THE JOURNAL OF INVESTIGATIVE DERMATOLOGY

Vol. 57, No. 1
Printed in U.S.A.

INTERCELLULAR SPACES OF THE HUMAN EPIDERMIS AS DEMONSTRATED WITH LANTHANUM*

KEN HASHIMOTO, M.D.

ABSTRACT

In normal human epidermis, lanthanum penetrated into the basal lamina-basal cell interspaces, including half-desmosome areas, non-specific intercellular junctions, desmosomes, close approximations and most of the occluding zonules and macules. The majority of what were previously claimed to be tight junctions were permeated by lanthanum, revealing that they were actually gap junctions. Maculae occludentes which follow desmosomes, for instance, were found not to be tight. On the other hand, the interspace between the stratum granulosum and stratum corneum was only rarely permeated. Lanthanum filled the intercellular channels between the lateral plasma membranes of granular cells but was stopped near the exit of the channel into the interspace. Truly tight junctions (zonulae occludentes or the *distal junctions of the stratum granulosum*) were found to be responsible for this occlusion. Intercellular spaces between the horny cells were not commonly permeated by lanthanum.

The ultrastructure of intercellular spaces of human epidermis has previously been investigated by the present author and his collaborator in relation to its abnormal changes, such as dissolution occurring in pemphigus vulgaris (1). With development of new electron microscopic techniques such as the ruthenium red stain (2) and lanthanum infiltration (3, 4), the intercellular spaces in general can be demonstrated much more precisely and adequately than before. The new methods were applied to guinea pig skin (5) and to normal (6) and abnormal human skins (5, 7, 8). These early studies demonstrated that in human epidermis specialized intercellular junctions were stained or permeated and some of the junctions previously believed to be tight were shown to be patent (5, 6). The present investigation was undertaken with improved methods and techniques to explore more fully the details of extracellular spaces of normal human epidermis.

MATERIALS AND METHODS

Normal skin of the right leg of an 11-month-old white boy (specimen №1), normal skin of the left anterior axillary fold of a 24-year-old Negro male (specimen №2) and normal skin of the toes of a 48-year-old Negro male (specimen №3) were obtained by punch biopsy under 1% procaine anesthesia. The epidermis of all specimens was immediately sliced into approximately 0.2 mm thick flakes with sharp razor blades cleaned with an acetone-chloroform mixture. The tissues were fixed at 4°C in 2.5% glutaraldehyde in cacodylate buffer (pH 7.2) containing 1% lanthanum nitrate. The fixation took 3 days for the first specimen, overnight for the second and 3 days for the third. The tissue flakes were rinsed in the same buffer containing 1% lanthanum nitrate for 6 hours, post-fixed for 1 hour with 1% osmic acid in Veronal buffer, pH 7.4, containing 1% lanthanum nitrate and then rinsed for 2 hours in the same Veronal buffer containing 1% lanthanum nitrate. After dehydration with 50% ethanol containing 1% lanthanum nitrate, the tissue was stained for 30 minutes with 1% uranyl acetate and 1% lanthanum nitrate in 50% ethanol. Dehydration was carried out with higher concentrations of ethanol, 60% through 90%, containing 1% lanthanum nitrate and 100% alcohol and propylene oxide without lanthanum nitrate. All tissues were embedded in Araldite. The above method was a modification of the method described by Overton (4). Thin sections were cut with a Porter-Blum MT-2 Ultramicrotome at 400–600A, picked up on Formvar-coated grids, stained with 1% uranyl acetate in 50% alcohol and then, while half-dried, restained with Reynolds' lead citrate (9). Stained sections were observed in an Hitachi HU-11C High Resolution Electron Microscope at 100KV.

Two modified staining methods were also used. 1) The tissue was fixed in the same solutions and rinsed in the same buffers containing 1% lanthanum, but 1% lanthanum was omitted from dehydrating alcohols 50% through 90%. 2) The same fixative, buffers and graded dehydration ethanols were used without 1% lanthanum nitrate. The lat-

Received November 3, 1970; accepted for publication March 5, 1971.
Supported in part by V.A. Part I Designated Research Funds and Medical Investigatorship Award of V.A. Career Development Program.
* From The Veterans Administration Hospital, Memphis, Tennessee, and The Departments of Medicine (Dermatology) and Anatomy, The University of Tennessee College of Medicine.

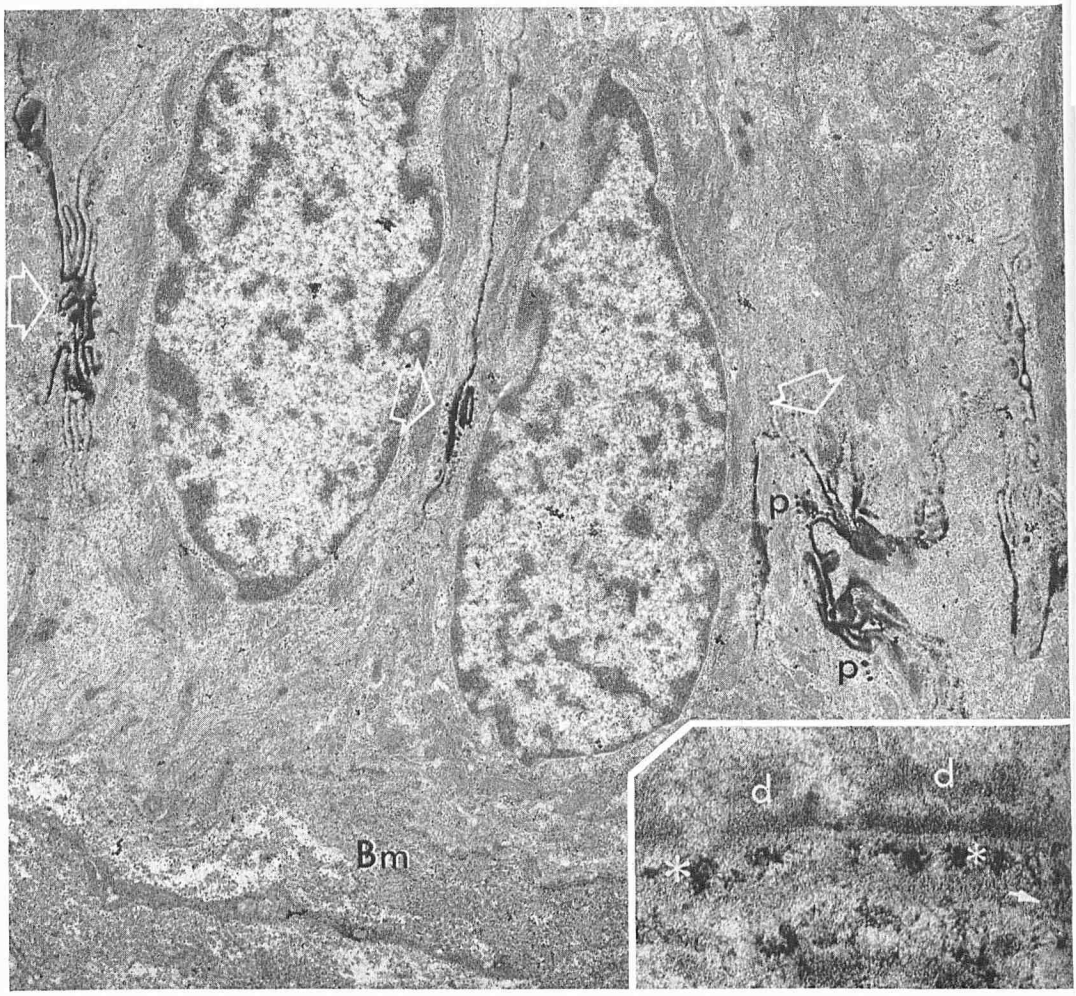


FIG. 1. Lanthanum complex heavily infiltrated the upper dermis, crossed the basal lamina (small arrow) and formed a number of aggregates in the lamina lucida (*). Lateral intercellular spaces between the basal cells retained lanthanum only in the upper portion (large hollow arrows); no lanthanum was retained near the basal lamina. The lamina lucida was permeated in the half-desmosome area (d). Pinocytotic vesicles (p) contain some lanthanum. Bm: basement membrane. Specimen #1: × 7,615. Insert: × 99,975.

ter served as a control. Embedding procedures and staining of thin sections were the same.

Although control specimens prepared without lanthanum should serve this purpose, in some specimens post-osmication, tissue block straining with uranyl acetate and thin section staining with uranyl acetate and lead citrate were either avoided or slightly done by shortening the staining time in order to determine what density is due to lanthanum penetration and what is due to other staining.

RESULTS

The pattern of permeation of the lanthanum was the same in all three specimens prepared with the various methods mentioned above. The following descriptions of the results, therefore, apply to all three. The degree of penetration varied from one block to another. In the following illustrations, unless specifically mentioned, the density of the infiltration is not so important as the fact that a certain junction was permeated. The density depended upon the degree of initial permeation, which was greater at the surface of the block, and the ease of leaching out of lanthanum once permeating, which was also greater at the periphery of the block. Where desmosomes and gap junctions were frequent,

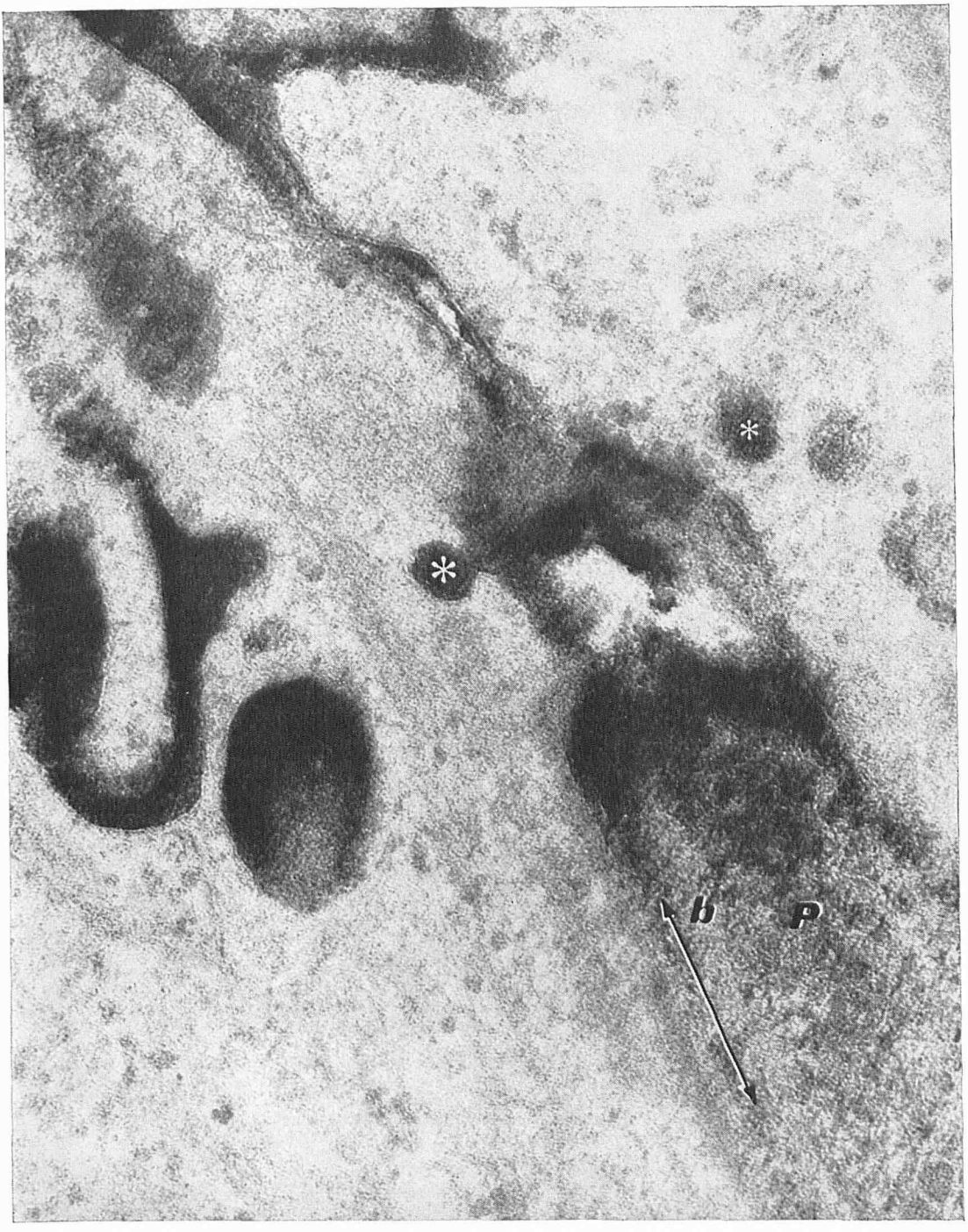


FIG. 2. Papillary dermis (P), basal lamina (b) lamina lucida (arrow) and intercellular spaces between the basal cells were continuously permeated by lanthanum. This specimen was fixed with glutaraldehyde alone and not stained with uranyl acetate and lead. Notice that cytoplasmic organelles were practically unstained but the intercellular spaces were densely stained with lanthanum. *: pinocytotic vesicle. Specimen ⅋1: × 133,750.

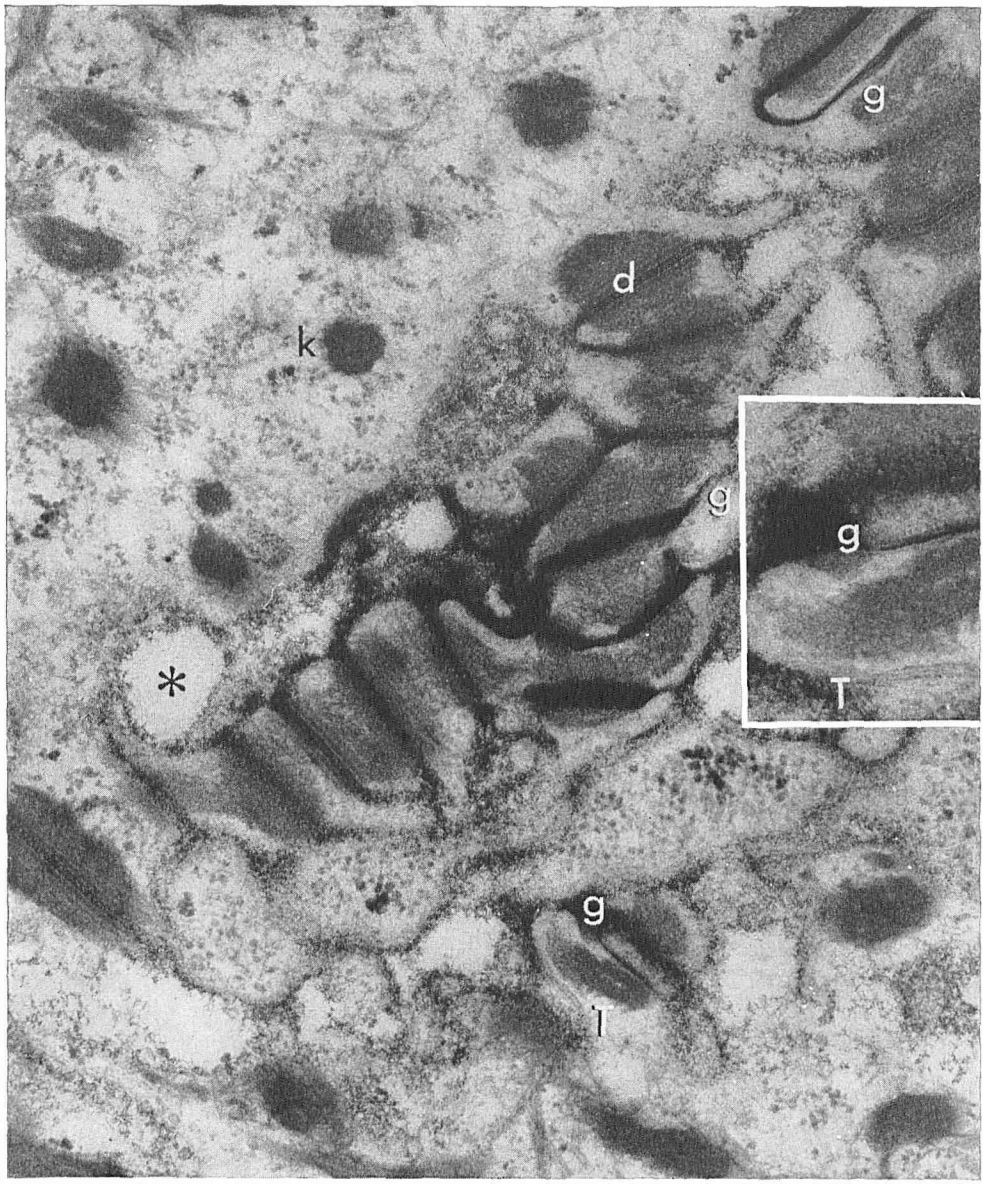


FIG. 3. Between upper Malpighian cells and a keratohyalin (k)-containing granular cell, lanthanum permeated gap junctions (g), desmosomes (d), non-specific junctions, and one presumably truly tight junction (T) (enlarged in the insert). Although an adjacent gap junction (g) was heavily infiltrated by lanthanum, this tight junction was not permeated. *: space produced by artefact. Specimen #1: × 41,750. Insert: × 102,500.

the permeated lanthanum was retained well. It has been postulated that, in the heavily infiltrated peripheral zone of the block, lanthanum might damage the junctional complex (10). In this study the peripheral, middle and central zones of each block were surveyed to avoid errors due to regional differences.

Control specimens prepared without lanthanum infiltration showed no significant staining of the intercellular spaces except for an occasional electron-dense substance and the discharged membrane-coating granules in the upper layers. Specimens stained with lanthanum alone demonstrated the intercellular spaces clearly in

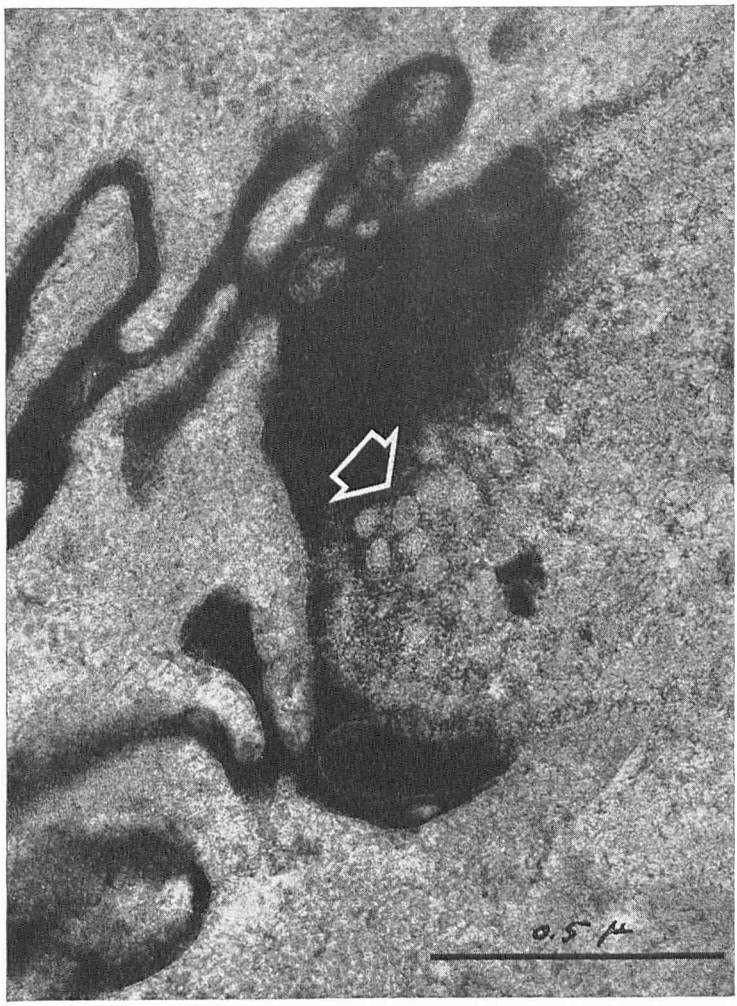


FIG. 4. Within what one may assume to represent a tangentially cut non-specific junction, penta- or hexagonal clear spaces surrounded with trilaminar membrane are seen (arrow). Specimen #1: × 78,400.

contrast to unstained surrounding structures (Fig. 2).

Basal Lamina-Basal Cell Interspace
(Lamina Lucida)

Lanthanum penetrated from the dermis into this space, crossing the basal lamina. The basal lamina was often stained with lanthanum (Fig. 1). The filling of the lamina lucida, however, was not always uniform, with formation of irregular aggregates of lanthanum (Fig. 1). Half-desmosome areas of the lamina lucida could also be permeated (Fig. 1, insert). In some sections the penetration of lanthanum could be followed continuously from the papillary dermis to the basal intercellular spaces (Fig. 2).

Intercellular Spaces

1. Usual intercellular space. The major portions of the cell circumferences of the adjoining cells of the epidermis were apposed to each other with a distance of from 150A to 200A. Lateral intercellular spaces of the stratum basale were also 150–200A wide and remained open to the lamina lucida. Lanthanum was often washed out from the basal intercellular spaces, leaving stringy, lanthanum-stained intercellular substance or cell surface coat in them. Large open spaces were occasionally found without lanthanum in the center (Fig. 3). Most of these were interpreted as representing artefacts due to fixation and dehydration. When a usual intercel-

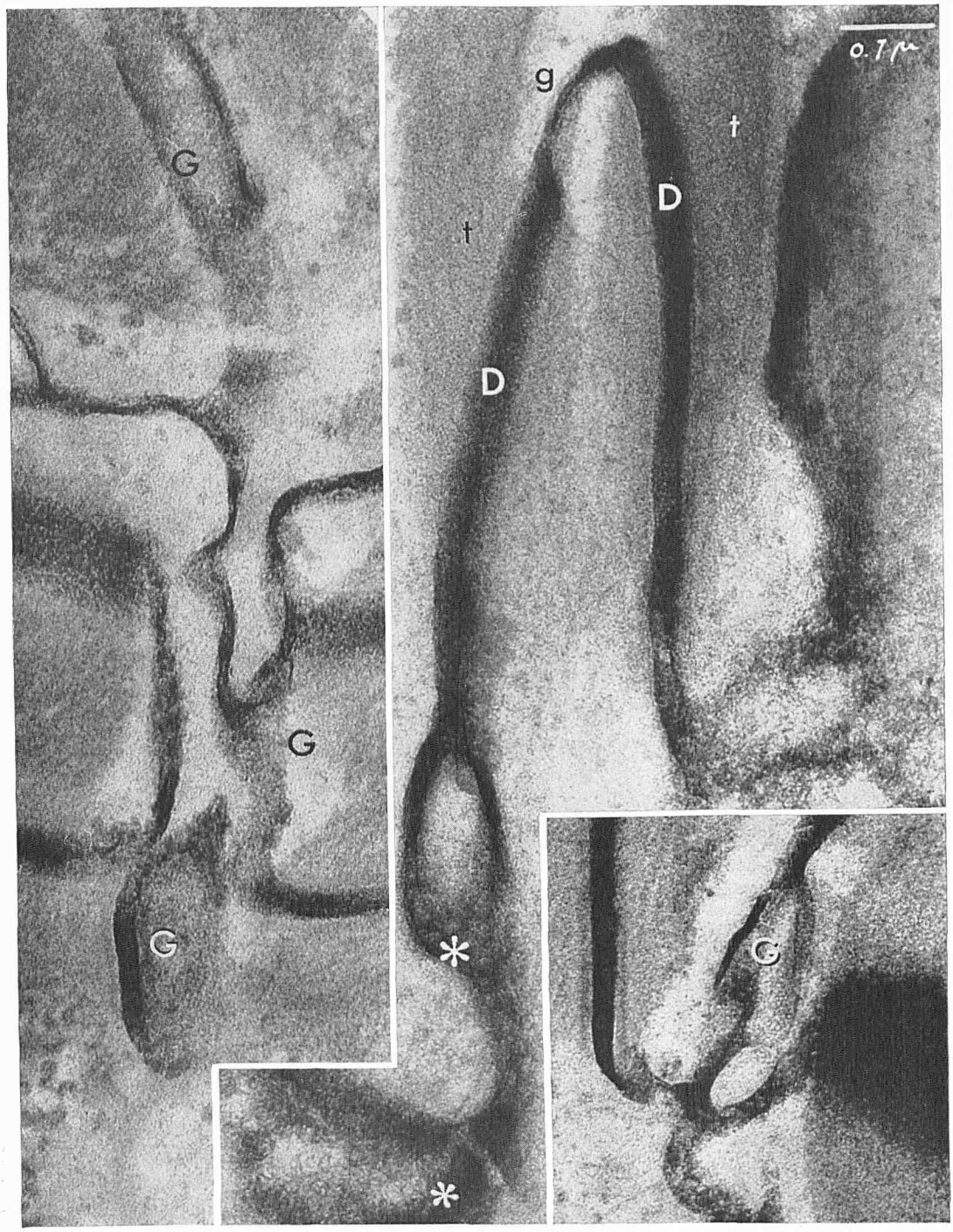


FIG. 5. Round and polygonal subunits similar to those shown in Fig. 4 are seen in two areas (*) which seem to be tangentially sectioned non-desmosomal intercellular junctions because of the absence of attachment plaques and converging tonofibrils. A focal gap junction (g) juxtaposed to a desmosome was permeated by lanthanum. Extended gap junctions (G) were cut tangentially and revealed arrays of negatively stained small subunits. D: lanthanum-permeated desmosome with converging tonofibrils (t). Middle—Specimen #1: × 134,587. Left and right-lower—Specimen #3: × 106,215.

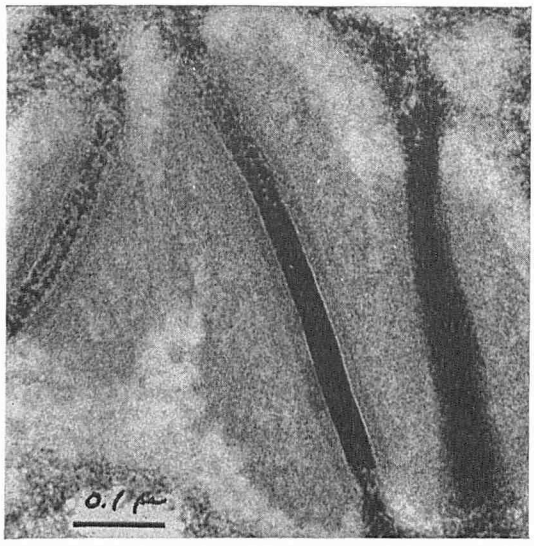


FIG. 6. Three desmosomes were permeated by lanthanum. The one on the left shows two lateral stained layers in contact with the outer leaflets of the apposed plasma membranes, the middle one shows a similar staining pattern in the upper portion but uniform permeation in the lower part, and the one on the right was cut tangentially. Specimen #1: × 120,713.

lular space was tangentially cut, negatively stained, round, pentagonal, hexagonal, and polygonal structures were occasionally revealed in aggregation (Figs. 4, 5). The longest diagonal dimension of these structures varied between 400–650A. This variation was partially influenced by the angle through which they were cut (Figs. 4, 5). These structures were delimited with trilaminar membranes.

2. Desmosome. At desmosomes the outer leaflets of the plasma membranes of the adjoining cells were separated by a distance of 225–325Å. Most of the spaces were densely filled with lanthanum (Fig. 6). In some desmosomes the central layer was less densely stained than the lateral layers (>40Å) directly in touch with the outer leaflets of the plasma membranes (Fig. 6).

3. Tight junctions and gap junctions. Among what might be called tight junctions in the control specimens (Fig. 7), the lanthanum method distinguished two classes. The first was the truly tight junction in which the fusion of the outer leaflets of the apposed plasma membranes was genuine and no permeation of lanthanum took place. The true tight junction was rare in the stratum basale and stratum Malpighii (Fig. 3). It was common in the stratum granulosum and

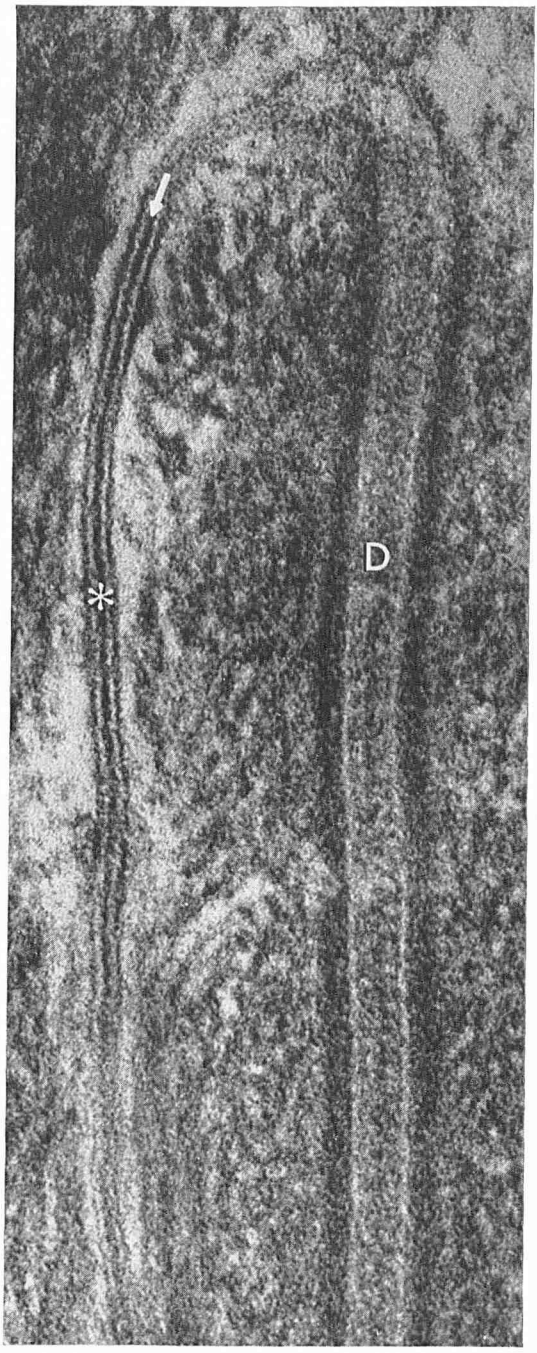


FIG. 7. Without the benefit of lanthanum, the extended junction (*) following a desmosome (D) appears to be tight. However, as shown with the lanthanum method (cf Figs. 3, 13, 14), most of this type are gap junctions. This section was rather heavily stained with uranyl acetate and lead to delineate the center ("fusion") layer (arrow) of the "tight junction". Specimen #3: × 250,403.

almost regularly present at the distal ends of the lateral junctions of the granular cells (Figs. 8, 9). These tight junctions will be referred to as the *distal junctions of the stratum granulosum*. In some junctions the fusion of the outer leaflets was not continuous, but a few punctate fusions occurred in a series (Figs. 8, 9). When the first focal fusion was circumvented through channels presumably present at a level different from that of the section, the second fusion usually prevented lanthanum from leaking into the interspace. In very heavily infiltrated junctions, slight leakage of lanthanum across the junction into the interspace between the stratum granulosum and stratum corneum was noted. Proximal to the true tight junction there were one or two desmosomes and in less heavily infiltrated specimens lanthanum did not infiltrate beyond one of these desmosomes (Fig. 10). Specimens prepared without lanthanum staining showed suggestions of such membrane fusion (Figs. 11, 12). However, without the aid of the lanthanum method, it was not certain whether such junctions were truly tight.

The second type proved to be a typical gap junction which in non-lanthanum treated control specimens appeared tight (Fig. 5). With lanthanum stain it was found to be patent and to allow penetration of lanthanum (Figs. 3, 5, 13, 14). It was either focal (Figs. 5, 14) or stretched over ½ μ (Figs. 3; 13, insert). This type should be called gap junction although the gap between the apposed outer leaflets was somewhat irregular in width. In some the gap distance fluctuated along the length of the same junction (Fig. 14). The average gap dimension, however, fell into the range reported in other organs (18–30Å) (3, 11, 12). The typical focal gap junction was seen in series with desmosomes (Fig. 5) and corresponded in position to that described as *macula occludens* by Farquhar and Palade (13) in amphibian skins. The long-stretched one was seen either alternating with a desmosome (Fig. 13), or independently (Fig. 13, insert); in the latter case corresponding to the nexus, also described in the frog epidermis by Dewey and Barr (14). When tangentially sectioned, a lanthanum-permeated gap junction demonstrated negatively stained arrays of subunits of 70–80Å (Figs. 5, 15).

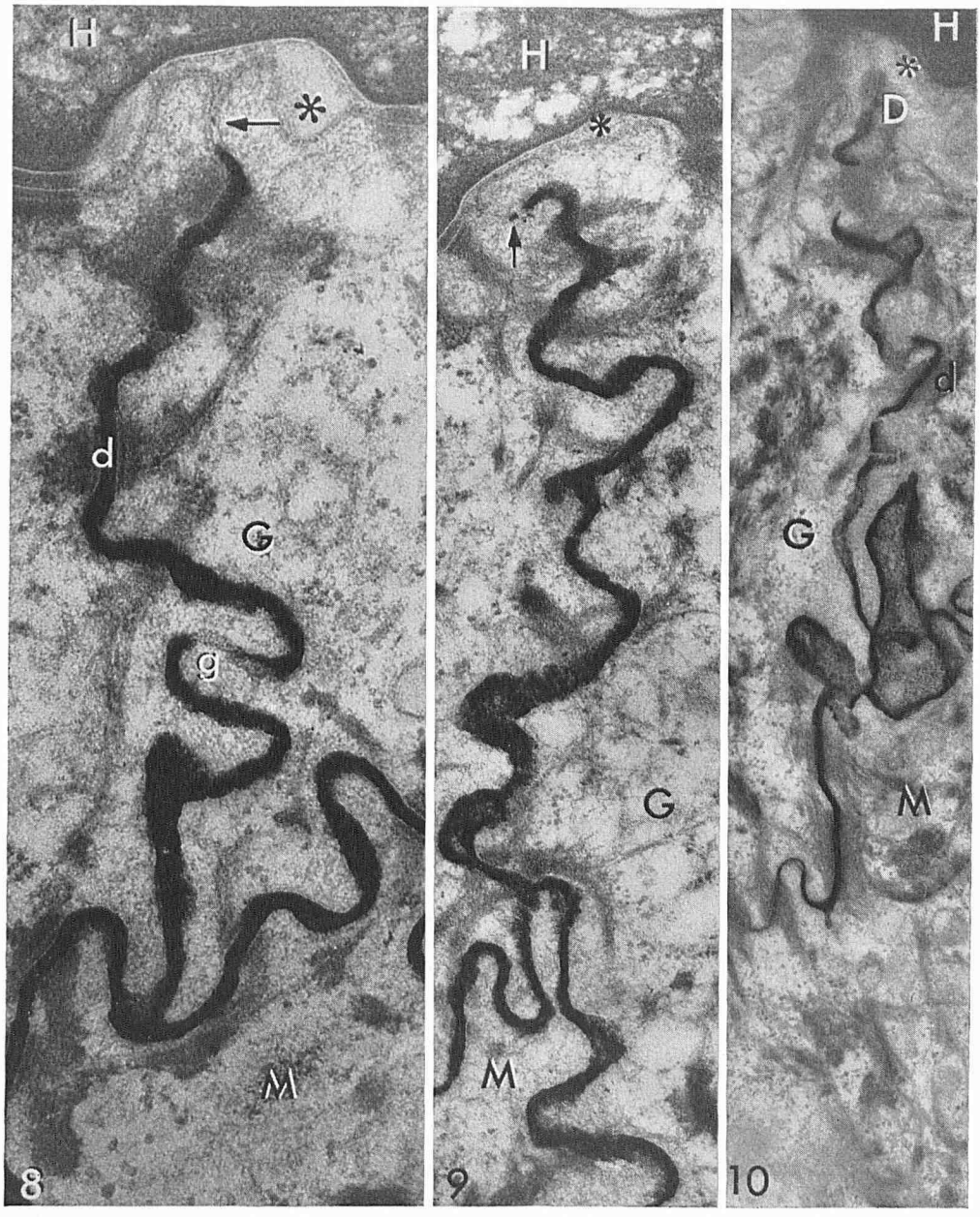
Intercellular spaces of the stratum corneum were only occasionally permeated by lanthanum, either from below or from above. Even in very heavily infiltrated areas, such as near the periphery of the block, most of these spaces remained free from lanthanum. It is not unreasonable to suspect that the intercellular cement substances of the stratum corneum might block the passage of lanthanum from above, although typical tight junctions such as those demonstrated in frog skin by Farquhar and Palade (13) have not been seen, neither along the lateral nor the vertical junctions of the horny cells.

4. Laminated bodies. Laminated bodies, both opened to and present in the intercellular spaces (Figs. 16, 17, 18), and also entirely included within the cytoplasm at the plane of the section, were permeated. Electron lucent layers (30–36Å) of the body were not stained, whereas the alternating dense layers (20–26Å) showed increased density (Figs. 17, 18). The total dimensions of these bodies and widths of the electron-lucent and electron-dense layers were compatible with those of the body known as Odland's body (15), membrane-coating granule (16), keratinosome (17), cement granule (18) or cementsome (19). Many of these granules were discharged in fairly intact form and some of them remained intact in the intercellular spaces (Figs. 16, 17, 18). In a few exceptional specimens in which lower layers of the stratum corneum were infiltrated by lanthanum, intact granules could be detected in the interspaces of horny cells.

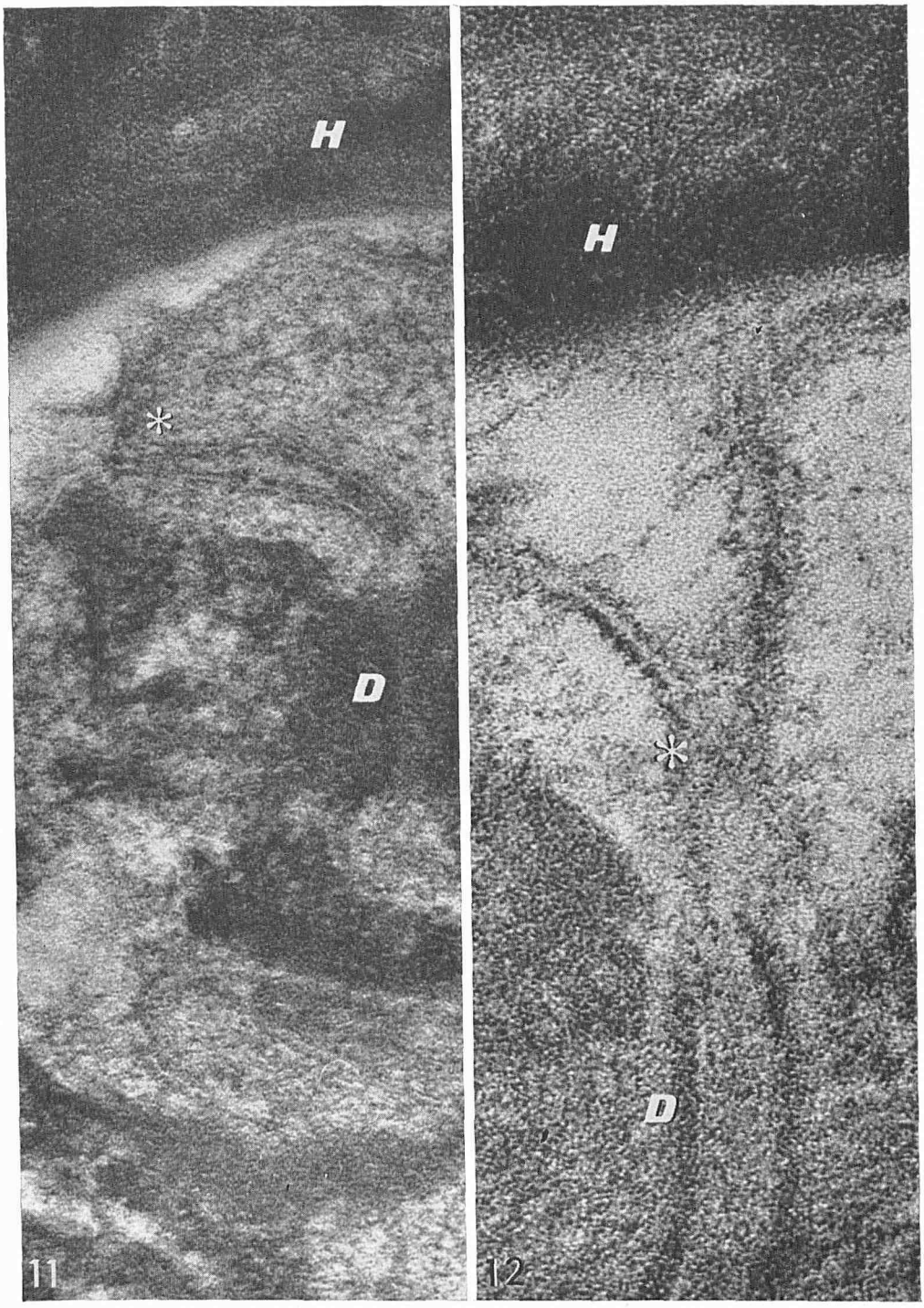
DISCUSSION

In this study, biopsied tissues were immersed in lanthanum-glutaraldehyde mixture and hence there is a possibility that some of the gaps and pathways demonstrated may represent fixation artefacts. In the following discussion it must, therefore, be understood that this is a limitation which most studies of this type possess.

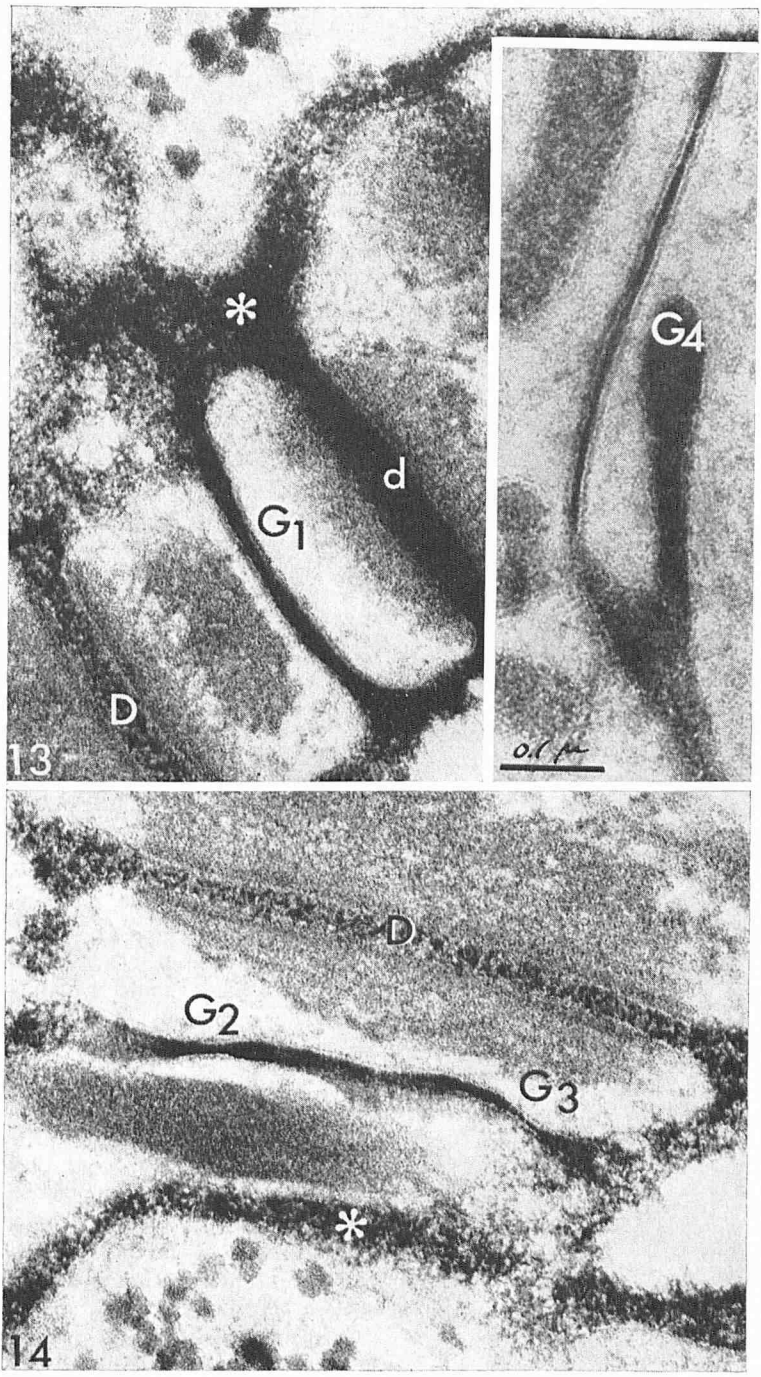
Permeation of lamina lucida. Although lanthanum must have had access to the epidermis on all sides of the tissue blocks, the preferred route seemed to be through the dermis, crossing the basal lamina. Thus, in contradistinction to the finding of Wolff and Schreiner (5), the *lamina lucida* was infiltrated. Lanthanum moved upward from this space to the upper level of the *stratum granulosum via the intercellular* channels. The permeation of the *lamina lucida*, however, was not as complete as in other spaces. It is possible that the anchoring filaments which criss-cross this space (7, 20, 21) prevented a free



FIGS. 8, 9, 10. In spite of the continuous permeation of the lateral interspaces between the upper Malpighian cells (M) and granular cells (G) including desmosomes (d), the distal junctions of the stratum granulosum are truly tight to the permeation of lanthanum. In Fig. 8 and Fig. 9 the first fusion of the plasma membrane was circumvented, with a leakage of lanthanum (arrows), but the second fusion stopped the penetration of lanthanum. In Fig. 10 lanthanum did not infiltrate beyond a distally located desmosome (D). Thus, the interspaces between the stratum granulosum and stratum corneum (*) are free from lanthanum. g: tangentially cut gap junction showing arrays of subunits. H: horny cell. Specimen ※1: Fig. 8 and Fig. 9: × 39,500. Fig. 10: × 25,750.



FIGS. 11, 12. Distal junctions of the stratum granulosum (*) appear to be tight, with the outer leaflets of the apposed plasma membranes being fused. D: desmosome. H: horny cell. Specimen #3: Fig. 11: × 147,250. Fig. 12: × 284,000.



FIGS. 13, 14. Fluctuation of the gap dimension is not always due to the angle of sectioning: G_1 (75A) is definitely wider than G_2 and G_3 (20–30A). G_4 stretches over $\frac{1}{2}\mu$ and the gap distance fluctuates between 30–75A. Three pictures were taken from adjacent areas of specimen #1 at the same magnification. D: desmosome. d: tangentially sectioned desmosome. *: usual intercellular space. × 138,750.

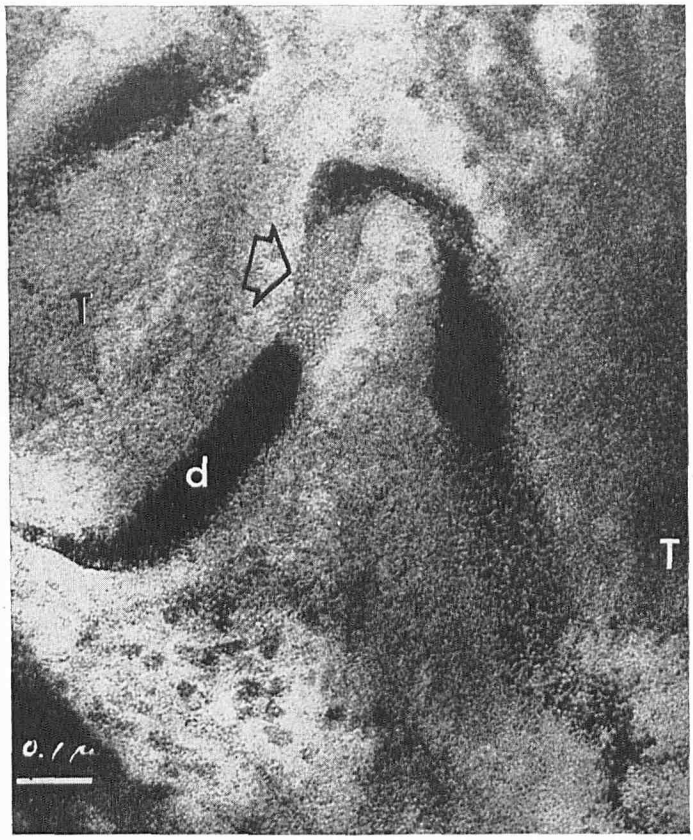


FIG. 15. Small subunits in a registered array (arrow) are located between two permeated desmosomes. The area seems to correspond to a gap junction juxtaposed to one of the desmosomes (d). T: tonofibrils converging to desmosomes. × 109,500.

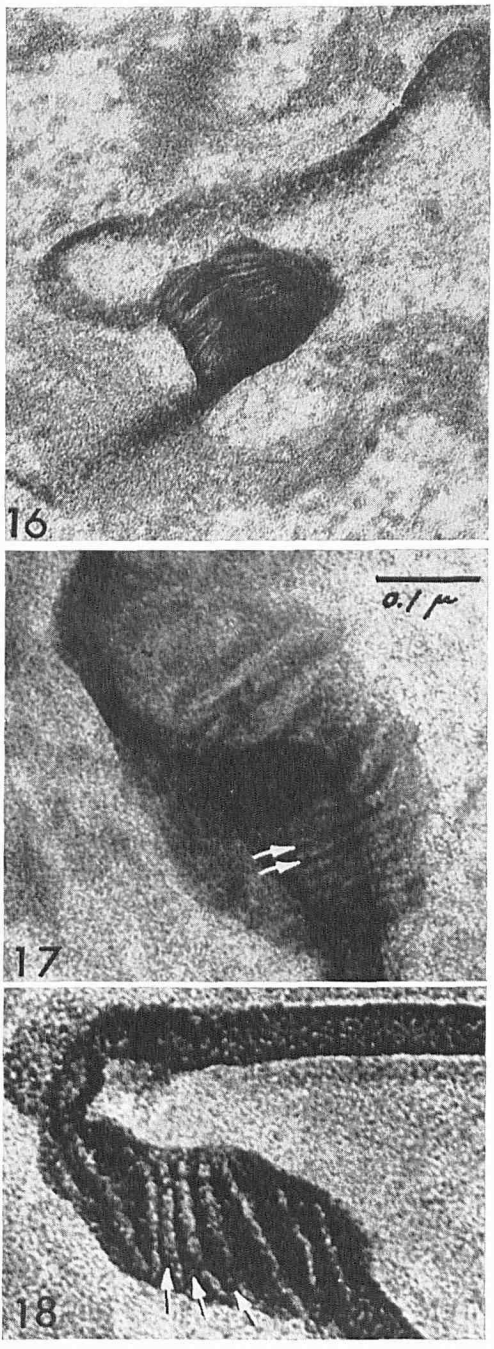
penetration of lanthanum. It is also possible that lanthanum once completely filled this space (Fig. 2) and was washed out during subsequent steps of specimen preparation because of the lack of a sufficient number of deterring devices such as desmosomes. The same effect of leaching out of lanthanum seemed to apply to the basal intercellular spaces (Fig. 1).

Extracellular compartment of the epidermis. Tight junctions aided by desmosomes seal off the intercellular spaces below the level of the stratum granulosum (at least to lanthanum penetration). The significance of this may be twofold. First, it may prevent free flow of exogenous material into the viable portion of the epidermis. The stratum corneum is not a perfect barrier for many noxious substances such as heavy metals (22), and it is presumably much less perfect in damaged skin. This barrier, therefore, as a second line of defense, seems to be important in the selective percutaneous absorption of exogenous materials whose route is mainly through the intercellular spaces.

Secondly, since the true tight junction of the epidermis may be impermeable even to small ions (13), these junctions assisted by desmosomes and gap junctions may compartmentalize the extracellular spaces of the viable epidermis to create an osmotic pressure gradient. This compartment is open to the dermis through the coarse filter of the basal lamina and is closed to the stratum corneum by the *distal tight junctions of the stratum granulosum.* Within this main compartment, subcompartments are formed by desmosomes, gap junctions and occasional tight junctions. Although the first two were demonstrated to be permeable to molecules as large as that of peroxidase (molecular weight: 40,000) (3), these specialized junctions probably interfere with the free passage of sol-

utes. Even water is thought to pass with difficulty a channel narrower than 1000Å because it develops an ice-like organization (23, 24). It may be postulated that ions and other solutes secreted by the keratinocyte into these sealed spaces create osmotic pressure and pull water with dissolved nutrients up to the higher levels. If the concentration of such ions and solutes gradually increases toward the upper layers of the epidermis by the factors of dehydration, discharge of membrane-coating granules and secretion of more concentrated ions and solutes by the dehydrating keratinocytes, the osmotic pressure gradient should also increase in those pools of the upper epidermis. Electrochemical potential gradients created in the sealed spaces may also help the transport of charged materials. A strong pumping system is indeed expected to exist since very rapid diffusion of intradermally injected materials into the upper layers of the epidermis has been observed. A remarkable rapidity of spread of injected material through intercellular spaces was reported by Wolff and Schreiner (25, 26) who found intradermally injected peroxidase filling the intercellular spaces of the upper layers of the stratum granulosum "immediately" after the injection.

Close approximation, close apposition, gap junction and tight junction. The regions of close membrane apposition of the frog epidermis, in which the outer leaflets of the apposed plasma membranes remain separate, was called close approximation by Farquhar and Palade (13). Similar seven-layered "close apposition" has been demonstrated in various other tissues (27, 28, 29, 30). With new techniques using uranyl acetate and/or lanthanum in tissue blocks, it has been revealed that certain junctions which appear tight with routine staining are actually open, i.e.; similar to "close approximation" or "close apposition". They showed a patent intercellular gap of 20–30A, into which lanthanum penetrated. These have been reclassified into "gap junctions" (3, 11). In lanthanum-permeated gap junctions, when sectioned tangentially, densely packed hexagonal subunits of 70–75Å were frequently observed (3, 11, 12). Similar structures were also demonstrated in the epidermis by this study (Fig. 15). Arrays of relatively large hexagonal structures of 300–650Å, found in the tangentially cut usual



FIGS. 16, 17, 18. Laminated bodes are seen in the intercellular spaces of the upper Malpighian cells. Electron-lucid layers alternate with electron-dense layers (arrows). Fig. 16: Specimen ⅋3: × 104,000. Fig. 17: Specimen ⅋1: × 229,250. Fig. 18: Specimen ⅋3: × 230,500.

intercellular spaces have not been described previously. They may be structures similar to those found in the gap junctions or simply represent tangentially sectioned irregular indentations of plasma membrane.

Old and new terminologies. In the normal human epidermis some junctions which had been believed to be tight in analogy to similar structures in the frog skin (13, 14) were found to be patent to lanthanum (5). However, no attempt has been made to analyze which types of junction previously thought to be tight were actually patent. In this study most of the short focal fusions usually present following desmosomes, i.e., maculae occludentes of Farquhar and Palade (13), were found to be permeable to lanthanum. A long variety seen alternating with desmosomes, which corresponds to nexus as described by Dewey and Barr in the frog skin (28), was also found not tight. These junctions should, therefore, be reclassified with "focal" or "extended gap junctions" respectively. On the other hand, the junctions present at the distal ends of the lateral junctions of the granular cells, which were called zonulae occludentes by Farquhar and Palade (13) in the frog skin and assumed to be tight, were indeed found in this study to be impermeable to lanthanum. These *distal junctions of the stratum granulosum* have not been described previously in the human epidermis.

Dewey and Barr (14) noticed a great variation of the dimensions among what they thought to be tight junctions, called nexuses, of various tissues including frog skin. They thought that factors such as types of fixation, differences in chemical composition of the membranes in different tissues, etc., caused variations. The present investigation showed that, even in a single section, dimensions of the "gap" are different (Figs. 13, 14). Also, as shown in Figure 13 and Figure 14, the focal, narrow variety can occur within the long, wide variety. In the future it may become necessary to subdivide the "gap junctions" of the epidermis if, for example, the functional significance of each becomes more precisely known. In the meantime, we may either use the term "gap junction" of the epidermis more liberally by defining it as lanthanum permeable intercellular slits of various widths (~115Å) and lengths (~½ μ), or elect to use a new term such as "slit junction" in order to avoid confusion with the periluminal type of gap junctions (3, 10, 12) which usually occur in a series (31).

REFERENCES

1. Hashimoto, K. and Lever, W. F.: An electron microscopic study on pemphigus vulgaris of the mouth and the skin with special reference to the intercellular cement. J. Invest. Derm., *48:* 540, 1967.
2. Luft, J. H.: Electron microscopy of cell extraneous coats as revealed by ruthenium red staining. J. Cell Biol., *23:* 54a, 1964.
3. Revel, J. P. and Karnowsky, M. T.: Hexagonal array of subunits in intercellular junctions of the mouse heart and liver. J. Cell Biol., *33:* C_7, 1967.
4. Overton, J.: A fibrillar intercellular material between reaggregating embryonic chick cells. J. Cell Biol., *40:* 136, 1969.
5. Wolff, K. and Schreiner, E.: An electron microscopic study on the extraneous coat of keratinocytes and the intercellular space of the epidermis. J. Invest. Derm., *51:* 418, 1968.
6. Hashimoto, K.: Lanthanum staining of Langerhans' cell. Arch. Derm., *102:* 280, 1970.
7. Hashimoto, K. and Lever, W. F.: An ultrastructural study of cell junctions in pemphigus vulgaris. Arch. Derm., *101:* 287, 1970a.
8. Lever, W. F. and Hashimoto, K.: The etiology and treatment of pemphigus and pemphigoid. J. Invest. Derm., *53:* 373, 1969.
9. Reynolds, E. S.: The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol., *17:* 208, 1963.
10. Matter, A., Orci, L. and Rouiller, C.: A study on the permeability barriers between Disse's space and the bile canaliculus. J. Ultrastruct. Res., Suppl. *11:* 5, 1969.
11. Brightman, M. W. and Reese, T. S.: Junctions between intimately apposed cell membranes in the vertebrate brain. J. Cell Biol., *40:* 648, 1969.
12. Goodenough, D. A. and Revel, J. P.: A fine structural analysis of intercellular junctions in the mouse liver. J. Cell Biol., *45:* 272, 1970.
13. Farquhar, M. G. and Palade, G. E.: Cell junctions in amphibian skin. J. Cell Biol., *26:* 263, 1965.
14. Dewey, M. M. and Barr, L.: A study of the structure and distribution of the nexus. J. Cell Biol., *23:* 553, 1964.
15. Odland, G. F.: A submicroscopic granular component in human epidermis. J. Invest. Derm., *34:* 11, 1960.
16. Matoltsy, A. G. and Parakkal, P. E.: Membrane-coating granules of keratinizing epithelia. J. Cell Biol., *24:* 297, 1965.
17. Wilgram, G. F.: Das Keratinisom: ein Faktor in Verhornungsprozess der Haut. Der Hautarzt, *16:* 377, 1965.
18. Hashimoto, K.: Ultrastructure of the human toenail. IV. Cell migration, keratinization and formation of the intercellular cement. Arch. Derm. Forsch., *240:* 1 (197).
19. Hashimoto, K.: Electron microscopy and histochemistry of pemphigus and pemphigoid. J. Dental Res. In press.
20. Kobayasi, T.: Electron microscopy of the elastic fibers and the dermal membrane in normal human skin. Acta Dermatovener., *48:* 303, 1968.

21. Susi, F. R., Belt, W. D. and Kelly, J. W.: Fine structure of fibrillar complexes associated with the basement membrane in human oral mucosa. J. Cell Biol., *34:* 686, 1967.
22. Silberberg, I.: Percutaneous absorption of mercury in man. I. A study by electron microscopy of the passage of topically applied mercury salts through stratum corneum of subjects not sensitive to mercury. J. Invest. Derm., *50:* 323, 1968.
23. Hanniker, G.: Depth of the surface zone of a liquid. Rev. Mod. Phys., *21:* 322, 1947.
24. Derjaguin, B. V.: Recent research into the properties of water in thin films and in microcapillaries. Symp. Soc. Exp. Biol., *19:* 55, 1965.
25. Wolff, K. and Schreiner, E.: Aufnahme, intracellulare Transport und Abbau exogenen Proteins in Keratinocyten. Eine elektronenmikroskopische-cytochemische Studie mit Peroxidase als Markierungssubstanz. Arch. Klin. Exp. Derm., *235:* 203, 1969.
26. Wolff, K. and Schreiner, E.: Uptake, intracellular transport and degredation of exogenous protein by Langerhans cells. An electron microscopic-cytochemical study using peroxidase as tracer substance. J. Invest. Derm., *54:* 37, 1970.
27. Coggeshall, R. E.: A fine structural analysis of the ventral nerve cord and associated sheath of *Lumbricus terrestris L.* J. Comp. Neurol., *125:* 393, 1965.
28. Reger, J. F.: The fine structure of neuro-muscular junctions and contact zones between body wall muscle cells of *Ascaris lumbricoides* (*var. Suum*). Z. Zellforsch., *67:* 196, 1965.
29. Takahashi, K. and Hama, K.: Some observations on the fine structure of the synaptic area in the ciliary ganglion of the chick. Z. Zellforsch., *67:* 174, 1965.
30. Hamilton, D. W.: Perilymphatic fibrocytes in the vestibules of the inner ear. Anat. Rec., *157:* 626, 1967.
31. Hashimoto, K.: Demonstration of the intercellular spaces of the human eccrine gland by lanthanum. I. The secretory coil. J. Ultrastruct. Res. In press.