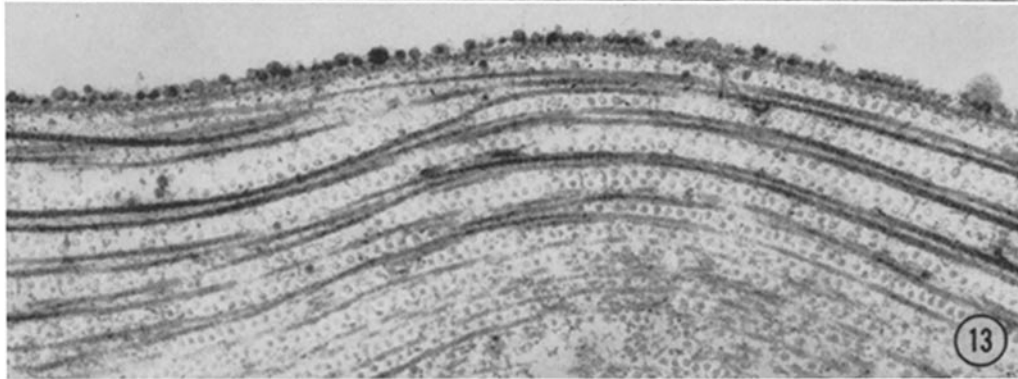
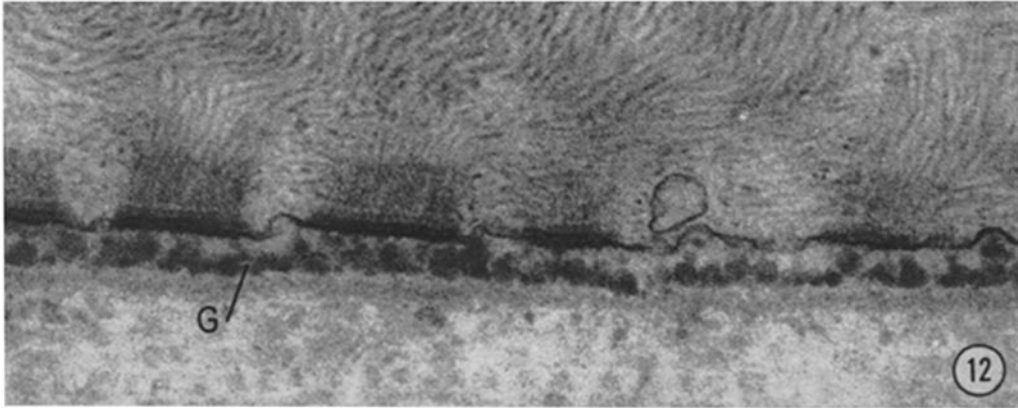
When epidermis is stripped from the tailfins of larval newts, using the drying/lens paper technique, the plane of cleavage invariably results in the retention of the globular layer, firmly attached to basal lamina and exposed to the surrounding medium. Weiss and Ferris (1956) noted this same

FIGURE 12 The basal epidermal region from an OsO_4 -fixed, but otherwise unstained preparation. The globular layer (*G*) displays a higher degree of osmiophilia than any neighboring structures, with perhaps the exception of hemidesmosome plaques and the cell membrane. This pattern is in contrast to that of Fig. 11, where the globular layer appears lighter than its surroundings after heavy metal staining. $\times 67,000$.

FIGURE 13 Adepermal components from which the overlying epidermis has been stripped. The globular layer has been exposed and has remained firmly attached to the basal lamina despite rinsing with pond water prior to fixation. OsO_4 fixation, lead citrate/uranyl acetate stain. $\times 33,000$.

FIGURE 14 Denuded adepermal components similar to those in Fig. 13. Treatment of the surface with chloroform prior to fixation has dispersed the globular layer (right). In a few places (left) large residual droplets of the globular layer have been left. OsO_4 fixation, lead citrate/uranyl acetate stain. $\times 22,500$.

FIGURE 15 Denuded adepermal components after a 10-minute prefixation treatment with 2.0% lipase. The globular layer appears dense, but otherwise unaffected. OsO_4 fixation, lead citrate/uranyl acetate stain. $\times 33,000$.



effect, but over much less extensive areas in the vicinity of induced epidermal wounds. Such exposed globules are not dislodged from their position by rinsing the surface with pond water (Fig. 13). In the limited number of experiments in which such exposed globular layers have been treated with enzymes or other agents prior to fixation and examination, alpha- and beta-amylase, hyaluronidase, and Pronase did not alter the morphology, position, or number of globules. Collagenase also left the globular layer intact, although the globules appeared slightly "fuzzy" and individually less distinct as circular profiles. A brief treatment with chloroform, however, totally abolished the globular layer over most of the exposed area (Fig. 14). After this treatment, large round profiles of what was judged to be coalesced residual globular material could be found scattered infrequently on the basal lamina surface. An even more effective removal of the globular layer was obtained by application of the nonenzymatic mucolytic agent, *N*-acetyl cysteine. After this procedure, not even residual profiles could be found along the cleanly denuded basal lamina.

Contrary to initial expectations, repeated applications of lipases for varying durations and in varying concentrations failed to provoke any disruption in the constitution of exposed globular zone (Fig. 15). This result leads to the suspicion that the globules are not lipid (or at least not a lipid responsive to the lipases used), or that their lipid content is in some manner protected from lipase attack. Evidence favoring the second explanation may be derived from micrographs of

the intact, unstripped adepidermal region after fixation with osmium tetroxide solutions containing the dye, ruthenium red (Fig. 16). These images clearly show a densely stained coat enveloping the globules and providing a continuous matrix attaching the globules one to another and to the basal lamina. Additionally, the stained material can be seen, in places, to bridge the wide adepidermal lucent zone and continue as a well defined component in or on the basal plasma membrane outer leaflet.

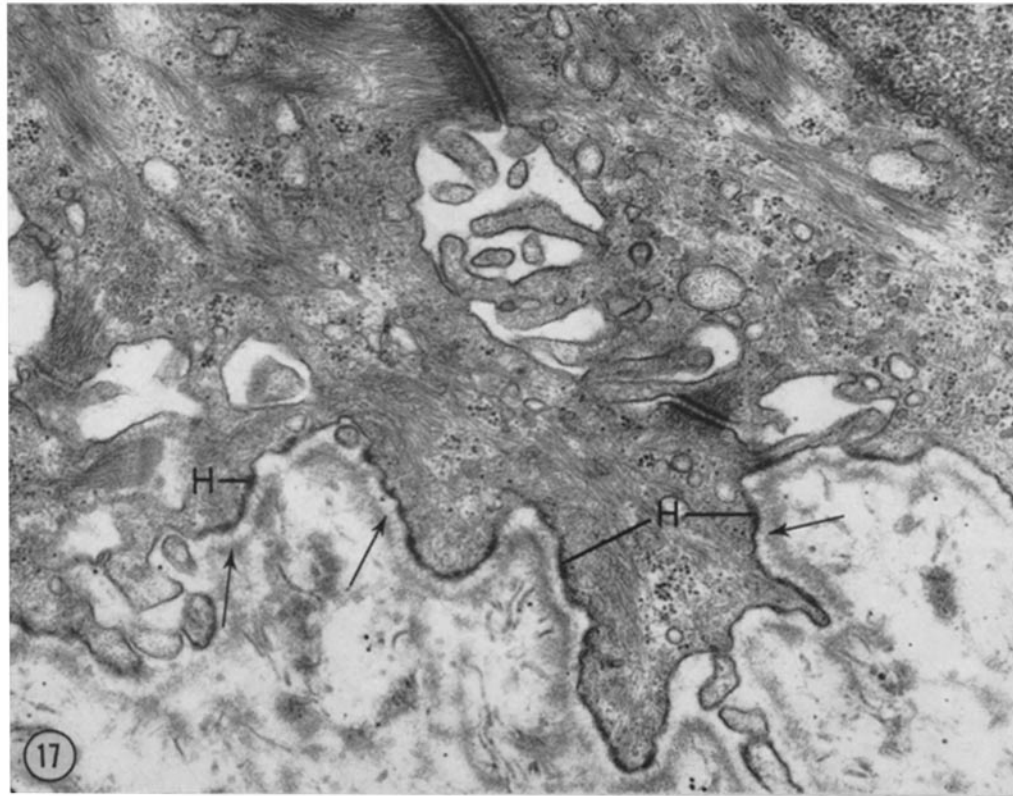
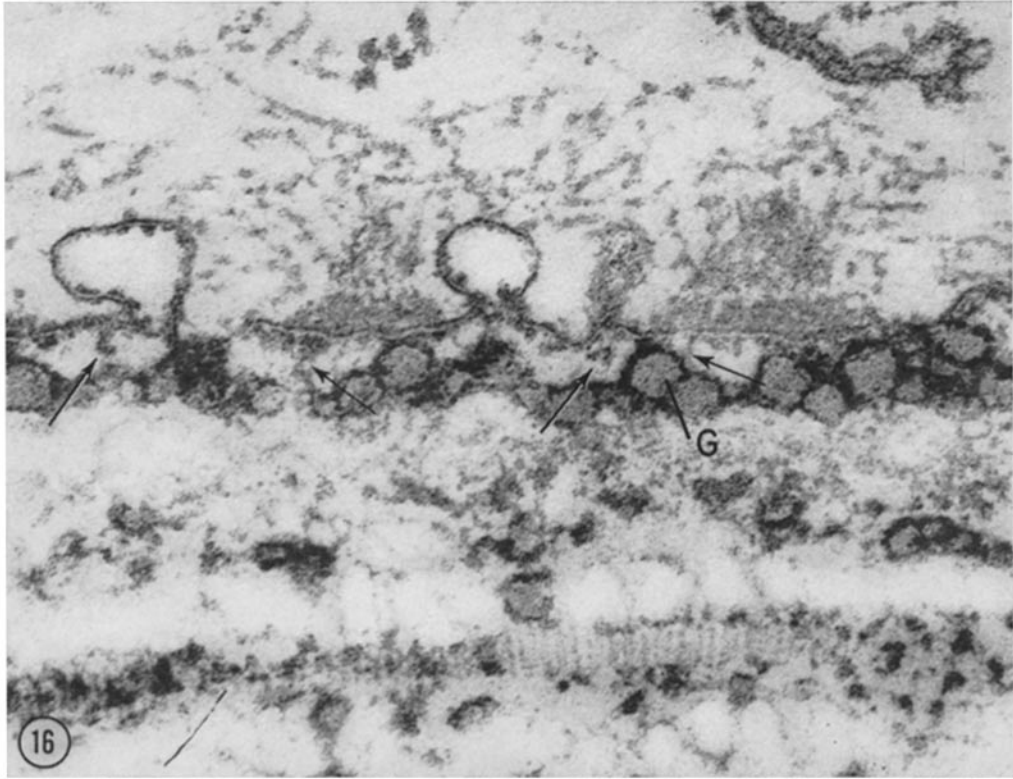
Changes in Adhesion Sites and Adepidermal Structure at Metamorphosis

The manifold morphological changes in amphibian integument which accompany metamorphosis and the assumption of semiterrestrial life include keratinization of outer epidermal cells, increase in the number of epidermal cell layers, loss of Leydig cells, development of both mucous and granular skin glands, and gross alterations in the subepidermal connective tissue (*see* Kemp, 1961, 1963; Voute, 1963; Venable, 1964; Usuku and Gross, 1965). Within the framework of these fairly obvious changes, the present study has disclosed two apparently simultaneous events involving the adhesive and adepidermal structures described above.

The adepidermal globular layer is present in tailbud embryos prior to hatching and in larvae of all ages. However, in animals examined after their emergence to terrestrial living (normally after 3 to 4 months in the laboratory), the globular layer was found to be totally lacking; its dis-

FIGURE 16 A very thin section of the basal epidermal region from a larval tailfin fixed in an OsO_4 solution to which the dye ruthenium red has been added. The dye has darkly stained a mucopolysaccharide coat which surrounds the presumed lipid components of the globular layer (*G*). Strandlike profiles can be seen (arrows) extending between the mucopolysaccharide coat and the outer leaflet of the cell unit membrane. Hemidesmosomes are faintly visible along the unit membrane between caveolae. Lead citrate/uranyl acetate stain added after sectioning. $\times 134,000$.

FIGURE 17 The basal epidermal area from the tail of a newt fixed 3 to 4 weeks after metamorphosis. The epidermal-dermal border is irregularly folded and no regular collagenous lamellae are present. There is no globular layer between the cell membranes and the basal lamina although strands (arrows) can be seen connecting these two components. Hemidesmosomes are only vague densities (*H*) along the cell membranes. This is in contrast to the large, complex desmosomes attaching adjacent epidermal cells (right center, top center). OsO_4 fixation, lead citrate/uranyl acetate stain. $\times 25,000$.



appearance apparently occurs within the few days when the grosser morphological changes take place. Fig. 17 shows an area along the epidermal-dermal junction in an animal fixed 3 to 4 weeks past emergence. By this stage, the junction has been thrown into an irregularly plicated border, beneath which is found a collagenous substrate no longer layered and highly ordered (see Kemp, 1961, 1963; Usuku and Gross, 1965). Globular structures in the wide adepidermal lucent zone are absent. The basal lamina is thicker, in some areas reaching or exceeding 1000 Å, and it appears still to possess fine strands bridging the lucent zone to reach the plasma membranes.

Fig. 17 also illustrates the fact that hemidesmosomes are much less complex and distinct following metamorphosis. In fact, the only evidence of their continued existence are vague densities which populate the basal plasma membranes, a configuration similar to hemidesmosomes in adult skin of other amphibian species (e.g., see Voute, 1963; Bani, 1964), and even less distinct than those found in mammalian skin (see, e.g., Odland, 1964, p. 242).

By contrast to these striking changes in basal presumptive adhesive sites, the desmosomes arranged on other surfaces of postmetamorphic epidermal cells show no diminution over their premetamorphic configuration (Fig. 17). If anything, the number and extent of these desmosomes seems augmented following metamorphosis. In keratinized outer cell layers, the desmosomes display more densely packed tonofilaments and an apparent increase in the size and electron opacity of intercellular material along the midplane density (see also the recent observations of Snell [1965] concerning accumulation of intercellular material in desmosomes of mammalian keratinizing epidermis).

DISCUSSION

Desmosomes and Hemidesmosomes

Both the large desmosomes and hemidesmosomes of larval newt epidermis appear to represent highly organized, presumed adhesive mechanisms, as judged from the large number of filaments involved, the intricacy of their arrangement in and near the plaque, and the amount of associated intercellular material (summarized in Figs. 18 and 19). These arrays may, in fact, represent something of an extreme in complexity when

compared to the desmosomes of many other systems. It is perhaps practical to think in terms of a spectrum of desmosome morphology in nature which ranges from those complex forms described here, or the nearly continuous desmosome chains described recently by Jurand (1965), to much simpler varieties which incorporate fewer or no tonofilaments, or finer filaments, or which, as exemplified by postmetamorphic hemidesmosomes, are discernible only as vague densities along apposed or approximated plasma membranes. The simpler end of a desmosomal spectrum might conceivably include intermediate junctions, and finally even tight junctions or nexuses (Dewey and Barr, 1964; Farquhar and Palade, 1963, 1964, 1965) by sequential exclusion of the plaque, intracellular filaments, and the amount of extracellular material between apposed plasma membranes (although these "simpler" junctions are generally broad or "belt-like" in their extent). Hemidesmosomes offer one example of the convertibility, with age and differentiation, of a complex attachment into a simpler one.

The recent literature concerning desmosomes seems divided between authors who have implied termination of filaments at the plasma membrane or in the plaque, and others who, perhaps more cautiously, have described only convergence of filaments toward the plaque. The outer networks of tonofilament loops along newt epidermis desmosomes do not seem to terminate, in the sense of there being an actual end to any one filament. Rather, each loop represents an excursion of a tonofilament past the plaque. It would appear likely that, at the point of nearest approximation, each loop should have some mode of attachment toward the plaque, but details of this relationship are not provided by the available micrographs. The few tonofilaments which seem to approach the plaque perpendicularly and proceed directly into the plaque may indeed terminate there, but the stereoscopic evidence seems more suggestive that they, too, loop, although much more tightly.

Looping of tonofilaments near the plaque, while clear in this epidermal system, may not be so obvious, or necessarily a basic rule, in all desmosomes. In a sense, looping is a necessary configuration conferred in epidermal desmosomes by two factors: (1) the fact that most of the filaments which participate in the desmosomes are members of an extensive tonofilament array which courses primarily in the periphery of the cell, parallel to

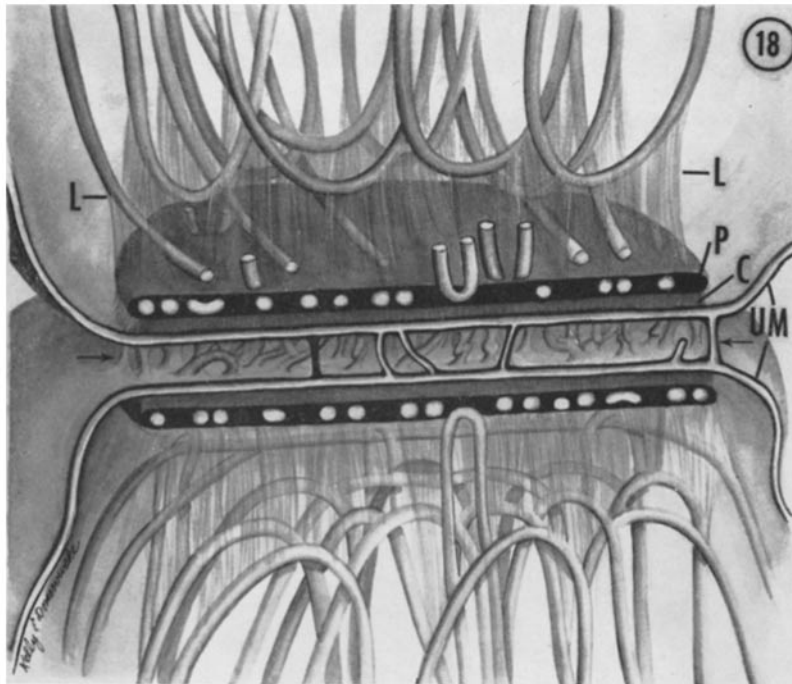


FIGURE 18 A schematized drawing depicting a cut-away view of a large epidermal desmosome. Central components include the unit membranes (*UM*) of two adjacent cells and a dense plaque (*P*) within each cell. Each plaque is adjoined to a cell membrane inner leaflet by a cement layer (*C*). The intercellular space is bridged by materials which are continuous with or deposited upon outer cell membrane leaflets. Many configurations of this material are shown. They are representative of various possibilities suggested by evidence presented in the text. Criss-crossing, overlapping, adjoining, or wrinkling of these configurations are interpreted as explanation for the discontinuous midplane density often seen in desmosomes at the plane marked by the arrows. The majority of tonofilaments approaching each plaque loop at plane *L-L*. Others may loop within the plaques or at intermediate levels. A connecting component between outer loops and the plaques is suggested by density in micrographs and indicated appropriately in this drawing, although an exact morphological interpretation is premature.

the general cell surface contour; and (2) the presence in the system of wide intercellular compartments which dictate that the course of filaments to and from the desmosomes must be via lengthy excursions within the confines of the narrow cellular extensions which bridge the compartments. Lack of this second factor in a system will de-emphasize the outer loops. Fawcett (1961) and Jurand (1965) and others have described desmosomes in which tonofilaments are nearly straight and oriented parallel to the plane of the plaque. Such may well be the general rule where no wide extracellular compartments exist, and where the predominant filament skeleton of the cell consists of peripheral tracts coursing parallel to the cell membranes.

Not all cells, however, display predominant peripheral, membrane-parallel filament populations. Many systems emphasize filaments which course across the cytoplasm rather than along its periphery. In such cases, desmosomes may primarily incorporate filaments approaching perpendicular to the plaque, and if looping does exist it may be much more tight and perhaps within the substance of the plaque.

The fact that both desmosomes and hemidesmosomes in this epidermal system, and many desmosomes in micrographs of other systems, seem to possess populations of filaments approaching both perpendicularly and parallel to plaques suggests a simple basis for variation in desmosome morphology; namely, that the mechanical require-

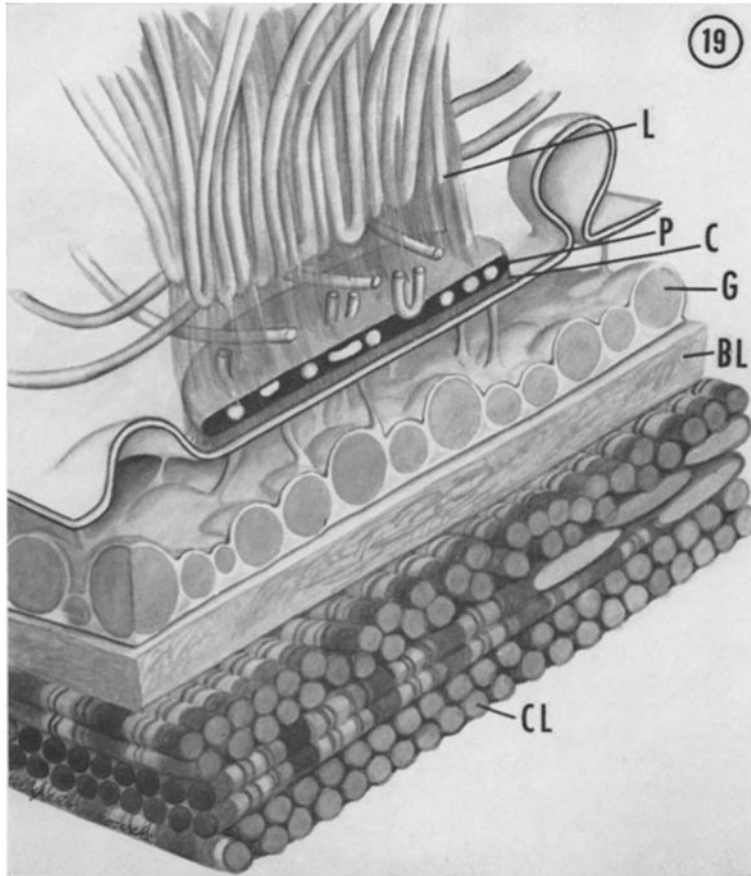


FIGURE 19 A similar schematization for an epidermal hemidesmosome and the adepidermal components and collagenous lamellae which underlie the basal cell membranes. The hemidesmosomal plaque (*P*) and its associated cement layer (*C*) are ellipsoidal and more narrow than those of a desmosome. The outer loops of tonofilaments (*L*) form a much tighter bundle. Adepidermal components overlying the collagenous lamellae (*CL*) include the basal lamina (*BL*) and the globular layer (*G*) with its associated coat. Strands of extracellular material extend between this coat and the cell unit membrane. Caveolae are seen along the unit membrane in areas between hemidesmosomes.

ments of a given system relate to an emphasis at the desmosomes of an approach of filaments oriented either perpendicular to or parallel to desmosomal plaques, or if appropriate, a precise combination. The large desmosomes in newt epidermis appear to emphasize a somewhat warped version of the parallel approach as the filaments loop past the plaque. Perpendicular filaments are much less numerous.

Most of the tonofilaments which approach hemidesmosomes, on the other hand, do so from well within the cell cytoplasm and, therefore, tend to approach steeply. Incidentally, some or most of these filaments may arch from plaque to

plaque, as noted by Chapman and Dawson (1961). Still, the approach to a given plaque is more nearly perpendicular and the final looping at the hemidesmosome (if looping is indeed present) must be very tight. Parallel filaments in the hemidesmosome region are relatively sparse.

With these points in mind, it is not difficult to visualize many desmosome variations related not only to the extent or strength of intercellular adhesion, but also related to internal cell architecture and prevailing mechanical stresses (*see* also Charles and Smiddy, 1957).

Abundant evidence has been brought to bear on the concept that no continuity of cytoplasmic

elements between adjacent nondividing cells is afforded by the presence of desmosomes (*see* Fawcett, 1961; Farquhar and Palade, 1965). This is particularly true in regard to filament continuity, for which no adequate demonstration has been presented. Since most desmosome-related tonofilaments in newt epidermal cells are seen to loop, rather than course into the plaque, the possibility of filaments bridging these desmosomes is correspondingly reduced. Furthermore, since the plasma unit membranes made obvious by ruthenium red display an uninterrupted, lucent middle leaflet over long distances through the desmosome, it appears even more doubtful that many, if any, filaments penetrate the membrane (Fig. 10). The same image, incidentally, serves to demonstrate the cell membrane as a recognizable and continuous unit as it extends from nonattached areas through the desmosome (*see* also Farquhar and Palade, 1965).

Of one accepts as fact the lack of cytoplasmic or filament continuity across desmosomes, then the adhesive characteristics attributed to desmosomes must be accounted for largely in terms of the properties and structure of the intercellular material. Ruthenium red staining appears to be the result of binding of the dye to polysaccharides possessing acid groups (Luft, 1964, and unpublished data), and it is likely that such reactions account for the added clarity with which the outer cell membrane leaflet and desmosome intercellular materials are seen in micrographs such as Fig. 10. The stained material resides either within or closely applied to the outer leaflet, and forms the borders of pillars and partitions which bridge the intercellular gap.

Those few observations (inset, Fig. 10) suggesting continuity of the lucent middle leaflet of the cell membrane with the interior of bridging pillars or partitions raise an interesting possibility that deserves further study. Current concepts as to the structure of the septate desmosomes of invertebrates (Wood, 1959; Locke, 1965) recognize the outer leaflet as the basic component which is confluent across the gap in a very regular pattern. In the sense of the outer leaflet alone, an intercellular continuity is thus established across a space of approximately 140 Å. A similar suggestion concerning outer leaflet participation in the bridging of a much larger (ca. 300 to 400 Å) gap might be made for epidermal desmosomes. However, further observations are needed to

justify such a proposal. An alternate possibility, that the stainable material only resides upon the outer leaflet and is itself the substance of the pillars and partitions between the cells, remains equally plausible.

Ruthenium red-stainable material is not only more heavily concentrated in desmosomes than in noncontact areas, but it also participates in the formation of the extracellular density lying midway between the apposed plasma membranes ("intercellular contact layer" of Odland, 1958). Several previous workers have noted the presence of extracellular materials in epidermal systems and their concentration at attachment points (reviewed by Odland, 1958; Snell, 1965), and Matoltsy and Parakkal (1965) have shown evidence for the origin of extracellular materials as membrane-bounded packets from keratinizing epidermal cells. Odland (1958) visualized the "intercellular contact layer" in human epidermis as a continuous sheet or plaque within this extracellular material. That situation may be more similar to the intercellular density seen in desmosomes from outer keratinizing cells of postmetamorphic newt skin. The observations on larval skin seem to indicate that a corresponding midplane density is discontinuous, particularly when fairly thin sections are studied (Fig. 7), and even less obvious or lacking in very thin (200 to 400 Å) sections (Fig. 10). A logical interpretation for these properties is that the density is not a continuous structure or sheet, but rather an increased density midway along individual pillars or partitions. Its precise location may be due to an overlap in the midline of extracellular elements from each side. The image of many such overlapping elements would tend to fuse into a single dense line in thicker sections, become discontinuous in thinner sections, and disappear in the thinnest sections. Many types of wrinkling, overlapping or adjoining configurations of extracellular material can be envisioned (Fig. 18) to account for such a discontinuous density located near the midplane, without invoking the concept of a midplane plaque.

Adepidermal Components

Rarely have structural components been demonstrated within the usually lucent extracellular compartment that separates cell membranes from an underlying dense basal lamina. The globular zone and its apparent associated

coats which are found during late embryonic and larval life under the epidermis of this and other amphibian species may represent a unique example of an extensive structural array within the lucent area. In view of the regular appearance of the globules after the variety of fixation procedures used in this study, and those used by previous workers, there is little doubt about the occurrence of an adepidermal globular zone in many and perhaps most amphibian larvae. It is, however, a zone that is susceptible to distortion or damage in methacrylate sections, judging from some earlier micrographs (e.g., Hama, 1960; Chapman and Dawson, 1961; Singer and Salpeter, 1961), and this fact may account for some interpretations of the zone as primarily filamentous rather than globular. It is also interesting that a globular zone can be discerned in the micrographs of some previous authors (e.g., Kemp, 1961, 1963; Usuku and Gross, 1965) who did not otherwise explore the matter beyond the original interpretations (Weiss and Ferris, 1954; Porter, 1954).

An assessment of the nature of this layer is still difficult, although the following seems plausible on the basis of observations reported to date. The globules themselves are quite likely lipid, as Weiss and Ferris (1954) first proposed, and judging by the rapid dissociation of exposed globular zone when treated with a lipid solvent such as chloroform. The globules appear to be embedded in, and coated with, an acid mucopolysaccharide material which probably serves to anchor the globules to the basal lamina. The coating is made visibly more apparent with ruthenium red, and is not dissolved by treatment with amylase, hyaluronidase, collagenase, or Pronase. It is, however, sensitive to a nonenzymatic mucolytic agent, *N*-acetylcysteine, which appears to cause dissolution of the coat and the enclosed globules. The failure of lipases to disrupt the globular zone may be due to a protective effect provided by the coat. Such a protective effect, however, apparently does not inhibit the action of chloroform. Further substantiation of this interpretation seems desirable before final judgments are made.

Because of their morphology and their subsequent disappearance at metamorphosis, it seems doubtful that the lipid elements of the globular zone play a definitive role in adhesion of epidermis to the underlying strata. Some regulatory function in skin physiology during aquatic, larval stages would seem more plausible, though largely con-

jectural at present. In adult frog skin a normal transepidermal potential appears to rely in part upon the presence of intra- and extracellular compartments and the maintenance of sodium and potassium ion gradients between these compartments (*see* discussions of Ussing, 1963–1964; Farquhar and Palade, 1964, 1965). It is interesting that, at least in the bullfrog, transepidermal potentials due to ion transport do not appear until the onset of metamorphosis (Taylor and Barker, 1965) when the globular zone disappears and other dermal and epidermal changes take place.

The lipid-mucopolysaccharide complex presented by the globular zone is also of possible significance in regard to proposals concerning the origin of the underlying collagenous lamellae as products of the epidermal cells (Hay and Revel, 1963; Hay, 1964). During larval life, when most of the lamellae are laid down, the globular zone stands interposed in the path of presumed epidermal products destined for collagen fibril formation. A similar interposition exists relative to whatever inductive (Kallman and Grobstein, 1965) or enzyme (Eisen and Gross, 1965) influences may exist between epidermis and underlying mesenchyme during this time. Loss of an interposed globular layer at metamorphosis may relate to ensuing morphogenetic changes.

Micrographs presented here (Figs. 11 and 16) reveal irregular strands of material bridging the light areas between basal plasma membranes and the globular layer or basal lamina. Ruthenium red staining discloses that these strands seem continuous with the mucopolysaccharide coat on the one side and a coating on or in the outer cell membrane leaflet on the other side. While not exclusively located opposite hemidesmosomes, there is definitely a greater abundance of the strands at those sites. Singer and Salpeter (1961), assuming the hemidesmosomal plaque or “bobbin” to lie in the plasma membrane, described these structures as hubs or stays from which a fibril population radiates intracellularly as the bodies of Eberth, and extracellularly as attachments to the “adepidermal reticulum” (basal lamina plus collagenous lamellae). While the strandlike profiles seen in the current investigation before and after metamorphosis seem too irregular to term fibrils or filaments, they may, if considered analogous to similar extracellular material in desmosomes, have significant adhesive properties.

Like the desmosomes, the hemidesmosomal region in this system gives no evidence for a continuity of either tonofilaments or extracellular strands through the plasma membrane. Adhesive properties probably relate to extracellular materials in both attachment mechanisms.

Concluding Comments

Desmosomes and hemidesmosomes in epidermal systems are not the only sites in which deposits of extracellular mucopolysaccharide are located. In fact, the general clarity which ruthenium red imparts to the unit membrane is likely due to the binding of this dye with coatings on the external leaflet (Luft, 1964). A possible morphological vehicle, therefore, exists for the general selective, weakly adhesive properties which cells are known to possess (Moscona, 1960; Steinberg, 1964). In view of the added accumulation of such extracellular material as pillars, partitions, strands, etc., as integral components of fixed desmosomes and hemidesmosomes, a structural basis can be envisioned for Steinberg's concept that these attachments are strong ones, supplementary to the general adhesive tendencies of a given cell (*see* also Overton, 1962). The participation of intracellular filaments in the formation of such strong attachment zones is probably not directly related to adhesion per se, but rather to structural support of the cell skeleton at the attachment sites. Effective supplementary attachment at a given point in a given system therefore involves the appropriate combination of two facilities which topographically are largely or totally separated by the cell unit membrane. These are: (1) adhesion of external cell surface; and (2) internal cell support. The architecture of either of these components appears variable over a wide morphological range to suit the mechanical requirements of any given attachment site.

While the observations and interpretations reported here emphasize presumed mechanical

roles, equal consideration must ultimately be given to physiological processes which may govern and depend upon attachment site morphology. In considering tonofilaments, for example, one need not assume that these are static struts rather than, or in addition to, being elastic, contractile, conductive, or of synthetic value. Desmosomes of adult frog skin have been considered as a possible cell-to-cell diffusion path for sodium ions (Ussing, 1963-1964). The current data on the involvement of septate desmosomes and tight junctions in cell-to-cell permeability (Farquhar and Palade, 1964, 1965; Loewenstein and Kanno, 1964; Wiener, Spiro, and Loewenstein, 1964; Loewenstein et al., 1965), or on synaptic or tight junction adhesive mechanisms governing impulse transmission (Robertson, 1964; Dewey and Barr, 1964) are also suggestive that significant physiological events may be dependent upon specific attachment structures.

In short, cells probably require proximity or attachment for a variety of reasons, both mechanical and physiological. Moreover, conditions of readjustment may prevail according to mechanical or physiological demand, or according to the state of differentiation. The architecture outlined for attachment zones and associated structures might now be considered in terms of these fluctuating parameters.

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