Brief Notes

Cell Interconnections in Normal Human Cervical Epithelium.* By H. E. Karrer. With the Assistance of Natasha Kent and R. Calame. (*From the Department of Pathobiology and Department of Surgery, School of Hygiene and Public Health and School of Medicine, Johns Hopkins University, Baltimore.*)‡

Several recent electron microscope investigations have been concerned with the fine structure of various cell interconnections, notably in epidermis (5, 11, 17), heart muscle (intercalated discs (18), corneal epithelium (6), and capillary endothelium (4). These studies established that desmosomes represent specialized zones of attachment between separate cells. Similarly specialized attachment zones appear in the form of "terminal bars" (3, 21). Recently, Odland described several specialized layers within the nodes of Bizzozero of human epidermis and presented a schematic diagram of these epidermal attachment zones (9). The same layers have been recognized in oral mucosa by Fasske and Themann (2), and in cervical epithelium by Vogel (19) who also demonstrated the continuity of the plasma membranes with some of the desmosome layers.

The present paper describes the fine structure of desmosomes in human cervical epithelium. A report on this subject seems in order because of the interesting relationship of the plasma membranes to different layers within the desmosomes. In addition, another type of a specialized cell interconnection will be described for the first time.

Materials and Methods

Biopsy specimens of normal human cervix were obtained through the cooperation of the gynecological outpatient department of the Johns Hopkins Hospital. The specimens, which usually consisted of epithelium and subepithelial connective tissue, were immediately fixed either in veronal buffered 1 per cent osmium tetroxide solution (10) containing sucrose (1) or sodium chloride (8), or in veronal-buffered 0.6 per cent potassium permanganate (7) to which polyvinylpyrrolidone (final concentration of 0.1 per cent) had been added for isotonicity.[1] Fixation was performed at about 4°C. for a period of 2 hours. Ascending concentrations of cold acetone were used for dehydration, allowing about 1 hour total dehydration time. The tissues were then impregnated in a mixture of three parts *n*-butyl- and one part ethyl-methacrylate containing 0.1 per cent of benzoyl-peroxide. They were subsequently embedded in a prepolymerized syrup of the same composition. Polymerization was carried to completion in a vacuum oven set at about 80°C., during a minimal period of 48 hours. Thin sections were cut with a Porter-Blum microtome using glass knives. Osmium-fixed preparations were stained with uranyl acetate for additional contrast (20), but permanganate-fixed specimens were left unstained. A Siemens and Halske Elmiskop I b electron microscope (15) was used with initial magnifications of about 37,000 to 51,000. A cooling stage (16) was sometimes used in order to minimize contamination of the specimen during the prolonged search for specialized structures.

OBSERVATIONS

Desmosomes:

The cervical epithelial cells are interconnected by means of specialized attachment zones (nodes of Bizzozero). Within these several strata can be recognized (Figs. 1 and 2). Five thin dense strata ("lines") usually appear in ideal transverse sections through osmium-fixed desmosomes (a, a', b, b', x). Each of these is about 3 to 5 mμ wide; these dense strata are separated by four layers of low density each about 6 mμ in width. In preparations showing well fixed plasma membranes, the pairs of dense strata, which are the plasma membranes of the two cells (p, Fig. 1), can be seen to be continuous with the strata pairs a/a' and b/b' of the desmosomes (arrow, Fig. 1). This becomes even clearer in permanganate-fixed desmosomes (arrow, Fig. 3). The median stratum x apparently has no continuity with any structure outside the

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region of the desmosomes. Unlike the plasma membranes and their derivative strata (a/a' and b/b') (Figs. 1 and 2), the median stratum x (Fig. 2) is not preserved in permanganate-fixed material (Fig. 3). Instead there appears a denser middle zone, about 22 mμ wide, which separates strata a' and b'.

After osmium fixation the cytoplasm of adjacent cells appears condensed along strata a and b in the form of a dense zone, about 16 mμ wide (c, Figs. 1 and 2). After permanganate fixation this zone is either not apparent at all, or it is only faintly outlined (as in Fig. 3). Beyond this dense zone the cytoplasm appears granular and dense over a width of about 40 to 120 mμ (t, Fig. 1); these areas represent bundles of cross-sectioned tonofilaments that run more or less tangent to the desmosomes. Cross-sections of tonofilaments are not recognizable in permanganate-fixed desmosomes (Fig. 3).

"Quintuple-Layered" Cell Interconnections:

In the same cervical epithelium another type of specialized cell interconnection can be recognized. It appears in the form of three parallel dense strata or "lines" which are separated by two layers of low density. The total width of this structure is about 20 mμ (Fig. 4). The outer two "lines" appear slightly denser and slightly thicker (~3 mμ) than the middle one (~2 mμ). This type of cell interconnection can be recognized in osmium-fixed material, but it becomes especially prominent after permanganate fixation (Fig. 4). There is good evidence that this structure is entirely homologous to the external compound membrane described in mesaxons of nerve fibers (12–14). Such evidence will be presented elsewhere.

DISCUSSION

Desmosomes:

Some investigators consider the fine structure of the desmosomes in cervical epithelium to be similar to that of epidermal desmosomes as described by Odland (9). However, analysis of the relationship between the cell membranes and the different desmosome strata results in an interpretation of these strata that differs from Odland's model. Odland assumed that the dense zones or "attachment plaques" (c, Figs. 1 and 2) were derived from the plasma membranes of the adjacent cells. Instead, it is the strata pairs a/a' and b/b' which derive from the plasma membranes. The two dense zones which accompany them (c, Figs. 1 and 2) must therefore be considered as "condensed" cytoplasm. It seems perhaps unnecessary to designate strata a' and b' (Figs. 1 and 2) by a new term (Odland's "intermediate dense layer" (9)) since these strata are simply portions of the two plasma membranes.

At present the origin of the median stratum x (Odland's "intercellular contact layer") is not clear, but it is found too regularly to be considered a fixation artifact. The stratum x appears to differ in composition from the plasma membranes (strata a/a' and b/b', Figs. 1 and 2) since, unlike these, it does not become visible after permanganate fixation. It might be that this median stratum represents a layer of "condensed" intercellular material, formed along the plane where the two plasma membranes meet.

"Quintuple-Layered" Cell Interconnections:

These specialized cell interconnections are distinct from the classical nodes of Bizzozero though they might serve a similar function and provide zones of attachment between cells—but this assumption has not been proved. They are regularly found in cervical epithelium and have also been recognized in striated muscle of lung blood vessels.[2] Thus it appears that this type of cell interconnection may be of general importance, and that careful studies would reveal its presence in a variety of tissues. The fine structure of this type of cell interconnection is obviously similar in appearance to the external compound membrane of mesaxons in nerve fibers (13, 14), although the total width of such a mesaxon was found somewhat smaller (~15 mμ) than the ~20 mμ width of the cell interconnection. There is, however, one important difference: in mesaxons the compound membrane results from the fusion of the infolded portions of one plasma membrane belonging to one and the same Schwann cell (13, 14), whereas the "quintuple-layered" cell interconnection is the fusion product of two plasma membranes belonging to two different cells.[2] In a way, the "quintuple-layered" cell interconnection is homologous with one lamella (12, 13) of myelin. That this type of structure occurs in at least three different tissues (myelinated nerve fiber, cervical epithelium, and muscle[2]) is highly interesting, but

[2] Personal observation (H. E. K.), to be published.

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the significance of this fact is not fully apparent at the present time.

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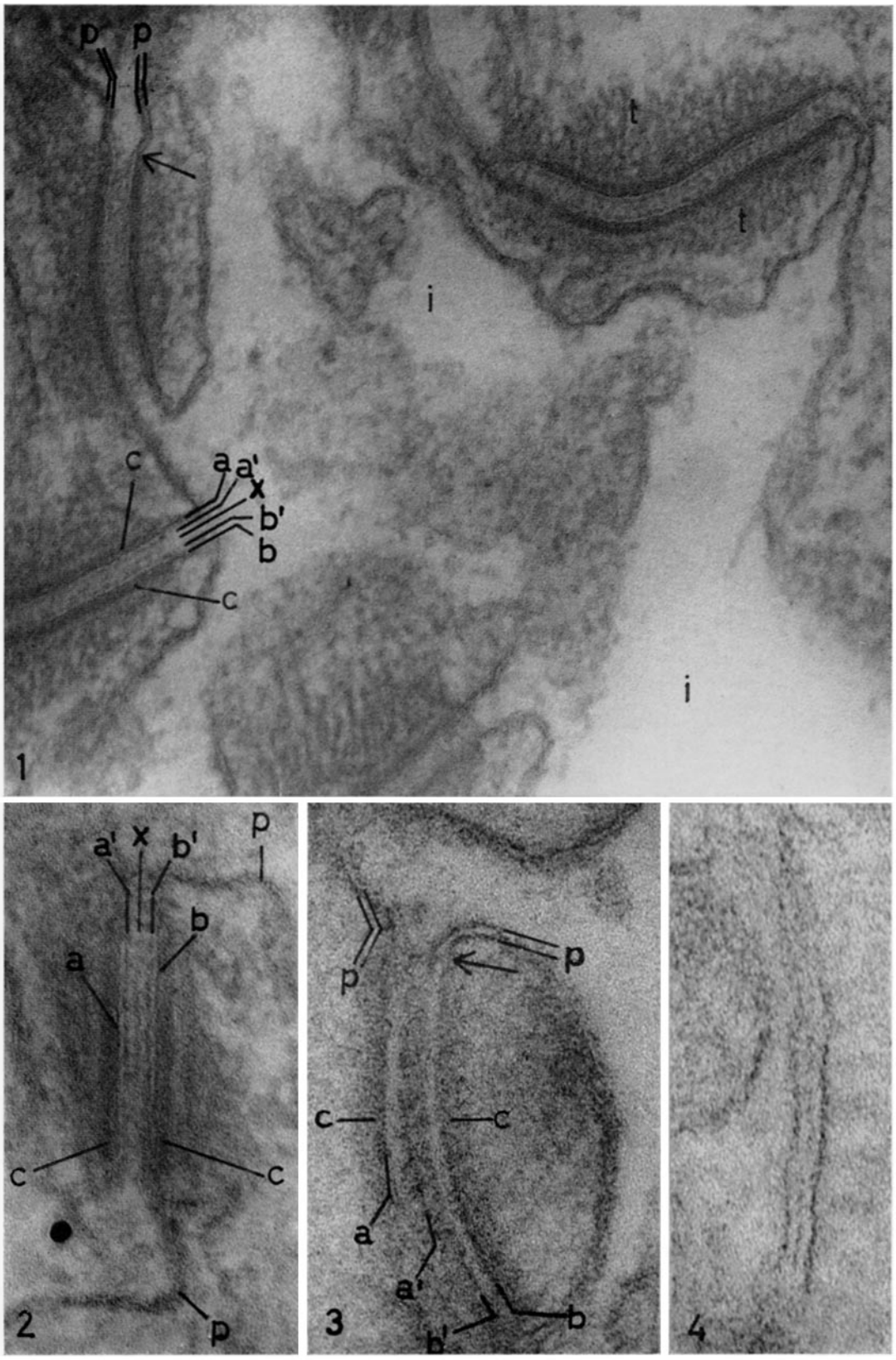
EXPLANATION OF PLATE 97

FIG. 1. Cross-section through four desmosomes. Only small portions of the cells are seen within the frame of this figure. In between the contact points (desmosomes) the intercellular space is greatly widened and appears structureless (*i*). The desmosomes show a specific structure consisting of five parallel dense strata which appear as dark lines (*a*, *a'*, *b*, *b'*, *x*) and which are separated by four layers of low density. The two outermost dense strata (*a* and *b*) are each accompanied by a condensed zone of cytoplasm (*c*). The tonofilaments insert themselves tangentially within these condensed zones of cytoplasm and, therefore, appear in cross-section in this micrograph. The total mass of such cross-sectioned tonofilaments appears as a dense, oval-shaped mass surrounding each desmosome (*t*). The relation of the cell plasma membranes (*p*) with the different strata of the desmosomes is demonstrated at the arrow. It is obvious that the paired dense layers which make up the plasma membranes of the adjacent cells continue within the desmosomes in the form of strata *a*/*a'* and *b*/*b'*. On the other hand, the median stratum *x* has no apparent continuity beyond the area of the desmosomes. Osmium tetroxide fixation. × 125,000.

FIG. 2. Cross-section through a desmosome. This figure shows more clearly the different strata. However, their relation to the plasma membranes cannot be made out in this electron micrograph. The plasma membranes (*p*) do not show their pairs of dense strata possibly because of less favorable fixation. The different dense strata (*a*, *a'*, *b*, *b'*, *x*) fade out at either end of the desmosome. This effect is probably produced by less-than-ideal transverse sectioning. Osmium tetroxide fixation. × 170,000.

FIG. 3. Cross-section through a desmosome. Permanganate fixation. This figure clearly illustrates (arrow) the continuity of the two dense strata of each plasma membrane (*p*) with the respective strata (*a*/*a'*, *b*/*b'*) of the desmosome (see also description of Fig. 1). The permanganate-fixed desmosome appears different from the osmium-fixed one: the median dense stratum *x* is invisible; the interspaces within strata pairs *a*/*a'* and *b*/*b'* appear wider and less dense than in osmium-fixed material; the condensed zones of cytoplasm (*c*) are less dense than in osmium-fixed desmosomes; the cross-sections of tonofilaments are not recognizable. × 180,000.

FIG. 4. "Quintuple-layered" cell interconnection (not related to nodes of Bizzozero). It consists of three dense strata separated by two zones of low density. The two outer dense strata appear thicker (~ 3 mμ) and denser than the middle one (~ 2 mμ). For further discussion see text. Permanganate fixation. × 235,000.



(Karrer: Cell interconnections in cervical epithelium)