

CHANGES IN TIGHT JUNCTIONS OF THYROID EPITHELIUM WITH CHANGES IN THYROID ACTIVITY

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The tight junction (zonula occludens) is a membrane specialization which occurs at some regions of close contact between cells and appears in thin sections as an area of fusion of the outer leaflets of the plasma membranes of adjacent cells (5, 8). In thyroid follicles it is located at the apical end of the

lateral plasma membranes of the epithelial cells bounding the follicular lumen (5). It has been assumed to inhibit the passage between thyroid follicular cells of thyroglobulin from the lumen of thyroid follicles.

The fine structure of the tight junction has been

studied in freeze-fracture replicas of several tissues (liver [2, 7], small intestine [3, 6], epididymis [6], exocrine pancreas [6], stomach [3], gallbladder [3], urinary bladder [3], kidney tubule [3], and mammary gland [10], among others). We describe here the appearance of this membrane specialization in rat thyroid follicular cells and the changes in its morphology that occur in response to changes in thyroid activity.

MATERIALS AND METHODS

Thyroids were obtained from male Fischer rats 3–4 mo of age, weighing 200–300 gm, which had been fed Purina Laboratory Chow since weaning. Six rats were untreated, eight rats were hypophysectomized for 1 or 20 days, seven rats were injected intravenously 5–60-min earlier with 0.05 IU of mouse thyroid-stimulating hormone (TSH), and eight rats were fed a low-iodine diet (Remington diet, General Biochemicals, Inc., Chagrin Falls, Ohio), containing 0.25% thiouracil (TU) for 7 or 14 days. The glands were fixed either by immersion for 1 h in 3% glutaraldehyde in 0.1 M phosphate buffer, or by perfusion with 2.5% glutaraldehyde in 0.05 M phosphate buffer followed by immersion in 3% glutaraldehyde in 0.1 M phosphate buffer, all at pH 7.4. After fixation, the glands were cut into 1-mm³ pieces and were transferred to a solution of ice-cold 20% glycerol in 0.1 M phosphate buffer, pH 7.4, for 2–3 h. The blocks were then rapidly frozen in liquid nitrogen, freeze-cleaved, and platinum-carbon shadowed in a Balzers' freeze-etch apparatus. The replicas were examined and photographed in a Philips EM 200 electron microscope. Observations were con-

finied to regions of the replica with recognizable thyroid follicles in which the geometrical relations were clear.

The widths of all tight junctions observed were measured, provided both apical and basal edges were included in the fracture face and the tight junction lay in a plane approximately perpendicular to the angle of view. The contributions of single elements with terminal loops extending from the main network of the tight junction were not included in width measurements, nor was the specialized meshwork at the juncture of three cells.

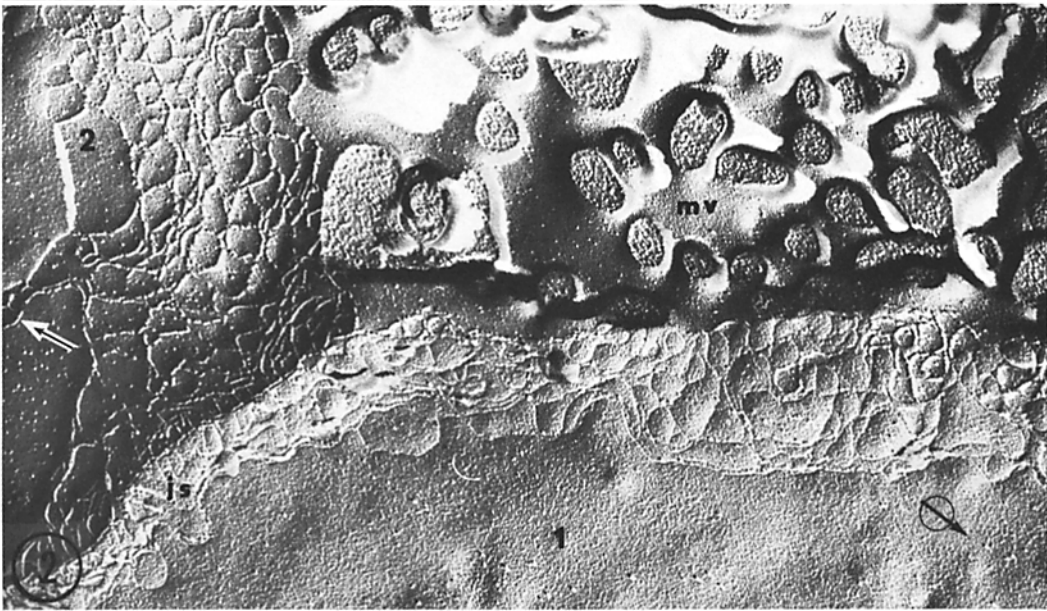
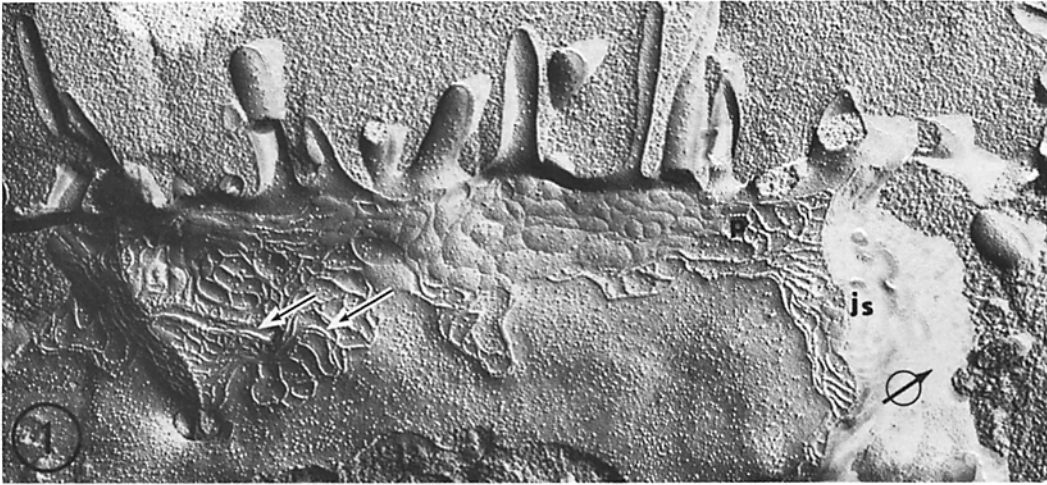
RESULTS

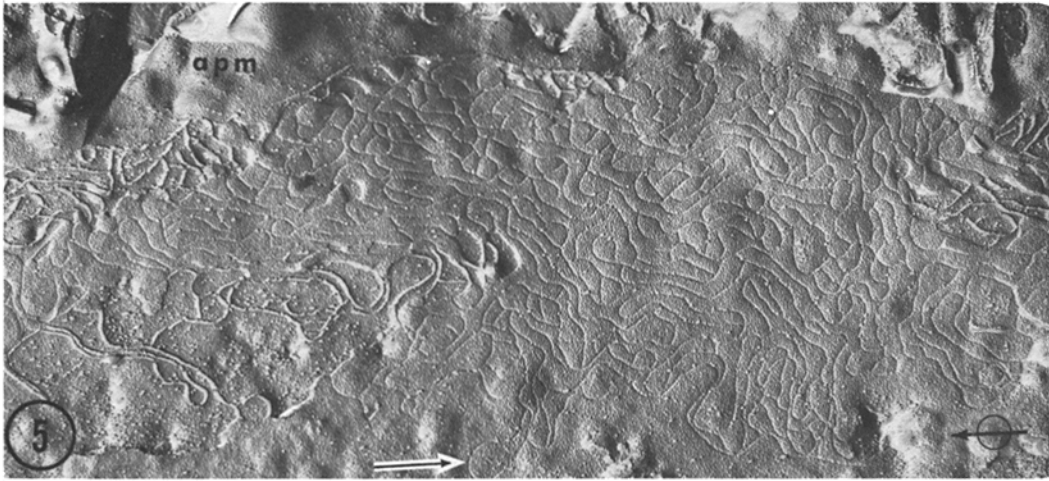
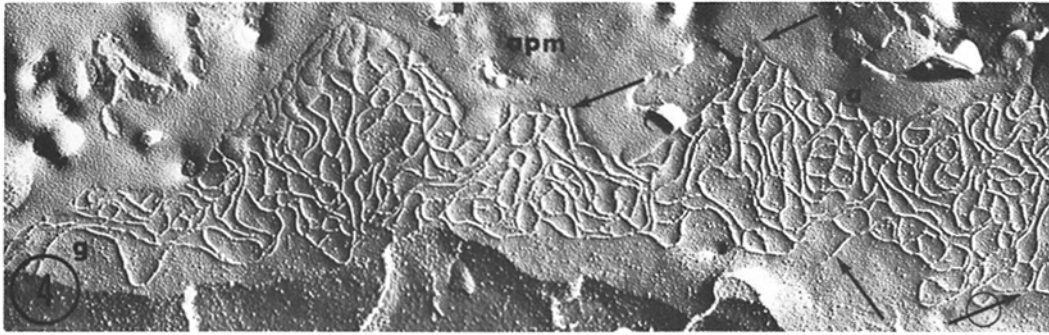
In a freeze-fractured replicas of thyroid epithelium, when the plane of fracture was within the plasma membrane, two fracture faces were exposed; one, the A face, facing the outside of the cell; the other, the B face, facing the interior. As in other tissues, the areal density of intramembranous particles on the A face was considerably higher than that on the B face. Tight junctions appeared as a more or less continuous band made up of a meshwork of ridges and furrows on the lateral plasma membrane just below the cell apex (Figs. 1–7). Furrows on the B face were usually continuous with ridges on the A face when transitions in the fracture plane from A to B faces occurred (Fig. 1). The ridges (or furrows) were usually single, but occasionally two of them closely paralleled each other (Fig. 1). The ridges usually appeared as continuous lines, but occasionally they contained gaps or were represented by rows of

FIGURE 1 Freeze-fracture replica of the apical part of a normal rat thyroid epithelial cell. Membranes of microvilli or broken stubs of microvilli (*mv*) are seen at the top of the micrograph. The fracture plane traverses the lateral plasma membrane where the high density of intramembranous particles characteristic of the A face is seen. The tight junction appears as an anastomosing network of ridges and furrows extending from 0.27 μm to 0.67 μm toward the base of the cell. Where transitions from ridges to furrows occur, these appear to be continuous. Some ridges are represented by rows of particles (*p*). Although most elements run an apparently random course, a few tend to parallel each other (arrows). At the right of the figure is the junctional specialization (*js*) where three cells meet, which resembles that seen in other cell types (11). Here the tight junctions are widened, forming a specialized, orderly array. At such points a pair of elements parallel the line of junction of two cells. These are joined by many closely spaced, divergent elements which extend from these parallel elements to the remainder of the tight junction network. $\times 46,500$.

FIGURE 2 In this replica the lateral plasma membranes of three cells are revealed at the bottom and left of the figure, while the A face of the apical plasma membrane fractured across the bases of microvilli (*mv*) is shown at upper right. The tight junction of one cell (1) has a relatively constant width of 0.23 μm , but the tight junction of the adjacent cell (2) is much wider (up to 0.85 μm) and varies in width. Note the presence of a basal loop (arrow). Again, note the junctional specializations (*js*) occurring where three cells meet. $\times 43,700$.

FIGURE 3 Tight junction from the thyroid gland of a rat 3 wk after hypophysectomy. Stubby microvilli (*mv*) are present at the top of the figure. This tight junction (B face) is relatively narrow (0.13–0.33 μm). Many of the ridges on the left of the figure run parallel to each other (arrows). $\times 42,300$.





particles (Fig. 1). The continuity of the furrows was sometimes interrupted by particles lying in the furrow (Figs. 1, 2). Usually, both apical and basal edges of the junctional array were relatively smooth in contour.

Although the tight junctions of all thyroid epithelial cells had certain morphological features in common, they differed quantitatively in their width and complexity, both from cell to cell (Fig. 2) and within different areas in the same cell (Fig. 1). In some cells from normal thyroids, the width of the tight junction was fairly uniform (Fig. 3), but in others it varied by a factor of 2 (Fig. 1). Still further quantitative variations were produced by the experimental treatments that the animals received.

In thyroids taken from rats hypophysectomized for 1 or 20 days, many tight junctions were indistinguishable from those of normal animals, but two very narrow junctions were encountered in rats hypophysectomized for 20 days. These junctions were relatively constant in width (Fig. 3). Many elements were closely apposed, with two elements running parallel to each other (Fig. 3). The cells had about the same number of elements (8–10) per tight junction as did the cells in normal animals, suggesting that their narrow width was due primarily to the small space between the elements rather than to their disappearance. In other cells, the distance between some elements was small while other elements retained relatively normal spacing.

The appearance of tight junctions did not change within 5 to 60 min after injection of TSH. However, in rats fed thiouracil for 7–14 days (Figs. 4–7), some tight junctions were wider (Fig. 5) than those from either normal or hypophysectomized rats, some were narrower (Fig. 6), and in many cells the junctions had both very narrow and unusually wide areas (Figs. 4 and 7). In contrast to the narrow junctions from hypophysectomized rats, narrow junctions or narrow junctional regions from thiouracil-fed animals had only one or a very few elements. Such junctions often had single elements with a terminal loop extending usually basally but sometimes apically from the remainder of the tight junction. In others, there were narrow gaps in the meshwork in which no elements were present (Fig. 4). Wide tight junctions contained many more elements (Fig. 5) than did junctions from unstimulated glands, but the distance between elements was usually not increased.

DISCUSSION

The tight junctions of rat thyroid epithelium resemble those of other epithelia in the general arrangement of their elements, in the occurrence of interruptions in their elements, and in the variable width of junctions from cell to cell. In proximal tubule cells of the kidney (1) and in beta cells of the pancreas (9), the width of tight junctions can be varied by experimental means, and in the mammary gland the width of tight junctions varies with its physiological state (10). In the latter tissue, the

FIGURE 4 This tight junction from a rat fed TU for 1 wk varies in width from 0.16 to 1.06 μm . The cell apex (*apm*) is at the top of the figure. Several unlooped elements are suggested at the apical end of this tight junction and one may be present basally (arrows). At the left of the figure, a gap (*g*) is present which is only two elements in width. Note apical loops (*a*) at upper right. $\times 37,900$.

FIGURE 5 This very wide tight junction from a rat fed TU for 2 wk is from 1.25 to 1.67 μm wide. The cell apex (*apm*) lies in the upper part of the figure. The number of elements in the tight junction is clearly greater than normal. Although the apical part of the tight junction has a relatively smooth boundary, a basal loop is clearly seen (arrow). $\times 30,625$.

FIGURE 6 This relatively simple tight junction is from a rat fed TU for 2 wk. The cell apex (*apm*) with its stubs of microvilli is at the upper right of the figure. The number of elements appears to be unusually small in this tight junction, which has a relatively simple meshwork. Basal loops are present (arrows). A junctional specialization (*js*) where three cells meet is present at the left of the figure. $\times 66,350$.

FIGURE 7 This tight junction from a rat fed TU for 2 wk varies in width from 0.30 to 0.81 μm . The A face of the apical plasma membrane (*apm*) is at the top of the figure. The wider portion of the junction at the right of the figure appears to contain many more elements than do tight junctions from normal rats. At the left of the figure, the meshwork is relatively simple. $\times 40,800$.

tight junction appears to remain intact despite considerable changes in the diameter or perimeter of cells at the level of the tight junction.

It is interesting that short exposure to TSH has no obvious effect on thyroid tight junctions, since TSH induces release of immunologically intact thyroglobulin from the gland (4), possibly via channels between follicular cells. The lack of obvious change in tight junction structure under these experimental conditions suggests either that such changes do not occur or are not detected, or that immunologically intact thyroglobulin can pass out of the thyroid follicles by an intracellular route.

Our findings show that the width and complexity of thyroid tight junctions can change after prolonged TSH stimulation. Major changes in thyroid tight junctions do not appear to be produced by changes in cell shape. Typical thyroid epithelial cells are flatter after hypophysectomy than in normal controls (often with an increase in perimeter), but tight junctions do not change appreciably when the cells flatten. In rats fed TU, when the thyroid epithelial cells increase appreciably in height, several changes in tight junction occur which are not easily explained by change in cell shape.

The first change is the occurrence of especially wide junctions. Some of the tight junctions in thyroids of TU-fed rats are so elaborate, with double or triple the number of ridges per width of tight junction, that there may have been formation of new tight junction material during TU feeding. Such increases in width are often limited to a restricted region of the tight junction, suggesting that initially such changes in width may be a localized phenomenon.

The second is the occurrence of very simple junctions or junctional regions of only one or two elements which often have several single looped elements associated with them. It may be that these are newly formed junctions. New formation of plasma membrane and tight junctions might be expected in these cells because the thyroid gland is growing. The total number of cells increases twofold within the 1st wk and fivefold in 2 wk (12, 13). It is entirely possible that the very simple, narrow tight junctions found in growing glands are at new cell surface, but there is no direct evidence for this.

A prominent feature in some cells in stimulated thyroids is the presence of a loop on the end of a

single element (Figs. 3-5). Such loops occur at the basal or, less frequently, the apical side of the tight junction, and occasionally can be recognized within the junction. The high incidence of loops associated with simple tight junctions only a few elements wide is consistent with the possibility that these simple junctions are newly formed and that loop formation is a stage in junction assembly or extension. However, the existence of wide and narrow junctional regions within the same cell suggests that wide junctional regions might also arise by transfer of junctional material from narrower areas. If this were true, the presence of loops at the end of single linear elements could indicate that a local unravelling of junctional material as rearrangement of elements takes place.

SUMMARY

The morphology of the tight junction of rat thyroid epithelium was examined in freeze-fractured material fixed in glutaraldehyde and briefly glycerinated. In normal thyroids the overall appearance of this junctional specialization resembled that of other cell types in many respects. Short-term changes in thyroid activity and hypophysectomy for 3 wk did not obviously affect the appearance of tight junctions. Feeding of the goitrogen, thiouracil, which stimulates secretion of thyroid-stimulating hormone, resulted in the appearance of some very narrow and some very wide, tight junctions or sometimes junctions with both wide and narrow regions within the same cell.

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