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Ultrastructural characteristics associated with the anchoring of corneal epithelium in several classes of vertebrates

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INTRODUCTION

Tangential thin sections of mouse corneal epithelium, cut in the plane of the basal plasma membrane and examined by electron microscopy (Buck, 1982) reveal hundreds of hemidesmosomes in a single micrograph. They have the form either of linear densities or of chains of smaller densities arranged in roughly parallel rows. The rows tend to lie parallel to the radial axis of the cornea. The only other report of hemidesmosomes being examined *en face* is that of larval newt tail-fin epidermis, studied by high voltage microscopy (Shienvold & Kelly, 1976), the hemidesmosomes appearing as punctate structures, with no tendency to be arranged into rows.

There are several studies of mammalian corneal epithelial hemidesmosomes, referred to later, but descriptions of them in other classes are few. Kaye (1962) finds that, in the frog, they resemble those he had studied earlier in mammals; Newsome (1979), investigating the development of corneal epithelium, illustrates hemidesmosomes of chick, frog, and tadpole corneas; Hay & Revel (1969) have observed the exceptionally irregular basal plasma membrane and its hemidesmosomes in chick cornea. The many other ultrastructural studies of non-mammalian corneas that have been made, particularly in fish, do not comment on the attachment of epithelium to stroma, and do not describe the structure of the hemidesmosomes.

The work reported here began in an effort to confirm in several other mammalian species the observations already made on mice. Then, while investigating in tangential sections the irregularity of the basal plasma membrane reported previously by Hay & Revel (1969), a very different form of hemidesmosome was observed; therefore, a variety of other animals has been examined.

MATERIALS AND METHODS

The present investigation was not a systematic study of the vertebrate cornea, but was rather a survey of a sample of species readily available locally or from commercial animal suppliers. The species studied are shown in Table 1.

Animals were killed by decapitation or overdose of barbiturate, the eyes enucleated and placed immediately in fixative consisting of 2% formaldehyde and 2·5% glutaraldehyde in 0·1 M s-collidine buffer at pH 7·3. After 4 hours fixation at room temperature, the corneas were excised with a razor blade, rinsed twice in 5·4% sucrose in the same buffer, and cut into quadrants. The specimens were post-osmicated for 1 hour in 1% osmium tetroxide in s-collidine buffer at 4 °C, and then immersed in a 1% aqueous solution of uranyl acetate for 1 hour. After dehydration through a graded alcohol series, they were embedded in Epon after a short transition in propylene oxide.

Table 1. *Hemidesmosome characteristics of various species*

Class and common name	Scientific name	Number of animals	Hemidesmosomes /μm^{2*}	% of basal surface*	Pattern
Mammalia					
Domestic cat	*Felix catus*	3	—	31	Chains
Domestic pig	*Sus scrofa*	2	—	47	Chains
New Zealand rabbit	*Oryctogus cuniculus*	6	—	25	Chains
Swiss mouse	*Mus musculus*	5	—	14	Chains
Avia					
White Leghorn chicken	*Gallus domesticus*	2	81	12	Rosettes
Japanese quail	*Coturnix japonica*	1	44	6	Rosettes
Reptilia					
Garter snake	*Thamnophis sirtalis*	2			Punctate
	Spectacle		8	13	
	Cornea		14	3·5	
American chameleon	*Anolis carolinensis*	3	43	8	Rosettes
Amphibia					
Green frog	*Rana clamitans*	2	69	14	Chains
American toad	*Bufo americanus*	2	43	9	Chains
African clawed frog	*Xenopus laevis*	3	46	6·4	Rosettes
Pisces					
Goldfish	*Carassius auratus*	3	0	0	

* Average of counts on hemidesmosome-covered parts of three prints of *en face* sections.

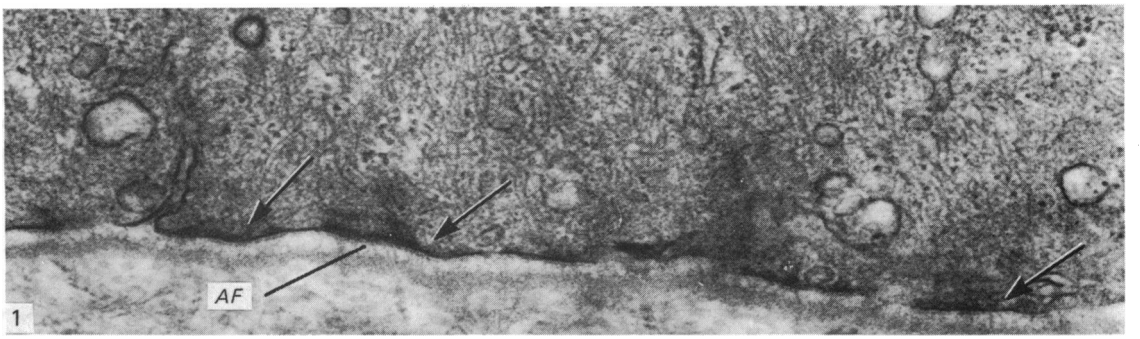
The embedded quadrants of cornea were reoriented after polymerisation so that the sections could be cut either normal or parallel (*en face* sections) to the surface. The sections were cut on a Porter Blum MT 1 microtome with glass knives, picked up on uncoated grids, stained with lead citrate, and examined in a JEOL 1000 CX or AEI 801 electron microscope at 80 kV. Blocks of corneas to be sectioned *en face* were first orientated in the microtome chuck with the edge of the block that had been directed towards the limbus facing upwards. Thus, *en face* sections were cut in the direction parallel to the radial axis of the cornea, and mounted as serial sections in a consistent axis of the grids, which had an orientation marker incorporated in their design.

Fig. 1. Hemidesmosomes (arrows) on the basal plasma membrane of pig corneal epithelium. Some of the tonofilaments of the cytoplasm converge towards the plaques. Traversing the lamina lucida to the basal lamina, numerous anchoring filaments (*AF*) are especially obvious at the sites of the hemidesmosomes. × 47500.

Fig. 2. *En face* sections of mouse corneal epithelium passing through the plane of the hemidesmosomes, and into the stromal collagen (*S*) and the basal cytoplasm (*C*), in which the tonofilaments have been transected. Hemidesmosomes appear as rows or chains of densities. The boundary between two cells follows the irregular course produced by interdigitating processes, some of which have a core of the dense hemidesmosome material, and others have filaments. The radial axis of the cornea runs vertically in this picture. × 36000.

Fig. 3. The cornea of the cat shows a pronounced parallel arrangement of the chains of hemidesmosomes when examined *en face* at low magnification, as in this picture. The interdigitations between two cells are seen. The relationship is readily seen between the parallel chains of hemidesmosomes and the radial axis of the cornea, which runs horizontally in this picture. × 10000.

Fig. 4. The corneal epithelium of *Bufo* examined *en face* shows discrete hemidesmosomes, often arranged in rows (arrows). Complex interdigitation between cells is characteristic. × 25300.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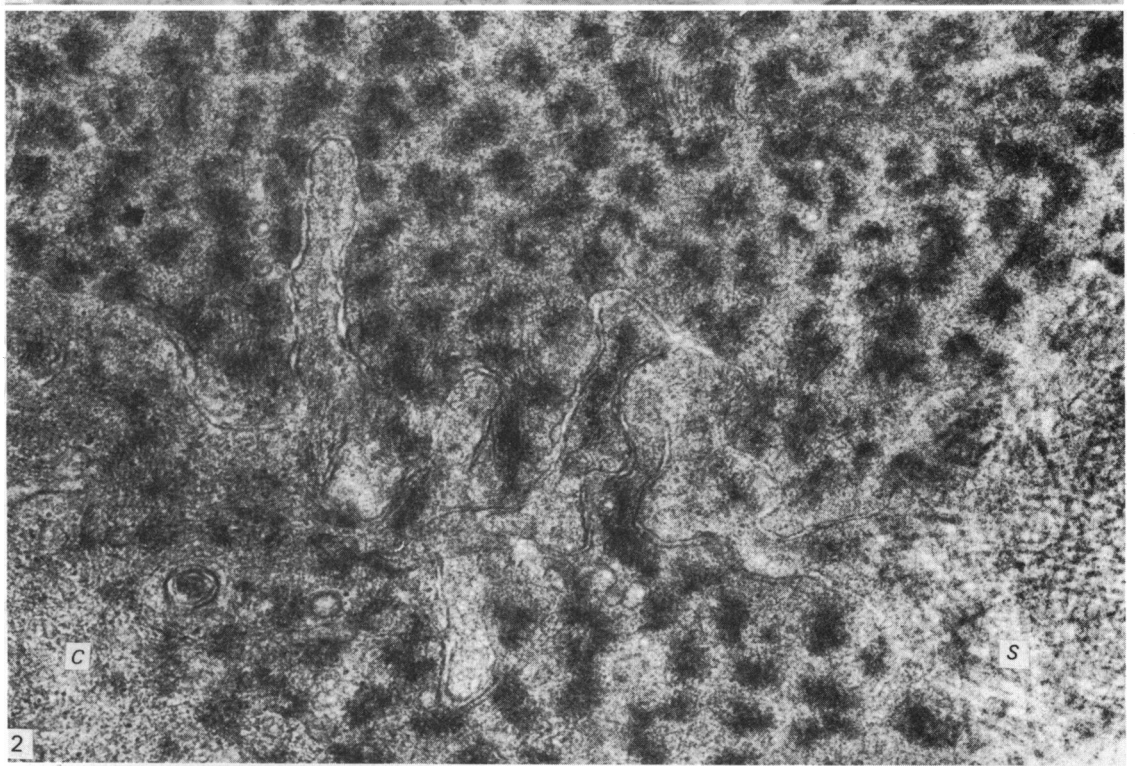
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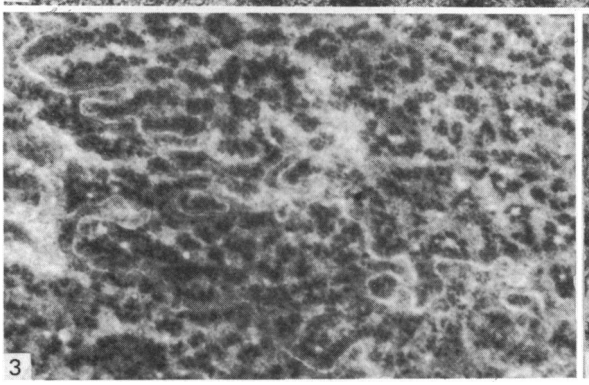
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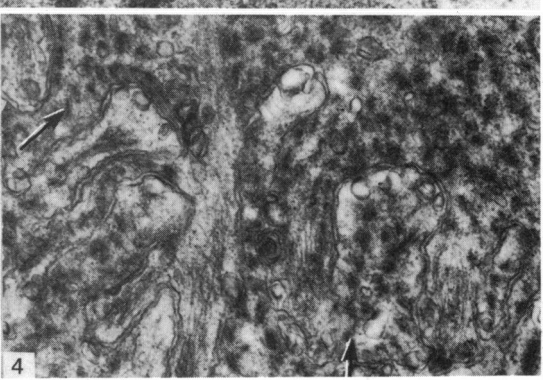
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Section orientation made possible the recognition of any possible relationship of the pattern of hemidesmosomes to the limits and radial axis of the cornea. This relationship was not studied by image analysis, because of the diversity and complexity of the patterns of the hemidesmosomes that were observed. In an earlier study, it was possible to carry out image analysis on the pattern of the hemidesmosomes of the mouse cornea (Buck, 1982). In the present study, the Zeiss image analysis apparatus was used to measure the proportion of the basal surface of the cells which consisted of hemidesmosomes.

Terminology

The clear description of various structures at the interface between epidermis and dermis given by Briggman, Dalldorf & Wheeler (1971) and Briggaman & Wheeler (1975) appears to have influenced others to adopt their terminology, and has been used in the present study. The epidermal-dermal junction has four components: (1) Basal cell plasma membrane with its attached hemidesmosome complexes. (2) An electron-lucid zone, the lamina lucida, traversed by anchoring filaments. (3) The basal lamina (lamina densa). (4) The sub-basal lamina fibrous components – anchoring fibrils, dermal microfibrils, and collagen fibres (unit fibrils of collagen).

RESULTS

As shown in Table 1, great variation was observed in the proportion of the basal plasma membrane occupied by hemidesmosomes. As a group, the mammals showed the highest proportion. It was not usually possible to define the limits of individual hemidesmosomes in the mammals, so that the number of hemidesmosomes per μm^2 could be counted only in the non-mammalian species, and among these there was a tenfold variation.

The pattern in which the hemidesmosomes were arranged is given in Table 1. Four types were recognised: (1) A pattern of chains (*Mammalia, Rana, Bufo*). (2) A pattern of rosettes (*Avia, Anolis, Xenopus*). (3) Punctate hemidesmosomes (*Thamnophis*). (4) Absent hemidesmosomes (*Carassius*).

Specific features of the different patterns of hemidesmosomes

Hemidesmosomes in chains

Many more specimens of mammals were studied than of the frog (*Rana*) or toad (*Bufo*). All five species of mammals showed the same relationship of the cells to the basal lamina. The appearance of this interface in a section cut normal to the surface

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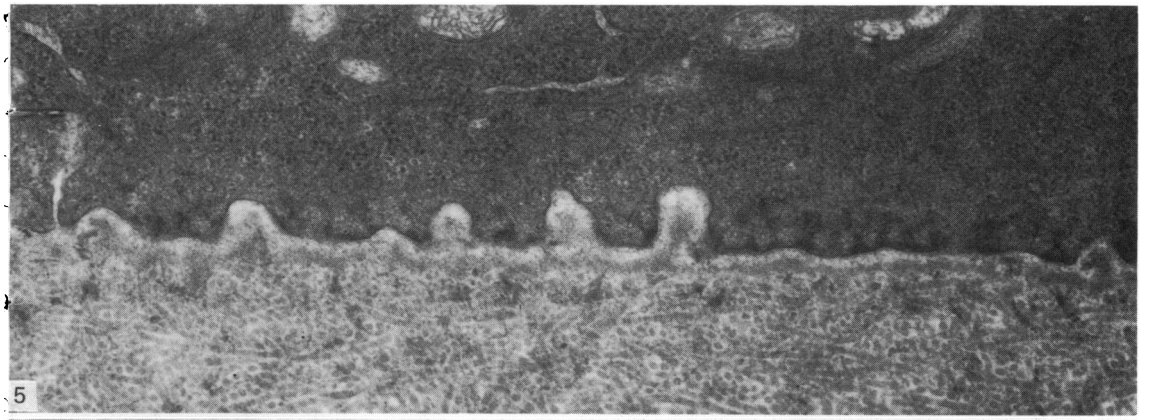
Fig. 5. The basal plasma membrane of the corneal epithelium of the quail is not smooth, as in mammals, but shows numerous pockets spaced at apparently irregular intervals. The lamina densa, as well as the lamina lucida, enters the pockets. The hemidesmosomes are small densities on the membrane between the pockets. They appear to extend up from the plaque in a conical shape, the apex joining a thin layer of filaments about 100 nm inside the cell. Anchoring filaments are seen at the sites of the hemidesmosomes. × 36000.

Fig. 6. This *en face* section through the hemidesmosomes and the plasma membrane pockets in the chicken shows the orderly arrangement of both structures. Each pocket contains a lamina densa centre surrounded by the pale ring of lamina lucida. Some of the pockets contain a few radially arranged filaments, possibly some of the anchoring filaments. Hemidesmosomes appear in the form of rosettes, with 7–9 elongated densities radially arranged about the pocket. Parts of three cells are seen, the meandering intercellular junction showing some of the processes terminating in pockets. × 39000.



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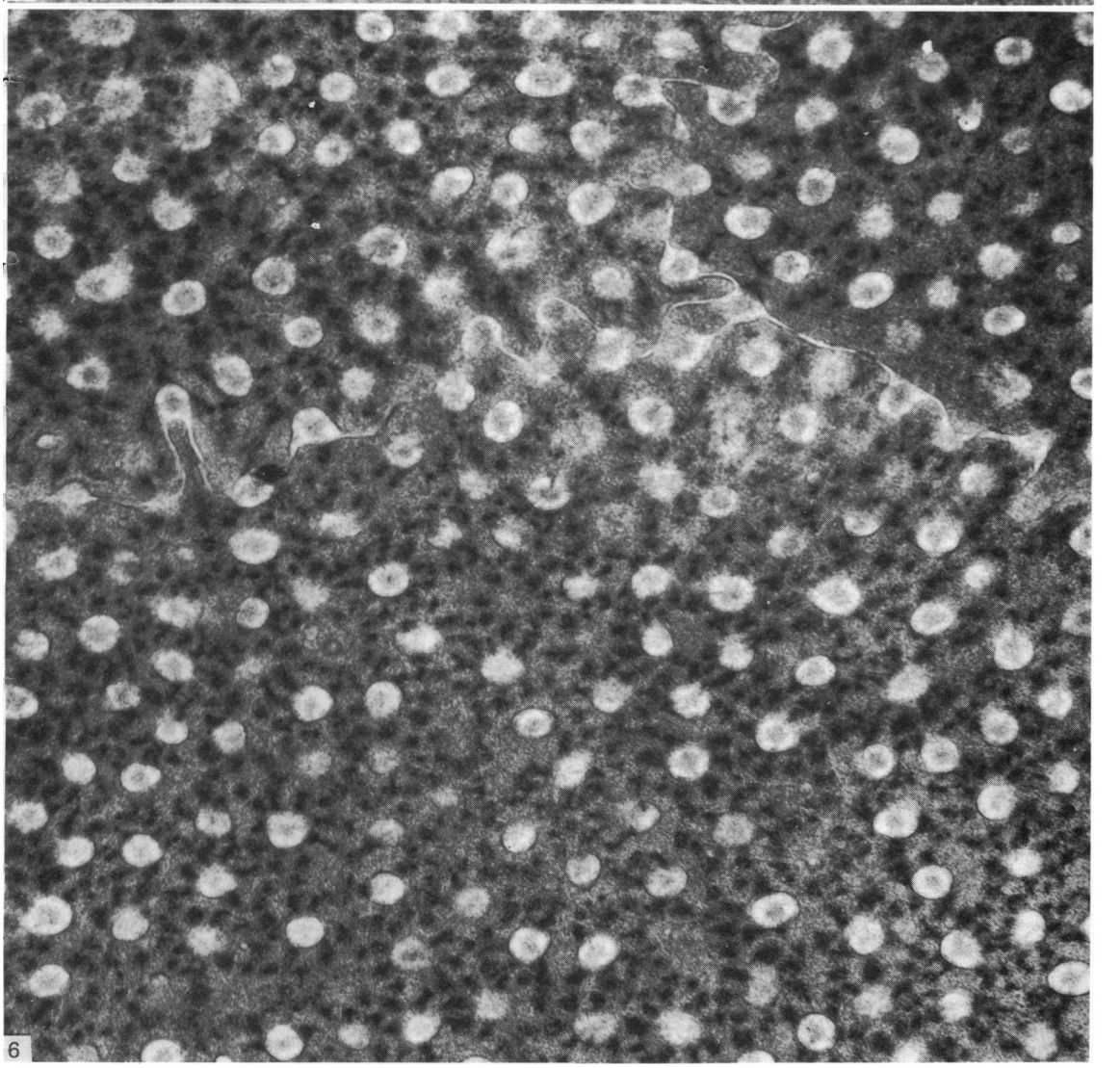

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is shown in Figure 1, which illustrates the typical dense hemidesmosomes of the pig cornea. Normally sectioned corneas of *Bufo* and *Rana* appeared essentially similar.

The hemidesmosomes, spaced at irregular intervals along the basal plasma membrane, consisted of a plaque that might appear as a single density on the membrane or as two densities, the second being separated from the first by a lighter zone. As described in the epidermis (Briggaman & Wheeler, 1975), tonofilaments and anchoring filaments were observed, as well as the increase in thickness and density of the basal lamina opposite the hemidesmosomes. Neither the unique anchoring fibrils (as distinct from anchoring filaments) seen in skin (Swanson & Helwig, 1968) and oral mucosa (Susi, Belt & Kelly, 1967), nor microfilaments of the type found in skin (Kohayasi, 1977) were observed in cornea.

En face sections showed that mammalian hemidesmosomes were much more profuse than might be expected from the normally cut sections. In all mammals they appeared as elongated densities or as chains of smaller densities lying very close together (Fig. 2). The parallel nature of these chains was more obvious in some species than in others, especially in the cat (Fig. 3) and the pig. In these species and in the mouse (the only ones measured) the chains were from 0·2 to 0·6 μm in length (average 0·36 μm). They had a remarkably uniform width of 0·10 to 0·15 μm. The chains of hemidesmosomes in the frog and toad differed from those in mammals in being composed of separate, although rather irregular, densities placed in less obvious rows than those of mammals (Fig. 4). The arrangement into rows was particularly apparent where they extended out along slender cytoplasmic projections.

Hemidesmosomes in rosettes

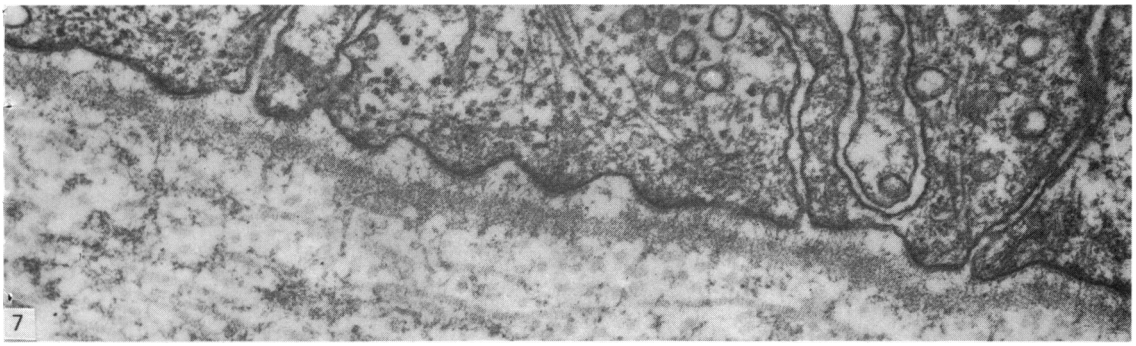
The pattern of hemidesmosomes in the American chameleon (*Anolis*) and *Xenopus* was virtually identical. Although in the form of rosettes around a pocket of the basal plasma membrane in both species, they were less orderly than in the rosettes of avian corneas. Moreover, although a great concentration of these pockets was seen in all three classes, their relation to the lamina densa and the shape of the hemidesmosomes were unique features of the avian corneas.

In the birds, sections cut normal to the surface (Fig. 5) showed an interface with serrations which appeared to be irregularly spaced. The basal lamina followed the contour of the plasma membrane into the serrations. Small densities of the hemidesmosomes were located mainly on those parts of the plasma membrane that projected towards the stroma. The densities did not spread along the membrane, as in all other species studied, instead, they extended up into the cytoplasm, where they appeared to join a thin layer of filamentous material lying about 100 nm from the plasma membrane.

Fig. 7. In *Xenopus*, projecting portions of the serrated basal plasma membrane are the sites of flat plaques of the hemidesmosomes. Tonofilaments sometimes radiate from the plaques. Anchoring filaments are largely confined to the hemidesmosome locations. × 52300.

Fig. 8. The basal surface of the chameleon (*Anolis*) cornea resembles that of *Xenopus* in being highly serrated. In this very thin section, the hemidesmosomes have little density. Thicker sections show that they occupy much of the outward-projecting parts of the plasma membrane. Anchoring filaments are prominent, and are not confined to the location of the hemidesmosomes. × 79800.

Fig. 9. *Xenopus* corneal hemidesmosomes appear in *en face* sections to form rosettes of a dozen or so densities surrounding pockets of the plasma membrane. The pockets contain only lamina lucida, and the tiny dots seen in some of the pockets may possibly represent cross sectioned anchoring filaments of this layer. Stroma (*S*); basal cytoplasm (*C*). × 33600.



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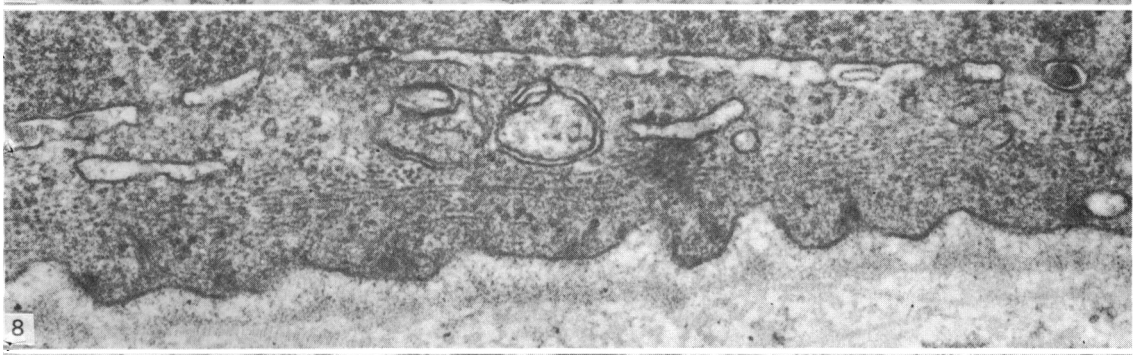

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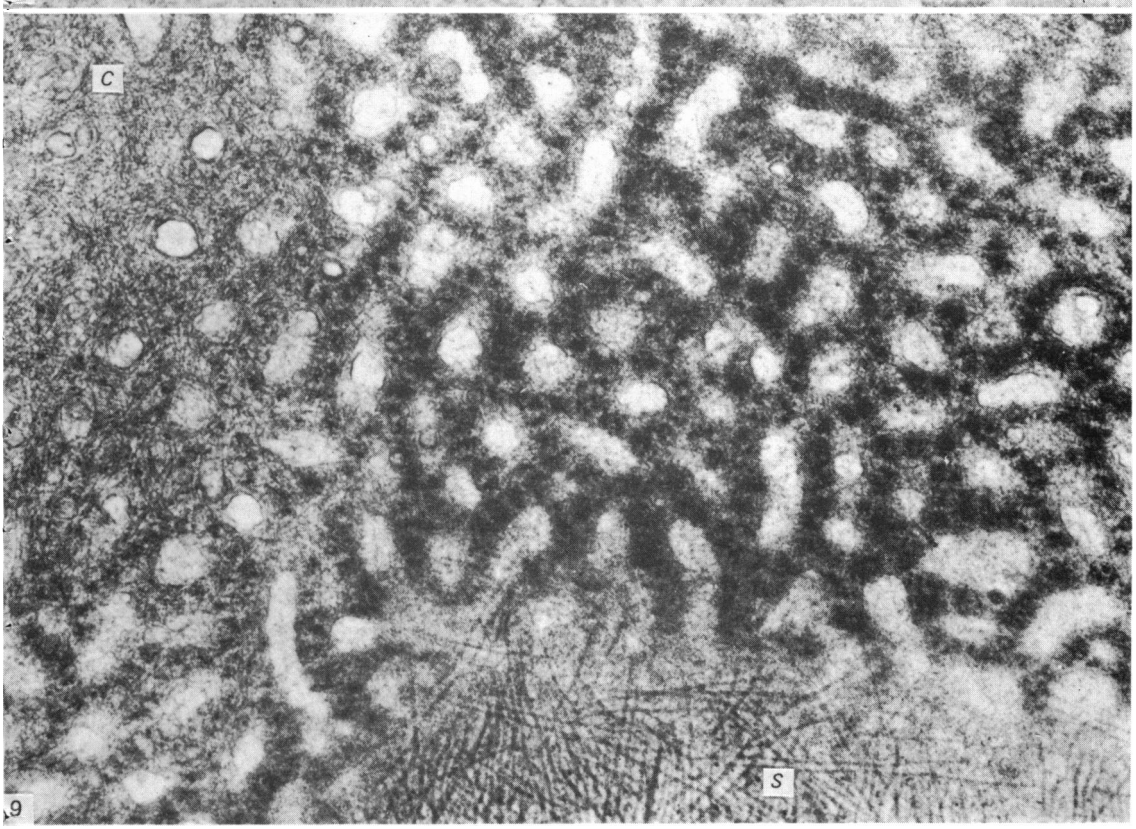

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When seen *en face*, it was found that the serrations of the interface were actually spaced remarkably regularly (Fig. 6). The pockets appeared as membrane-bounded circular profiles, each surrounded by seven to nine elongated hemidesmosome densities arranged radially. In the chicken, the pockets occupied about 17 % of the basal surface, more in the quail. The pockets enclosed a pale lamina lucida and a darker, central lamina densa or basal lamina. Interdigitating processes at junctions between cells frequently had one of the pockets at their tips.

In *Xenopus* (Fig. 7) and the chameleon (Fig. 8), the hemidesmosomes lay flat against protruding parts of the basal plasma membrane between the pockets. The lamina densa only very rarely entered the pockets. The pockets occupied about 19 % of the basal surface in *Xenopus*. Each rosette had about ten to twelve hemidesmosomes, some of them shared with adjoining rosettes (Fig. 9).

Punctate hemidesmosomes

In this pattern, seen only in the snake (*Thamnophis*), individual hemidesmosomes were not arranged into chains or rosettes. The cornea of the snake (and that of certain other vertebrates such as the sea lamprey) is covered by a spectacle of transparent skin, and a fluid fills the space between spectacle and cornea. The snake spectacle is of the tertiary type, the result of fused lids (Walls, 1942). It consisted of a great number of layers of fully cornified cells, the basal cells having prominent tonofilaments which terminated in punctate hemidesmosomes on a very flat basal plasma membrane (Fig. 10). The epithelium of the true cornea, in contrast to that of the spectacle, was only one or two cells thick, had few tonofilaments, and inconspicuous hemidesmosomes which had no special arrangement when seen *en face* (Fig. 11). The vesicles of the basal cytoplasm were conspicuous.

Absence of hemidesmosomes

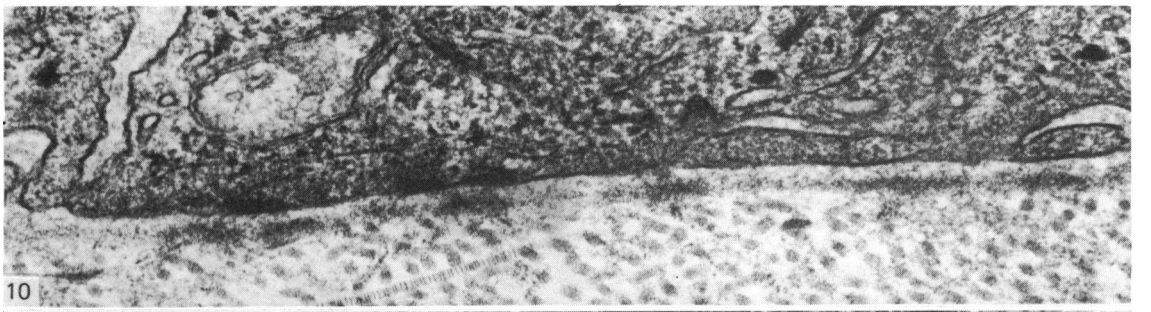
A very irregular course was followed by the lateral plasma membranes of contiguous basal cells of the goldfish (*Carassius*) cornea, but the basal plasma membranes were flat (Fig. 12). The basal cytoplasm was highly fibrillar. The cells rested on a flat, very thick, basal lamina. No hemidesmosomes were seen in sections cut normal to the surface. *En face*, the most basal part of the cytoplasm showed a finely stippled appearance, possibly the anchoring filaments in cross section (Fig. 13).

Fig. 10. The spectacle of the snake is highly keratinised, and apparently well anchored with prominent hemidesmosomes. The density caused by the anchoring filaments traversing the lamina lucida at the hemidesmosome sites is readily seen. The lamina densa is also much denser at these points. × 68400.

Fig. 11. The true cornea of the snake (and also its spectacle) has isolated, or punctate, hemidesmosomes lacking any special arrangement. The irregular densities of this true cornea are scattered on the plasma membrane between the numerous pinocytotic vesicles that open on to the membrane. Stroma (*S*); basal lamina (*BL*). × 52300.

Fig. 12. The goldfish cornea has a flat, uniform basal surface against a prominent basal lamina. No focal hemidesmosome densities are present, but the entire basal plasma membrane receives the insertion of anchoring filaments that seem to emerge from the lamina densa. Their great number gives the lamina lucida a much higher density than that of other animals. × 87500.

Fig. 13. Goldfish cornea in *en face* section shows no evidence of focal hemidesmosome densities as the plane of the section passes from the stroma (*S*) into the basal cytoplasm (*C*). A few vesicles and the dense array of tonofilaments (*T*) are present in the basal cytoplasm. The anchoring filaments cannot be positively identified in sections cut in this plane. × 35000.



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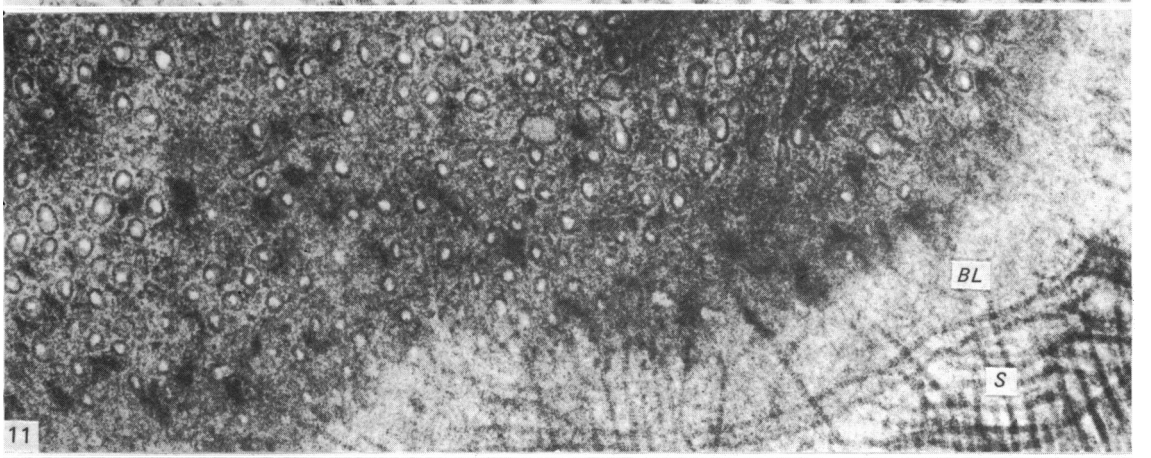

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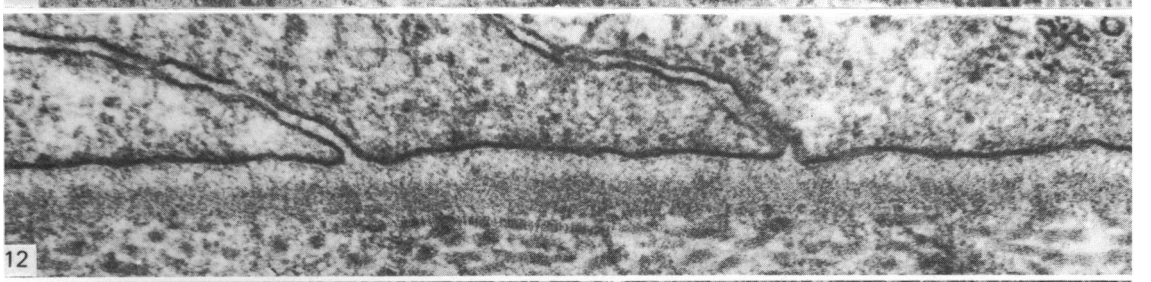

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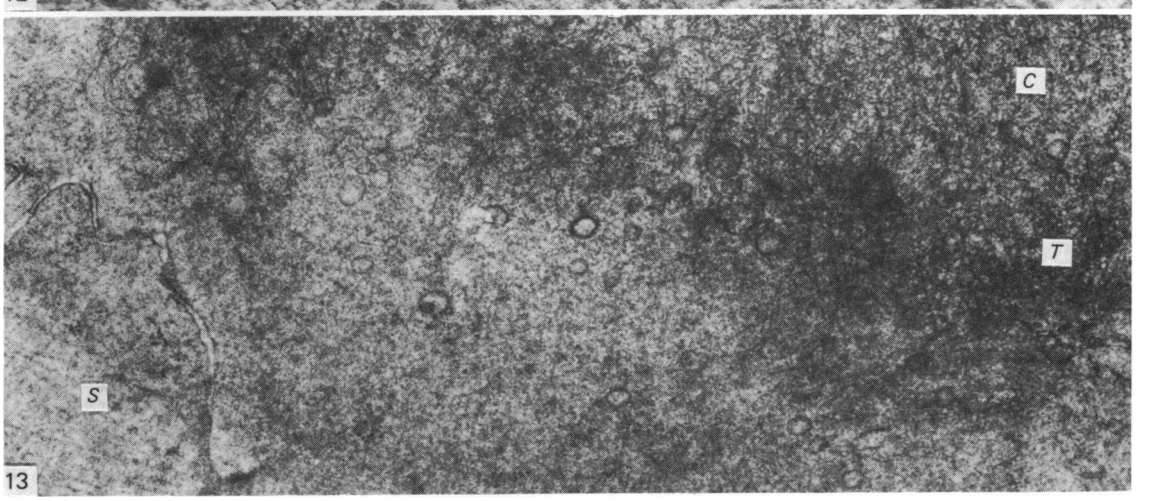

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Universal features of the basal attachment in all species

(1) The basal lamina was of variable thickness from species to species, but always consisted of a lamina densa separated from the basal plasma membrane by a pale lamina lucida (Figs. 1, 5, 7, 8, 10, 12). (2) There were vesicles in the most basal part of the cytoplasm (Figs. 4, 9, 11, 13) measuring 50–100 nm in diameter. They resembled pinocytotic vesicles described in other tissues, and at least some of them appeared to open on to the plasma membrane. The vesicles were much more apparent in sections cut *en face* than in corneas sectioned normally. (3) Anchoring filaments traversed the lamina lucida. Even in the goldfish, where hemidesmosomes were not observed, fine filaments disappeared into the plasma membrane (Fig. 12). Connections with tonofilaments of the cytoplasm were not seen. In other species, anchoring filaments tended to be associated with the hemidesmosomes, but not exclusively so in the mammals nor in the chameleon; in this species, the filaments inserted into the whole of the plasma membrane (Fig. 8), without preference for hemidesmosome sites. (4) Basal cytoplasmic filaments, or tonofilaments, were present in large numbers inserting into hemidesmosomes (Figs. 1, 2, 4, 7), except in the true cornea of the snake where they were scanty. The snake spectacle had large numbers of tonofilaments (Fig. 10). Usually, tonofilaments were better demonstrated *en face* than in sections cut normally. (5) Interdigitating processes were present (Figs. 2, 3, 4, 6). The basal cells of all corneas sent out foot-processes to underlie adjoining cells. The processes were often several micrometres in length and between 0·1 and 0·3 μm in width. They were especially prominent and complex in mammals, *Rana* and *Bufo*. Except in the goldfish, hemidesmosomes were present within the core or at the termination of the processes (Figs. 2, 6).

DISCUSSION

Although hemidesmosomes are numerous and prominent in a variety of epithelia, such as epidermis (Weiss & Ferris, 1954; Kelly, 1966; Briggaman & Wheeler, 1975) and urogenital sinus epithelium (Flickinger, 1970), as well as in corneal epithelium (Kaye, 1962; Buck, 1982), the pattern of their distribution on the interface with the connective tissue is difficult to determine except in cornea, because in most of the other situations the interface is too irregular to be cut in the plane of the hemidesmosomes. The larval tail-fin epidermis is also suitable, and has been much studied by Kelly and his co-workers (Kelly, 1966; Shienvold & Kelly, 1976; Kelly & Kuda, 1981). They have provided a detailed analysis of the filamentous insertions into hemidesmosomes, obtained by freeze-fracture methods as well as conventional and high voltage transmission electron microscopy. In addition, these authors describe the size and shape of the hemidesmosomes which are punctate structures measuring 0·08–0·13 μm in diameter, and are elliptical rather than circular, with the long axis of the ellipse parallel to the long axis of the tail-fin. They do not appear to be arranged in chains although the authors did not comment on this aspect.

The size of mammalian corneal epithelial hemidesmosomes, as seen *en face*, is indefinable because they are often joined together to form quite elongated densities. Measurements made on normally sectioned mammalian corneas may be unreliable unless the axis of sectioning in relation to the corneal radius is known. On the other hand, the width of the chains of hemidesmosomes is rather uniform, measuring 0·10–0·15 μm in all mammalian species studied. This is similar to the value of

0·15 μm, given by Whitear (1960), for the diameter of mouse corneal hemidesmosomes. White & Gohari (1978) have studied the size and distribution of hemidesmosomes of hamster cheek pouch epithelium, using stereological methods. It can be calculated from their data that the hemidesmosomes measure 0·23 μm in diameter on average; in another paper, these authors report a size of 0·2 μm (White & Gohari, 1981).

A function ascribed to hemidesmosomes, from the time of their first description in larval amphibian epidermis by Weiss & Ferris (1954), is that of anchoring the basal cells to the connective tissue through the intervening basal lamina. The tonofilaments radiate to desmosomes situated between basal cells and cells of a more superficial layer, so that the whole epithelium is held together and bound down with sufficient firmness to resist avulsion by the physical forces to which stratified epithelia are potentially or actually subjected.

In the cornea, the exposure to abrasion obviously depends mainly on the habitat of the different species, and on the amount of protection afforded the cornea by such adnexal structures as eyelids, nictitating membrane, or spectacles. If the epithelium is abraded, the amount of damage sustained depends on a number of structural features, variable from class to class, or even from species to species. These include the number of layers of epithelial cells which, in mammals, varies from 3–5 in the mouse to 12–18 in the blue whale (Ehlers, 1970), the degree of cornification of the superficial cells and desmosomes between cells. These features are outside the scope of the work reported here, which is concerned only with the attachment of the basal layer of cells.

The great variation seen in the basal attachment may also relate to the effects of abrasion on the integrity of the epithelium in the various classes studied. Specific features relating to the attachment of corneal basal cells include: (1) The size, number and pattern of the hemidesmosomes. (2) The number and prominence of the anchoring filaments. (3) The area of the interface, as affected by the presence or absence of pockets of the basal plasma membrane. (4) The interdigitation of foot processes extending out from the cells of the basal layer. Each of these factors is now considered in turn.

(1) *Size, number and pattern of the hemidesmosomes*

The hemidesmosomes in mammals occupy a higher proportion of the basal plasma membrane than do those in other animals studied (Table 1). In birds, chameleon and *Xenopus* no hemidesmosomes are present on the plasma membrane of the pockets. A few tiny hemidesmosomes are seen in the protected snake cornea, although they are more conspicuous in the spectacle. In the goldfish, hemidesmosomes are absent.

Patterns of hemidesmosomes seen *en face* also vary, and the mammals, and (in a less orderly way) *Rana* and *Bufo*, show an arrangement into linear chains. In the mammals, these chains are orientated parallel to the radial axis of the cornea. Although this relationhsip was only detected in the mouse cornea by image analysis of electron micrographs (Buck, 1982), it is strikingly obvious in some other species, such as the cat and pig. The rosette, another type of pattern, seen in birds, chameleon, and *Xenopus*, is not influenced by the corneal axis, but is related instead to the numerous pockets of the basal plasma membrane which the hemidesmosomes encircle. The location of hemidesmosomes on the plasma membrane between the pockets, rather than on the pockets themselves, seems to be necessary so that the anchoring filaments can insert into them.

(2) *Anchoring filaments*

Anchoring filaments are seen traversing the lamina lucida of all species examined, although they are not prominent in the snake cornea. The degree of adhesion required by the snake corneal epithelial cells is expected to be small because they are protected from abrasion by the spectacle.

A profuse array of anchoring filaments is seen in the goldfish which, lacking hemidesmosomes, has the entire basal plasmalemma permeated by these fine filaments. Apparently, the stiffening of the basal cell by the abundant keratin filaments, combined with the binding down of the whole interface by the anchoring filaments, provides the adhesion needed for these lidless eyes.

Chameleons share with the goldfish an abundance of anchoring filaments permeating the whole basal plasmalemma, although hemidesmosomes are present as well. A remarkable characteristic of the cornea of this reptile is the extreme thinness of the epithelium, which consists of only two layers of cells. Possibly, this feature makes it unusually vulnerable to injury, and the profusion of anchoring filaments has a special protective function.

(3) *Area of the interface*

Infoldings of the basal plasma membrane in sections normal to the epithelium of the chick cornea have been observed and illustrated by Hay & Revel (1969). They suggest that the irregularities of the plasma membrane would be expected to provide a firmer attachment than a plane surface. The pockets obviously produce a considerable increase in surface area, although they limit the number of hemidesmosomes, which are confined to the membrane between pockets. It is puzzling that a particularly firm adhesion would be required for the birds, as they have the protection of both a nictitating membrane and eyelids. The other animals with an irregular plasma membrane may be vulnerable to abrasion: the chameleon, as mentioned above, because of the thin epithelium, and *Xenopus* because, although possessing a nictitating membrane, it has no lids. In this respect *Xenopus* differs from *Rana* and *Bufo*, and it is remarkable that its corneal hemidesmosome pattern should also be so different from theirs, and so similar to that of the chameleon. Obviously, the study of additional species of amphibia might provide some insight into this problem.

(4) *Interdigitating foot-processes*

An additional factor affecting epithelial adhesion would be expected to be the extent to which foot-processes spread and project out on the basal lamina, to underlie the basal surface of adjoining cells. This is a feature of all corneas studied, and it has been observed in the mouse previously (Buck, 1982). Usually, in the mammals, a chain of hemidesmosomes is found in the core of these processes, and in birds, the chameleon, and *Xenopus*, the foot processes often terminate in a pocket of the plasma membrane surrounded by hemidesmosomes. Thus, in these animals, it is apparent that the foot processes are well anchored; although they do not increase the area of the epithelium contacted by the basal lamina, they have the effect of extending the region of contact well beyond the body of the cell.

In conclusion, there is no doubt that several factors have a role in providing adhesion for corneal epithelium, and that the relative importance of each may differ from one species to another. It is apparent that there is no absolute requirement for any particular type of hemidesmosome. Hemidesmosomes may be large or small,

they may be numerous, scanty or even absent, and may be arranged in chains, rosettes or may lack an arrangement. This variation does not diminish the importance of their function in anchoring the types of epithelium in which they are readily seen.

But if, as seems likely, hemidesmosomes have an anchoring function, this can only be mediated by the insertion into them of the anchoring filaments. It is apparent from the goldfish cornea that these ubiquitous filaments do not require focal hemidesmosome plaques for their insertion. Therefore, we should search for the filaments in other types of epithelium which lack obvious hemidesmosomes, but which still need a firm anchorage to the basal lamina. In fact, after fixation with tannic acid, filaments running transversely through the lamina lucida have been observed in association with pulmonary alveolar cells, which lack hemidesmosomes (Vaccaro & Brody, 1981). Epithelia subject to physical forces might be expected to show the filaments. Examples would be the epithelia of the colon and the rectum, and possibly those of the cervix, nasal cavity and trachea.

SUMMARY

The electron microscopic examination of the basal cells of corneal epithelium of certain species of *Mammalia*, *Avia*, *Reptilia*, *Amphibia* and *Pisces* was directed particularly towards the hemidesmosomes. Sections cut normal to the basal lamina and sections cut parallel to it were studied in order to establish the number, shape and distribution of the hemidesmosomes. Four basic types of hemidesmosome distribution were recognised among a limited representation of the classes studied. (1) Linear chains of hemidesmosomes (*Mammalia*, *Rana*, *Bufo*). (2) Rosette arrangement of hemidesmosomes surrounding pockets of basal plasma membrane (*Avia*, *Anolis*, *Xenopus*). (3) Punctate hemidesmosomes with no arrangement (*Thamnophis*). (4) Absence of hemidesmosomes (*Carassius*). All animals showed a basal lamina, basal pinocytotic vesicles, anchoring filaments, tonofilaments, and interdigitating foot-processes. It is suggested that anchoring filaments deserve to be studied more thoroughly in certain other types of epithelia which do not have focal hemidesmosomes, but require firm anchorage to a basal lamina.

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