A SPECIAL FIBRIL OF THE DERMIS

GEORGE E. PALADE and MARILYN G. FARQUHAR

From The Rockefeller University, and the Department of Pathology, University of California Medical School, San Francisco

ABSTRACT

A new type of extracellular fibril is described in the dermis of *Bufo marinus*, *Rana pipiens*, and *Amblystoma punctatum*. It is restricted in distribution to the dermal micropapillae and the region immediately below them in the *stratum spongiosum*. The fibrils (diameter = 200 to 750 A) are lateral aggregates of fine filaments and have a unique banding pattern characterized by absence of recognizable periodicity and by polarization in respect to the basement membrane. Their distal[1] ends are anchored in the basement membrane, and their proximal ends converge in knots located in the middle region of the micropapillae. These anchoring fibrils seem to secure the minute outfoldings of the basement membrane along the dermal-epidermal junction.

Comparable, but less frequent, fibrils are also encountered along the proximal aspect of the basement membrane in the skin, lingual mucosa, and mucosa of the gastric fundus in the rat.

In a previous study of the amphibian skin (1), we noted the occurrence of a special extracellular fibril anchored on the dermal side of the basement membrane of the epidermis. This paper presents the observations made so far on the structure, relations, and distribution of this new fibril.

MATERIALS AND METHODS

Observations were carried out on the following species and organs:

Toad (*Bufo marinus*): skin from the abdomen, chest, back, thigh, interdigital web, and nictitating membrane.

Frog (*Rana pipiens*): skin from the abdomen, chest, and back.

Salamander (*Amblystoma punctatum*): skin from the tail of larvae approaching metamorphosis.

Rat (*Sprague-Dawley*), adult: skin from the lip, ear, hind legs, and tip of nose—lingual mucosa, and mucosa of the fundic portion of the stomach; newborn (1- and 2-day-old): skin from the abdomen and the back.

Specimens were prepared for electron microscopy as in (1), except that some OsO_4-fixed tissues were stained in block, before dehydration, in 1 per cent Na phosphotungstate in acetate-Vernol buffer at pH 4.0 or in 1 per cent K phosphotungstate in the same buffer at pH 7.0 or 9.0.

OBSERVATIONS

The following description is based primarily on observations made on the skin of the toad and frog in which the fibrils were originally found and extensively studied.

Morphology

The fibrils with which we are concerned vary in diameter from 200 to 750 A (Figs. 1 and 2) and appear to be formed by the lateral aggregation of finer filaments (Fig. 4). Their length cannot be determined, for they branch and fuse frequently and seem to be organized in irregular tridimensional networks rather than in bundles of long

[1] *Distal* and *proximal* will be used in this paper in reference to the surface (distal) and center (proximal) of the body.

parallel fibrils. In addition, they usually follow a curved course and hence remain for only short distances in the plane of any section. The longest, unsplit fibril so far encountered measures ~ 400 mμ. The banding pattern of these fibrils, shown and described in detail in Fig. 2, is characteristic and unusual. As far as we know, it does not resemble any fibrillar striation yet recorded; it does not have a recognizable repeating unit, and it is "polarized" in relation to the basement membrane; *i.e.*, in most cases it shows the same band sequence on the distal[1] end of the fibril, the end that approaches or contacts this membrane (Figs. 1 and 2). The band pattern can be detected only in some of the thicker fibrils, presumably those lying in the plane of the section. No banding has been resolved in fibrils or fibril branches thinner than ~300 A. With our procedures, these fibrillar elements are stained more intensely than collagen fibrils by uranyl (Figs. 1 to 3) as well as phosphotungstate ions. The two "stains" reveal the same striation, but the relative density they impart to the bands is different, and contrast between light and dense bands is higher in uranyl-stained specimens. When treated with aqueous phosphotungstate at pH 4.0, the fibrils split into divergent filaments of $\lesssim$100 A and the banding pattern is lost.

Relations to Other Dermal Structures

In adult amphibian skin, the fibrils are restricted to the upper region of the *stratam spongiosum* of the dermis, more precisely to the miniature dermal protrusions previously described as micropapillae (1) and to the region immediately below them. Sections normal to the dermal- epidermal junction show that the fibrils are distributed at random along this boundary and approach the basement membrane normally or obliquely, usu-

General Abbreviations

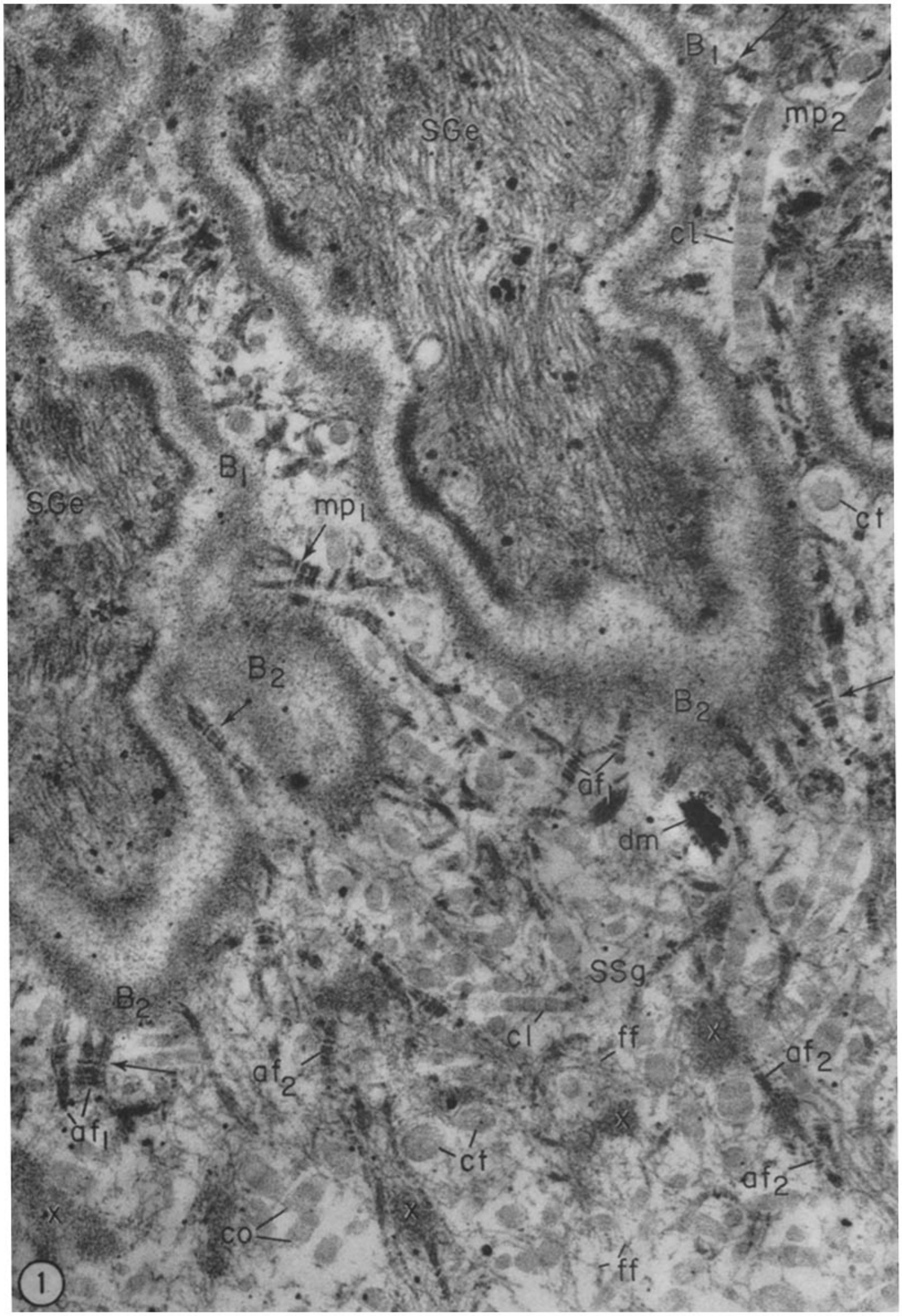
Epidermis, *stratum basale sive germinativum*	*SGe*	Fine fibrils (diam ≃ 100 A)	*ff*
		Collagen fibril	
Dermis, *stratum spongiosum*	*SSg*	in longitudinal section	*cl*
Basement membrane	*B*	in transverse section	*ct*
Anchoring fibril	*af*	in oblique section	*co*

All micrographs represent preparations of toad (*Bufo marinus*) skin fixed in 1 per cent OsO_4 in 0.1 M phosphate buffer (pH 7.4 – 7.6) for 2 hours at 0°, dehydrated in graded ethanols, and embedded in Epon, except for the specimen in Fig. 6 which was fixed in 2.5 per cent glutaraldehyde in 0.1 M phosphate buffer (pH 7.6) for 2 hours at 0° and subsequently treated as above. All sections were doubly stained in uranyl followed by lead (*cf.* 1).

FIGURE 1 Oblique section through the dermal-epidermal junction in abdominal skin of *Bufo marinus*. The epidermis (*Stratum basale sive germinativum*) is marked *SGe*, the dermis (*stratum spongiosum*) *SSg*, and two miniature dermal protrusions or micropapillae mp_1 and mp_2. The basement membrane is sectioned normally at B_1 and obliquely at B_2. Farther on, in the lower half of the field, this oblique section grazes or shaves off the tips of a whole series of interpapillary infoldings of the basement membrane, which appear as isolated patches of felt-like, moderately dense material (*x*).

Numerous anchoring fibrils can be seen in the dermis either approaching (af_1) the continuous basement membrane (B_1, B_2), or converging (af_2) on its shaved-off infoldings (*x*). Most of these fibrils have a stem that shows a characteristic banding pattern (short and long arrows). Some of them split into two or more branches (long arrows) that are apparently anchored in the basement membrane.

Fine filaments (*ff*) can be seen in the micropapillae and especially in the immediately subjacent dermal region. They occur either individually or in skeins and, like the anchoring fibrils, converge on the shaved-off tips of the basement membrane infoldings. Collagen fibrils, recognizable by their characteristic periodicity and moderate density, occur in longitudinal (*cl*), oblique (*co*), and transverse (*ct*) sections throughout the same regions of the dermis. The dense mass at *dm* is probably an anchoring fibril torn away from the rest of the tissue during sectioning. × 65,000.



B1
mp2
SGe
cl
B1
SGe
mp1
ct
B2
B2
af1
dm
SSg
B2
cl
ff
af2
af2
af1
ct
af2
co
ff
x
x
x
x
1

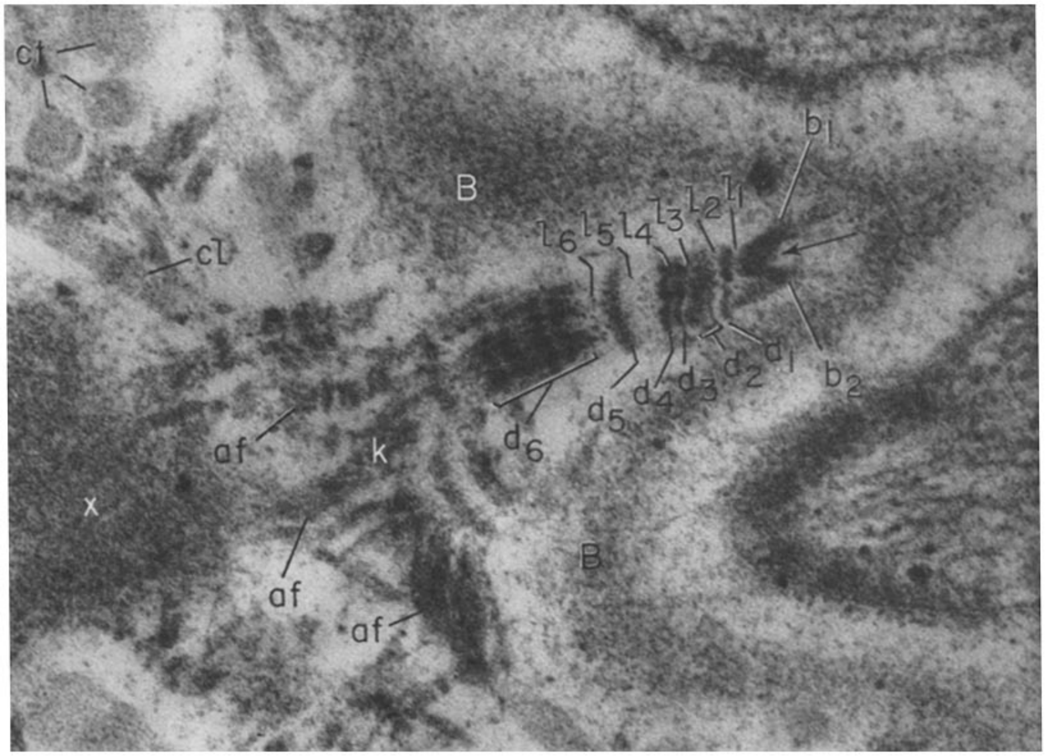


FIGURE 2 Longitudinal section through a dermal micropapilla showing, at a higher magnification, a special fibril with its banded stem (l_1 to d_6), branching point (arrow), and branches (b_1, b_2) that anchor it into the basement membrane (*B*). The characteristic banding pattern of the stem consists of the following sequence of light (*l*) and dense (*d*) bands numbered from the branching point towards the apparent proximal end of the fibril: l_1, ~80 A; d_1, ~80 A; l_2, ~80 A; d_2, ~240 A; l_3, ~50 A; d_3, ~80 A; l_4, ~65 A; d_4, ~80 A; l_5, ~400 A; d_5, ~160 A; l_6, ~240 A; and d_6, ~1260 A. The last dense band (d_6) is actually a complex of at least 6 secondary bands of moderate to high density. Note that there is no repetitive pattern in the banding.

A comparison with Figs. 1 and 3 to 6 shows the high frequency, if not the ubiquity, of this pattern in fibrils properly oriented in the plane of the sections. The sequence l_1 to l_5 appears to be the most constant part of the pattern. Note that the pattern is polarized: the same bands (l_1 to l_5) appear in the same sequence at the distal end of the fibrils. At *x* is the shaved-off tip of a basement membrane infolding, and at *k* a knot formed near the base of the micropapilla by 3 or 4 converging anchoring fibrils. × 130,000.

ally branching 70 to 100 mμ before reaching it. The branches measure 300 A or less in diameter and appear to enter the basement membrane and become part of its texture. It may be assumed that this insertion or anchoring involves an intertwining of the incoming branches with the matted filaments of the basement membrane proper. Anchoring points do not occur in any constant relationship to the half-desmosomes of the epidermis. Oblique or grazing sections through the micropapillae show that anchoring fibrils often converge from all directions on the infolded areas of the basement membrane which cover the interpapillary protrusions of epidermal cells (Fig. 3).

Relations at the proximal ends of the fibrils are less clear: most fibrils are simply lost from the plane of the section; others appear to end in knots formed by similar convergent elements (Figs. 4 to 6), or to enter small areas of felt-like material, which presumably represent patches of basement membrane shaved by oblique sectioning. These fibrils form, therefore, a network located under, and anchored in, the basement membrane. The network is particularly tight within the micropapillae (Fig. 4), where the fibrils connect one side of the outfold to the other by short cutting across (Fig. 5). Direct connections by single continuous fibrils are, however, absent or rare. Indirect connections involving knots of fibrils seem to be the rule. This structural arrangement suggests that the anchoring fibrils secure the outfolds of basement membrane that cover the micropapillae.

Individual collagen fibrils, reliably identified by their characteristic periodicity,[2] wave through the meshes of the network formed by the special fibrils (Figs. 1, 3); they appear as less dense, distinct elements frequently separated from the latter by a clear, narrow halo (Fig. 3).

Fine filaments, 50 to 100 A in diameter, of the type seen in connective tissue in many other locations (2–4) occur in large numbers in the dermis. They are particularly frequent in the region immediately below the micropapillae where they form irregular skeins around the special fibrils and the tips of the basement membrane infoldings (Fig. 1). Images suggestive of fusion of such filaments with anchoring fibrils are occasionally encountered, but, with the evidence available, it cannot be decided whether the two structures are entirely different or represent the same fibrillar component(s) in two different states of aggregation.

Distribution

Anchoring fibrils have been found in the skin of the toad and frog in all the locations mentioned under *Materials*, their frequency increasing with the frequency and size of the micropapillae. In general, they are more numerous in the toad than in the frog, which has a much less elaborately sculptured dermal-epidermal junction; least frequent in the toad in the dermis of the nictitating membrane, in which micropapillae are small and few in number; and only occasionally encountered in the dermis of *Amblystoma punctatum* larvae which is almost free of micropapillae.

Spotty observations indicate that anchoring fibrils are also present in the rat beneath the basement membranes of: (*a*) the skin epidermis,[3] (*b*) the lingual epithelium, and (*c*) the epithelium of the fundus of the stomach. They are, however, much less frequent and show a less distinct banding pattern than in the amphibian specimens mentioned. In all the sites listed in the rat, the basement membrane underlies a stratified, squamous, totally or partially keratinized epithelium.

DISCUSSION

The elements here described apparently represent a new entity among connective tissue fibrils. A survey of the literature indicates that they have not been recognized before, except for Brody (5), who mentioned the presence of "reticular filaments" under the basement membrane of the human epidermis, but did not further elaborate on their properties. One of his micrographs (Fig. 3) shows quite clearly, however, that he was dealing with the same type of fibril.

With the available information, it is useless to speculate about the chemical nature of these elements. It is clear only that they are morphologically distinct from native collagen fibrils as well as from other forms of tropocollagen aggregates studied *in vitro* (6–8). They are also distinct from the fibers, tactoids, sheets, or bodies described *in situ* (9–29) or in culture (30) in a variety of species, locations and conditions, and characterized by a truly periodic band pattern with a repeat of 700 to 1200 A. It is assumed in all these references (9–30) that such banded aggregates are comprised of tropocollagen,[4] but this assumption is reasonably well founded only in the case of Descemet's membrane of the cornea studied by Jakus (9, 10); in the other cases, it is based primarily on the demonstration of a periodicity resembling that found in Descemet's membrane.

In the skin, anchoring fibrils occur in the same location as the reticulin fibers of the light microscope literature (31, 32); yet, their identification as "reticulin" is unlikely since: (*a*) their diameter is definitely below the limit of resolution of the light microscope; (*b*) they are extremely rare in the skin of the newborn rat, in which reticulin fibers are abundant; (*c*) in other tissues, anchoring fibrils appear to have a distribution much more

---

[2] In fixed, embedded, and sectioned preparations, the repeat measures $\sim$ 530 A, *i.e.* considerably less than the 640 to 700-A period seen in isolated collagen fibrils.

[3] In addition, the basement membrane of the rat epidermis is anchored to the dermis by relatively large bundles of fine [diam $\simeq$ 100 A] filaments of the type described in this paper and in references (2–4).

[4] See, however, reference 28 which describes the occurrence of such masses in the cytoplasmic matrix of the sensory epithelial cells of the human macula.

restricted than that ascribed to argyrophilic reticulin fibers in light microscopy; and (*d*) according to Gross (33) and Bear (34), reticulin fibers are young collagen fibrils of small diameter but usual periodic structure.

It may also be premature to speculate on the function of these new fibrils. Yet, it is worthwhile pointing out that they seem to be of widespread occurrence among vertebrates (salamander, frog, toad, rat, and man (*cf.* 5)), and so far have been found beneath the basement membrane of stratified squamous epithelia which are subject to considerable friction and have a base provided with numerous, minute folds. Such epithelia are attached to their folded basement membrane by half-desmosomes (35), basal desmosomes (11, 12), or "bobbins" (36). The folds of the basement membrane seem to be secured by the underlying network of anchoring fibrils here described, and the whole complex linked to the rest of the dermis by the collagen fibrils that loop through this network.

This work was supported by research grants HE-05648 and AM-09090, from the United States Public Health Service, and by Public Health Service research career program award 1-K3-GM-25,109.

We gratefully acknowledge the technical assistance of Mrs. Lee Adinolfi and Mrs. Karin Taylor.

Addendum

Very recently, fibrils 150 to 200 A in diameter which are "connected to the lamina densa (*i.e.*, basement membrane) at right angles" and have "no definite periodicity" have been noted in the stroma of the human ectocervix by Younes *et al.* [37]. Their micrographs strongly suggest that they have been dealing with a type of fibril similar to the one here reported.

Received for publication, May 25, 1965.

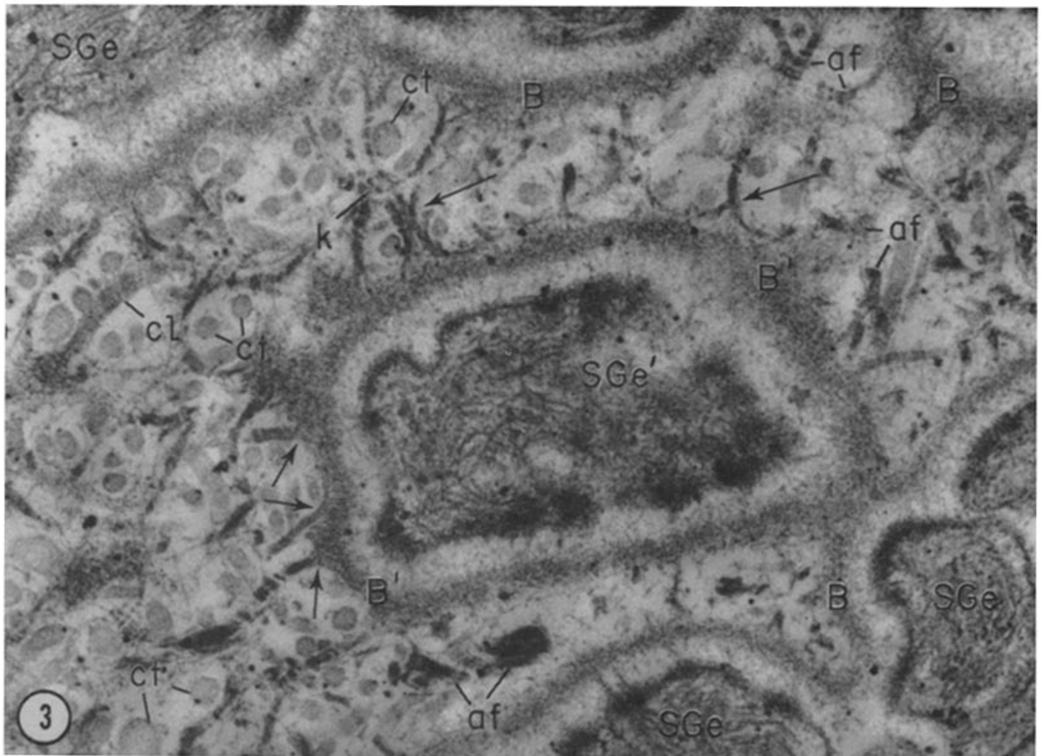
FIGURES 3 and 4 Oblique sections through the dermal-epidermal junction in the abdominal skin of the toad.

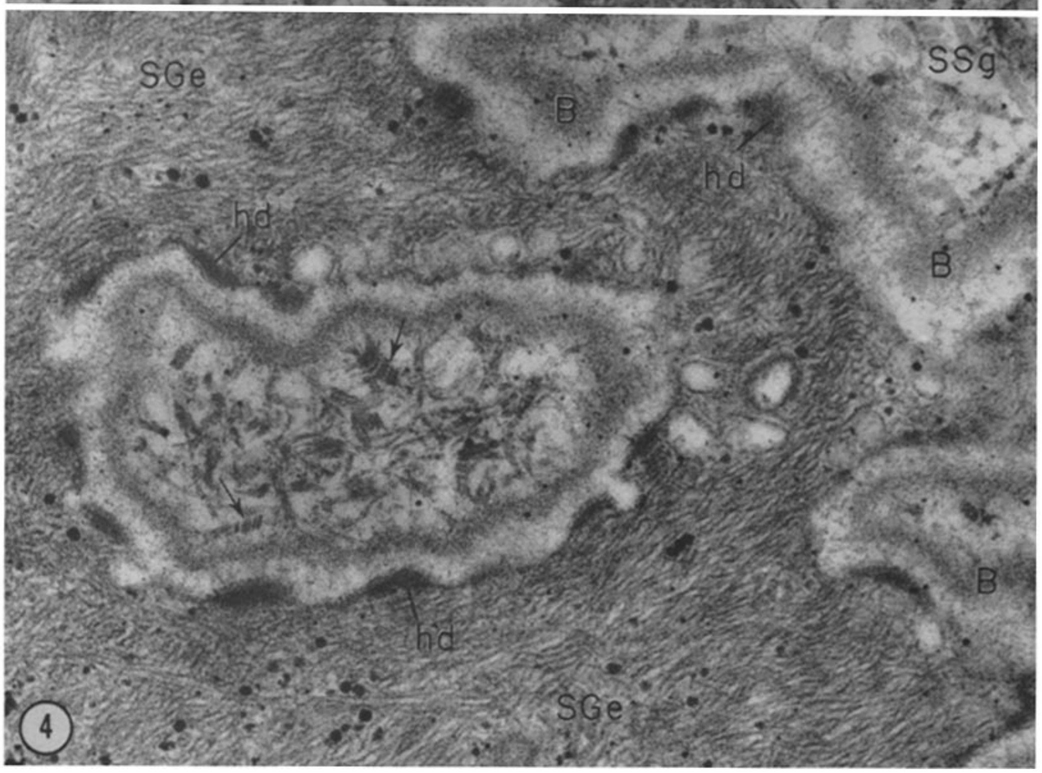
Fig. 3 shows parts of a number of epidermal cells (*SGe*), one of which (SGe′) appears as a completely detached island surrounded by a collar of basement membrane (*B′*) and beyond it by a moat-like micropapilla. Conversely, Fig. 4 shows an obliquely sectioned, subconical micropapilla which takes the form of a detached dermal island completely surrounded by the epidermis.

In Fig. 3, anchoring fibrils (*af*) converge from almost all directions on the basement membrane collar (*B′*) and attach to it either directly (short arrows) or after branching (long arrows). A knot formed by the convergence of the proximal ends of 5 to 6 anchoring fibrils appears at *k* in the middle of the moat-like micropapilla. At least 4 of these fibrils can be followed from the knot to the sites of their anchoring into the basement membrane. Note the arrangement of the collagen fibrils: most of them are located in the meshes of the network formed by the anchoring fibrils. The detail is particularly evident in places in which the collagen fibrils are transversely sectioned (*ct*).

FIGURE 4 demonstrates the complex network formed by the anchoring fibrils within a micropapilla. The characteristic polarized banding pattern of the fibrillar stem is visible in a few places (arrows). One of the fibrils shows, in addition, longitudinal striations (upper arrow) which suggest that these fibrils are formed by the lateral aggregation of finer fibrillar elements. Half-desmosomes are marked *hd*. In some places (upper right *hd*), they seem to be connected to the basement membrane by fine divergent filaments.

The "granules" of high density and variable diameter seen throughout the tissue in these and most other figures are present already in unstained sections. They may represent a precipitate (calcium phosphate?) formed during, or pre-existing, fixation. Fig. 3, × 56,000. Fig. 4, × 53,000.


SGe
ct
B
af
B
k
af
B'
cl
ct
SGe'
B'
B
SGe
ct
af
SGe
3


SGe
B
SSg
hd
hd
B
B
hd
SGe
4

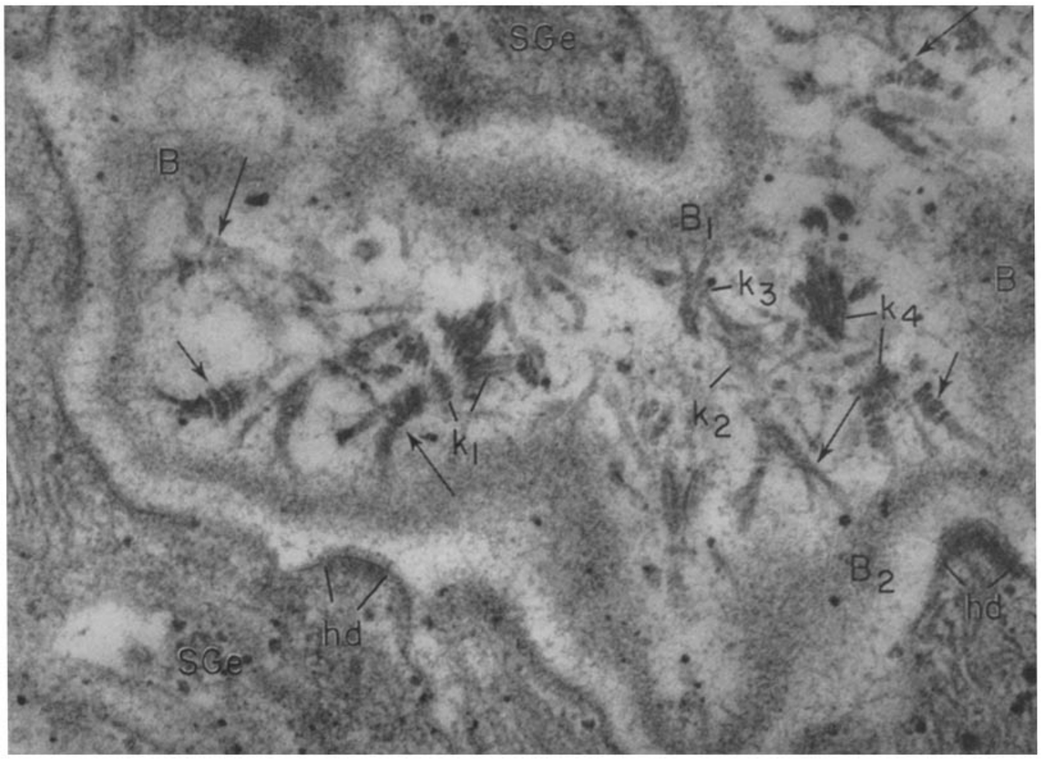


Figure 5 Oblique section through a dermal micropapilla in the abdominal skin of the toad. Parts of epidermal cells appear at *SGe,* half-desmosomes at *hd,* and the basement membrane at *B.*

Anchoring fibrils are encountered along the entire perimeter of this micropapilla. The usual banding pattern (short arrows) and the branches (long arrows) through which they attach to the basement membrane can be seen at their distal ends. The proximal ends of a number of fibrils converge into a series of knots (k_1 to k_4) along the middle region of the micropapilla. Note that, through the intermediary of knots k_2 and k_3, anchoring fibrils establish a continuous link that short cuts across the micropapilla to connect the two sides (B_1, B_2) of the fold formed by the basement membrane at this level. × 76,000.

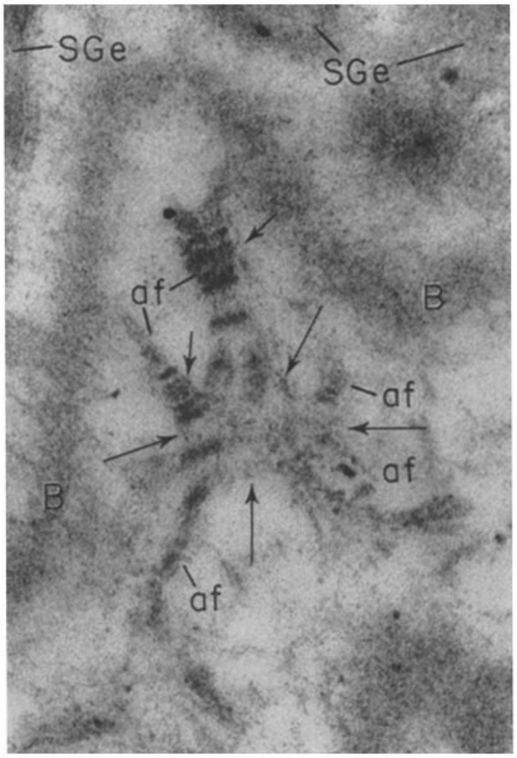


FIGURE 6 Longitudinal section through a derma micropapilla in the chest skin of the toad. Parts of epidermal cells appear at *SGe*, the basement membrane at *B*, and a few anchoring fibrils at *af*. The distal end of some of these fibrils shows the usual banding pattern (short arrows). Their proximal ends converge into a broad, complex knot, the limits of which are indicated by long arrows. Collagen fibrils do not show in this type of specimen which has been fixed in glutaraldehyde and postfixed in OsO_4. × 116,000.

REFERENCES

1. FARQUHAR, M. G., and PALADE, G. E., Cell junctions in amphibian skin, *J. Cell Biol.*, 1965, **26**, 263.
2. KARRER, H. E., The fine structure of connective tissue in the *tunica propria* of bronchioles, *J. Ultrastruct. Research*, 1958, **2**, 96.
3. LOW, F. N., Microfibrils, a small extracellular component of connective tissue, *Anat. Rec.*, 1961, **139**, 250.
4. PALADE, G. E., and BRUNS, R. R., Structure and function in normal muscle capillaries, *in* Small Blood Vessel Involvement in Diabetes Mellitus, (M. D. Siperstein, A. R. Colwell, Sr., and K. Meyer, editors), Washington, D.C., American Institute of Biological Sciences, 1964, 39.
5. BRODY, I., The ultrastructure of the tonofibrils in the keratinization process of normal human epidermis, *J. Ultrastruct. Research*, 1960, **4**, 264.
6. SCHMITT, F. O., GROSS, J., and Highberger, J. H., States of aggregation of collagen, *in Symp. Soc. Exp. Biol.*, 1955, **9**, 148.
7. SCHMITT, F. O., Interaction properties of elongate protein macromolecules with particular reference to collagen (tropocollagen), *Rev. Mod. Phys.*, 1959, **31**, 349.
8. HODGE, A. J., and SCHMITT, F. O., The tropocollagen macromolecule and its properties of ordered interaction, *in* Macromolecular Complexes, (M. V. Edds, Jr., editor), New York, Ronald Press, 1961, 19.
9. JAKUS, M. A., Studies on the cornea. II. The fine structure of Descemet's membrane, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 243.
10. JAKUS, M. A., The fine structure of the human cornea, *in* The Structure of the Eye, (G. K. Smelser, editor), New York, Academic Press, Inc., 1961, 343.
11. JAKUS, M. A., Further observations on the fine structure of the cornea, *Invest. Ophthalmol.*, 1962, **1**, 202.
12. JAKUS, M. A., Ocular fine structure, Retina Foundation Monographs and Conferences, Boston, Little, Brown and Co., **1**, 1964.
13. GARRON, L. K., FEENEY, M. L., HOGAN, M. J., and MCEWEN, W. K., Electron microscopic studies of the human eye. I. Preliminary investigations of the trabeculas, *Am. J. Ophthalmol.*, 1958, **46**, 27.
14. GARRON, L. K., and FEENEY, M. L., Electron

microscopic studies of the human eye. II. Study of the trabeculae by light and electron microscopy, *Arch. Ophthalmol.*, 1959, **62**, 966.

15. FEENEY, L. M., and GARRON, L. K., Descemet's membrane in the human peripheral cornea. A study by light and electron microscopy, *in* The Structure of the Eye, (G. K. Smelser, editor), New York, Academic Press, Inc., 1961.
16. HOLMBERG, A. C., Ultrastructure of the trabecular apparatus, *in* Glaucoma, (transactions IVth conference), (F. W. Newell, editor), New York, Macy Foundation, 1960.
17. CAUNAS, N., and ROSS, L. L., The fine structure of Meissner's touch corpuscles of human fingers, *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 467,
18. LEESON, T. S., and SPEAKMAN, J. S., The fine structure of extracellular material in the pectinate ligament (trabecular meshwork of the human iris), *Acta Anat.*, 1961, **46**, 363.
19. LUSE, S. A., Electron microscopic studies of brain tumors, *Neurology*, 1960, **10**, 881.
20. LUSE, S. A., ZOPF, D., and COX, J. W., An electron microscopic study of *in vitro* and *in vivo* long spacing collagen, *Anat. Rec.*, 1963, **145**, 254.
21. RAIMONDI, A. J., MULLAN, S., and EVANS, J. B., Human brain tumors; an electron microscopic study, *J. Neurosurg.*, 1962, **19**, 731.
22. ROHEN, J. W., Über das Ligamentum pectinatum der Primaten, *Z. Zellforsch.*, 1962, **58**, 403.
23. ROHEN, J. W., Experimental studies on the trabecular network in primates, *Arch. Ophthalmol.*, 1963, **69**, 335.
24. NAUMANN, R. A., and WOLFE, D. E., A striated intercellular material in rat brain, *Nature*, 1963, **198**, 701.
25. WETZSTEIN, R., SCHWINK, A., and STANKA, P., Pericapillargelegene periodische Strukturen im Subcommissuralorgan bei Ratten, *Naturwissensch.*, 1963, **50**, 137.
26. WETZSTEIN, R., SCHWINK, A., and STANKA, P., Die periodisch strukturierten Körper in Subcommissuralorgan der Ratte, *Z. Zellforsch.*, 1963, **61**, 493.
27. FREIDMANN, I., CAWTHORNE, T., MCLAY, K., and BIRD, E. S., Electron microscopic observations of the human membranous labyrinth with particular reference to Ménière's disease, *J. Ultrastruct. Research*, 1963, **9**, 123.
28. FRIEDMANN, I., CAWTHORNE, T., and BIRD, E. S., The laminated cytoplasmic inclusions in the sensory epithelium of the human macula. Further electron-microscopic observations of Ménière's disease, *J. Ultrastruct. Research*, 1965, **12**, 92.
29. PILLAI, P. A., A banded structure in the connective tissue of nerve, *J. Ultrastruct. Research*, 1964, **11**, 455.
30. GOLDBERG, B., and GREEN, H., An analysis of collagen secretion by established mouse fibroblast lines, *J. Cell Biol.*, 1964, **22**, 227.
31. SZODORAY, L., The structure of the junction of the epidermis and the corium, *Arch. Dermat. Syph.*, New York, 1931, **23**, 920.
32. ODLAND, G. F., The morphology of the attachment between the dermis and the epidermis, *Anat. Rec.*, 1950, **108**, 399.
33. GROSS, J., Connective tissue fine structure and some methods of its analysis, *J. Gerontol.*, 1950, **5**, 343.
34. BEAR, R. S., The structure of collagen fibrils, *Advances Protein Chem.*, 1952, **7**, 69.
35. PORTER, K. R., Observations on the fine structure of animal epidermis, *in* Proceedings 3rd International Conference on Electron Microscopy, (R. Ross, editor) London, Royal Microscopical Society, 1954, 539.
36. WEISS, P., and FERRIS, W., Electron micrograms of larval amphibian epidermis, *Exp. Cell Research*, 1954, **6**, 546.
37. YOUNES, M. S., STEELE, H. D., ROBERTSON, E. M., and BENCOSME, S. A., Correlative light and electron microscope study of the basement membrane of the human ectocervix, *Am. J Obst. and Gynec.*, 1965, **92**, 163.