

Ultrastructure of Intermediate Stages in Polarity Reversal of Thyroid Epithelium in Follicles in Suspension Culture

LUCIO NITSCH and SEYMOUR H. WOLLMAN

Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205. Dr. Nitsch's present address is Centro di Endocrinologia ed Oncologia Sperimentale del CNR, Istituto di Patologia Generale, II Facoltà di Medicina, 80131 Napoli, Italy

ABSTRACT Separated thyroid follicles can be maintained in suspension culture in Coon's modified F-12 medium in 0.5% calf serum. If the serum concentration is raised to 5%, the follicles undergo inversion in 3–5 d. During the process of inversion, epithelial cells can be observed in intermediate stages of polarity reversal. The earliest ultrastructural changes recognized are surface changes in which tight junctions and microvilli appear at the lateral margins of the cell near the medium. Later, changes in the distribution of intracellular organelles occur. The Golgi apparatus shifts towards the end of the cell facing the medium, and lysosomes shift toward the luminal end of the cell. The tight junctions and microvilli at the luminal end of the cell disappear sometime after the cytoplasmic organelles rearrange. The luminal colloid disappears only after the surface changes (loss of tight junctions and microvilli) occur at the luminal end of the cell. There appears to be some regulation of the order in which changes occur during polarity reversal of the thyroid epithelial cell.

Mauchamp and collaborators (2, 3) have reported that when separated hog thyroid cells are allowed to reaggregate in suspension culture they can form either of two stable structures, depending on the composition of the culture medium. They may form closed follicles in which the epithelial cells have normal polarity (with microvilli extending into the lumen) or inverted follicles in which the epithelial cells have reversed polarity.

We report here that in suspension culture using our standard medium (4), normal follicles invert slowly and that the rate of this inversion depends on the culture conditions. The process is slow enough so that one can observe intermediate stages in the reversal of polarity of the epithelial cells during the inversion process. In this paper we describe ultrastructural changes that occur in the thyroid epithelial cell during reversal of its polarity.

MATERIALS AND METHODS

Suspension cultures of separated thyroid follicles were prepared as previously described (4). In brief, thyroid glands from young Fischer rats were minced and dissociated by incubation with collagenase in complete tissue culture medium followed by passage through a pipette orifice. Clusters of epithelial cells, the remains of broken follicles, and possibly some intact individual follicles were separated from large aggregates of thyroid tissue and from single cells by differential filtration. Epithelial cell clusters were cultured in suspension without shaking in agarose-coated dishes in Coon's modified F-12 medium containing either 0.5 or 5.0% calf serum (Flow Laboratories, Inc., Rockville, Md.), 50 IU/ml of penicillin, and 50 µg/ml of streptomycin. Medium was changed every 3 d by centrifugation.

For examination by electron microscopy, the follicles in culture were collected by centrifugation, fixed for 15 min in 2.5% glutaraldehyde (Ladd Research Industries, Inc., Burlington, Vt.) in 0.1 M cacodylate buffer, pH 7.3, and postfixed for 20 min in 1% OsO₄ in the same buffer. Follicles were then stained for 1 h in

1% aqueous uranyl acetate and dehydrated in a graded series of ethanols and then directly embedded in Epon 812. Thick sections were stained with toluidine blue, thin sections with uranyl acetate and lead citrate.

RESULTS

When clusters of thyroid epithelial cells were incubated in suspension in culture medium containing 0.5% calf serum they formed spheroidal structures (Figs. 1 and 2) containing one or more lumens (4). The lumens contained a material that stained like thyroid colloid, and the epithelial cells surrounding the lumens had normal polarity. With the usual good batch of serum, almost all cells retained this polarity for several days.¹ However, if the concentration of the serum were changed to 5% the follicles gradually inverted, some being inverted by 1 d and almost all being inverted by 5 d (Figs. 3 and 4). Inverted follicles had a greatly enlarged lumen without colloid. The cells surrounding the lumen were sometimes cuboidal, but were usually very flat and attenuated. All showed reversal of polarity.

When cultures were examined 1 and 2 d after changing the serum concentration of the medium to 5%, many cells were observed in intermediate stages of polarity reversal by electron microscopy. It was possible to arrange profiles as sequential stages from minimal deviations from normal polarity to complete polarity reversal (Figs. 5–10). In all intermediate stages observed, the lumen of the follicle contained colloid (Figs. 5–8) and the cells possessed tight junctions in common with neighbor cells and microvilli at the luminal end of the cell. We

¹ With a few unusual batches of serum, follicles in a medium containing 0.5% serum inverted in 1 or 2 d.

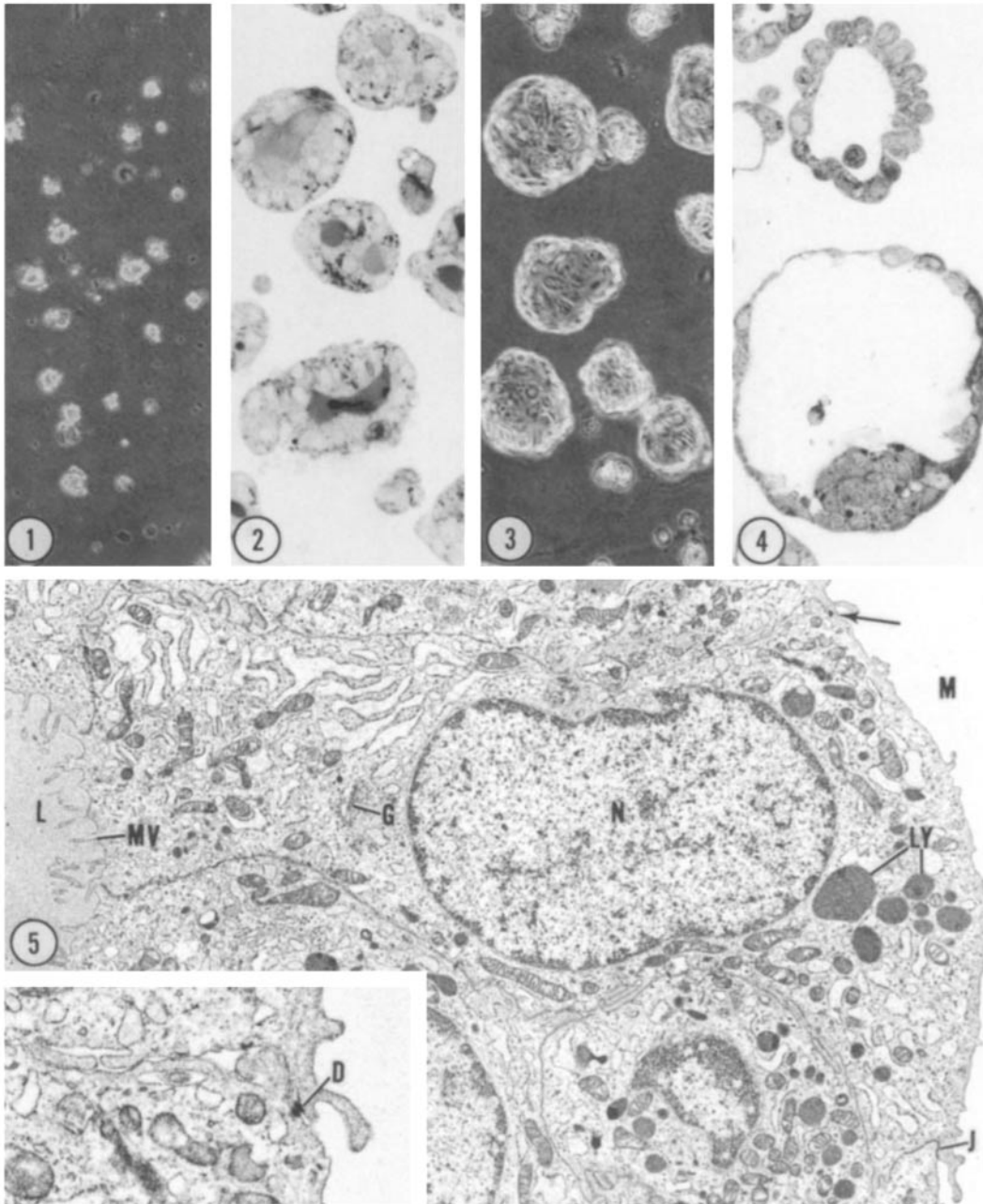


FIGURE 1 Thyroid epithelial cell clusters cultured for 3 d in 0.5% serum in agarose-coated dishes. The clusters have formed follicles in suspension. Phase contrast. $\times 82$.

FIGURE 2 Thick sections ($1.5 \mu\text{m}$) of follicles prepared as in Fig. 1. Electron-dense lumens are surrounded by cuboidal epithelial cells. Toluidine blue. $\times 330$.

FIGURE 3 Inverted follicles formed when follicles prepared as in Fig. 1 are cultured for an additional 5 d in 5.0% serum. Phase contrast. $\times 82$.

FIGURE 4 Thick sections of inverted follicles prepared as in Fig. 2. A layer of cuboidal or more frequently attenuated epithelial cells surrounds an electron-lucent lumen. Many inverted follicles were distorted during embedding. Toluidine blue. $\times 330$.

FIGURE 5 Ultrastructure of a thyroid epithelial cell that may be in an early stage of polarity reversal. This cell shows a minimal change from normal polarity in that there is a junction (*J*) present at the medium margin of the plasma membrane in contact with one neighboring cell but not at the contact with the other neighbor in the section (arrow). See *inset* for the latter margin where only a desmosome (*D*) is present. The nucleus (*N*) is basal, near the medium (*M*), as are the lysosomes (*LY*). Microvilli (*MV*) are present only at the lumen (*L*). Golgi apparatus, *G*. $\times 6,100$. *Inset*, $\times 24,000$.

observed that colloid was gone from the lumen only when tight junctions were no longer present at the luminal end of the cells (Figs. 9 and 10).

It is possible that the earliest step observed in the change of polarity was the appearance of a junction with only one of two neighbors in the section at the end of the epithelial cell facing the medium (Fig. 5). It was not possible to identify this junction as a tight junction or a gap junction unambiguously because in the few examples seen the orientation of the section did not

allow us to resolve the structure of the junction. At this stage there were no microvilli at the medium end of the cell. Cytoplasmic organelles were arranged as in cells with normal polarity. In the remainder of the cells that were reversing there were tight junctions at the medium end of the cell with both neighboring cells in the section, either before (Fig. 6) or after organelles changed position (Figs. 7-9). There is little question that these were tight junctions because there was no evidence for the occasional absence of a junction with a neighbor

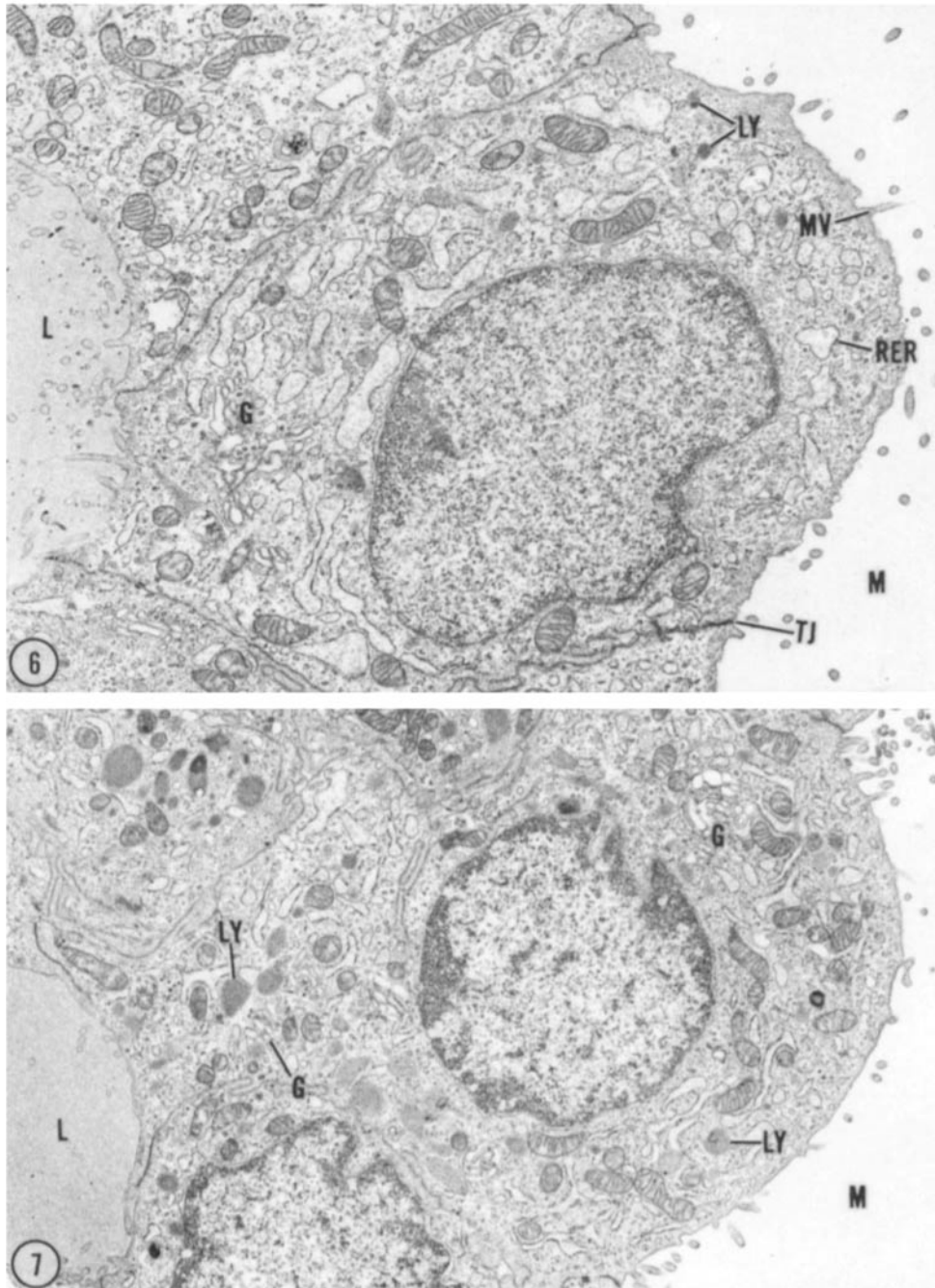


FIGURE 6 This cell is in a more advanced stage of polarity reversal than the one in Fig. 5. It appears to have a complete tight junction (*TJ*) and microvilli (*MV*) at the end of the cell in contact with the medium. The Golgi apparatus (*G*) is located near the lumen (*L*). *RER*, rough endoplasmic reticulum. $\times 9,900$.

FIGURE 7 This cell is in a somewhat more advanced stage of reversal than in Fig. 6 in that the Golgi apparatus (*G*) and lysosomes (*LY*) are found near both the lumen (*L*) and medium (*M*) ends of the cell. $\times 7,900$.

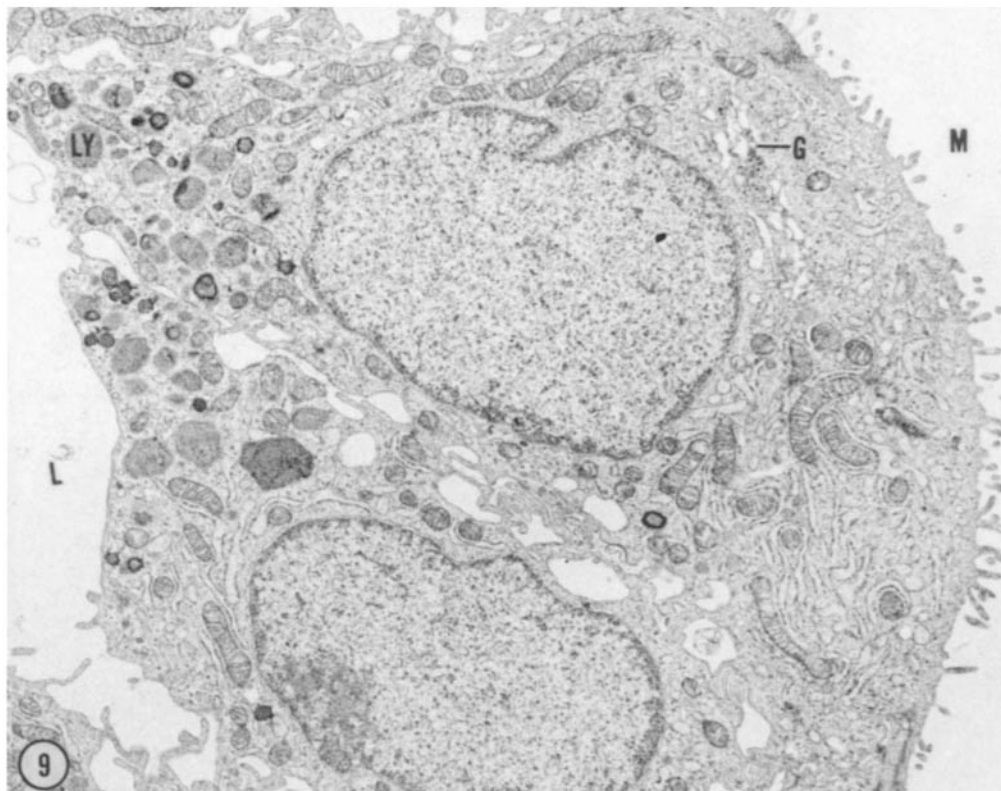
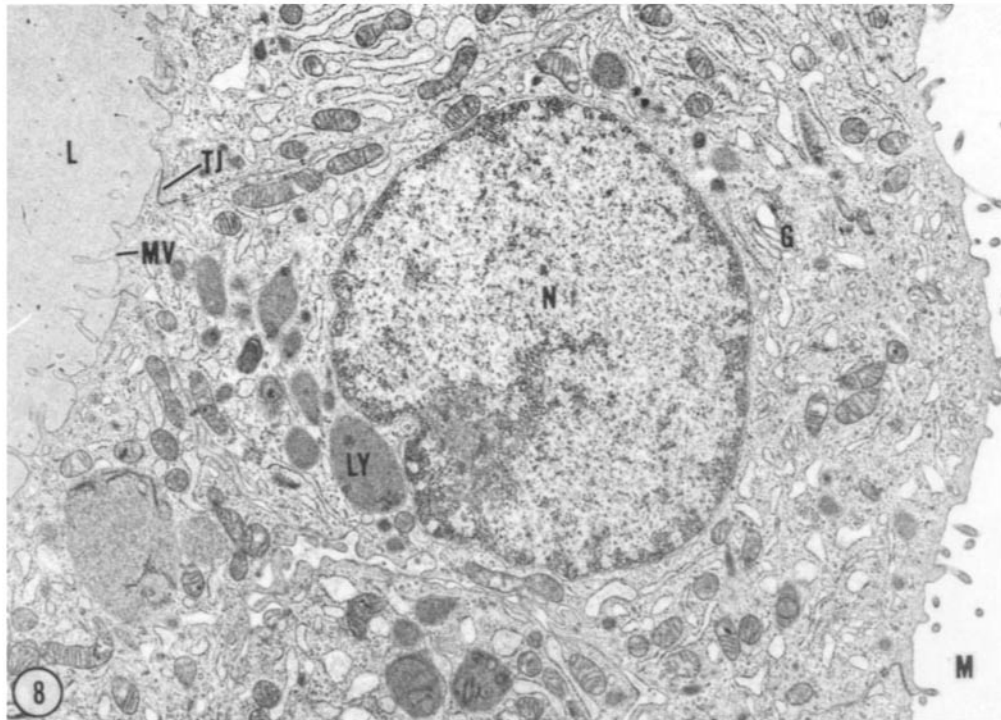


FIGURE 8 This cell has an advanced stage of polarity reversal in which the Golgi apparatus (*G*) is near the surface of the cell at the medium (*M*) and the lysosomes (*LY*) are between the nucleus (*N*) and the luminal surface. Note that the lumen (*L*) in this figure and Figs. 5-7 is electron dense and that microvilli (*MV*) and the tight junction (*TJ*) are still present at the luminal surface. $\times 9,000$.

FIGURE 9 This cell has completely reversed polarity. The microvilli and tight junction are missing from the surface near the electron-lucent lumen (*L*). Microvilli are now only present at the surface in contact with the medium (*M*). However, the cell is columnar and has a normal complement of cytoplasmic organelles. *G*, Golgi; *LY*, lysosome. $\times 7,200$.

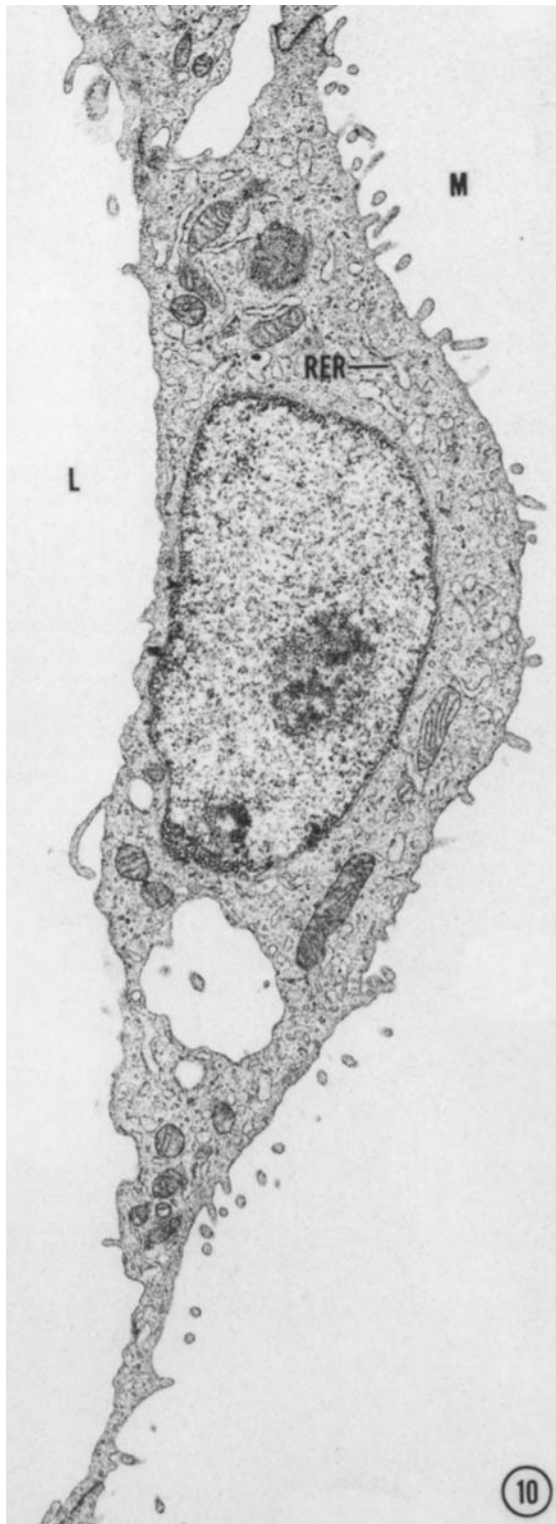


FIGURE 10 This cell is from a completely inverted follicle. Its surface has reversed polarity. The organelle complement is abnormal primarily in the gross decrease in the amount of rough endoplasmic reticulum (*RER*). *L*, lumen; *M*, medium. $\times 10,300$.

expected if the junctions were gap junctions. In these cells there were microvilli along the entire surface facing the medium (Figs. 6–10).

Cytoplasmic organelles were not observed to change their

characteristic location until there were tight junctions and microvilli at the end of the cell near the medium. The earliest changes detected in organelle position were those of the Golgi apparatus and lysosomes (Fig. 7). A stage was noted in which part of the Golgi apparatus had moved from its normal position close to and on the luminal side of the nucleus to a position on the side of the nucleus near the medium (Fig. 7) and a later stage in which the Golgi apparatus was observed only on the side of the nucleus toward the medium (Fig. 8). Similarly, a stage was observed in which, instead of their normal position, basal to the nucleus, many lysosomes were also found between the nucleus and the lumen (Fig. 7), and a later stage in which lysosomes were primarily on the luminal side of the nucleus (Fig. 8).

Two later stages were observed in which the follicles were completely inverted, tight junctions and microvilli were absent from the luminal surface of the epithelial cells, and colloid appeared to be absent from the follicular lumen. In what is interpreted as an earlier stage, the cell was cuboidal to low columnar in shape, and its complement of cytoplasmic organelles was normal (Fig. 9). Another type of profile was observed in which the epithelial cells were flat and attenuated, the rough endoplasmic reticulum was grossly decreased in amount, and the cell appeared to have dedifferentiated (Fig. 10).

The position of centrioles has not been observed during intermediate stages of polarity reversal. There was a change in position, however, from their position near the lumen in the cell with normal polarity (4) to a position near the medium (not illustrated) in the cell with reversed polarity.

DISCUSSION

The principal thyroid epithelial cell is polarized. Its apical and basal plasma membranes differ in morphology, and some of the organelles are located at characteristic levels along the axis between the apical and basal plasma membranes. The maintenance of this polarity is clearly important for efficient thyroid hormone synthesis because the polarity determines the direction of secretion of newly formed thyroglobulin (from the epithelial cell into the follicular lumen). This ultimately provides, in the lumen, a high concentration of thyroglobulin for iodination and thyroid hormone formation by the epithelial cell.

We have observed that the normal polarity of these cells reverses when follicles in suspension culture undergo inversion. During the polarity reversal, the cells remain attached to their neighbors. The surface features characteristic of the region of the cell next to the lumen (tight junction and microvilli) appear on the cell surface next to the medium before reversal of the positions of the intracellular organelles occurs. The tight junction and microvilli at the luminal surface do not disappear until after the intracellular organelles have reversed their positions.

The polarity reversal does not occur *in vivo* in the thyroid glands of intact rats or of hypophysectomized rats. Polarity *in vivo* is therefore not regulated by the presence or absence of thyrotropin, in contrast to what has been reported for follicles in suspension culture (3). Earlier in this century it was thought that polarity reversal could be observed *in vivo*, but more careful examination of the data by Gillman (1) indicated that it did not occur.

We have an approach to the determination of factors stabilizing follicles *in vivo* by comparing differences between the follicles *in vivo* and *in vitro*. The differences include: (a) the

presence of a basal lamina *in vivo* and its absence from our suspended follicles *in vitro*. It is possible that there is a non-specific aspect to the role of the basal lamina. Cells that adhere to a tissue culture dish have microvilli on their free surfaces (7). (b) A difference between blood plasma and tissue culture medium. Serum and plasma may differ in their content of a factor regulating the polarization of the epithelial cell.

Polarity reversal in follicles in suspension culture involves changes first in the surface features of the epithelial cell and then in the position of cytoplasmic organelles. Although the order of these changes appears invariable, in other situations the changes are not tightly coupled. Cytoplasmic organelles can change their position without a polarity reversal of the surface features. For example, the position of lysosomes in the cell can be changed from basal to the nucleus to apical by the administration of thyrotropin (4). The position and character of many organelles change during mitosis (5, 8). However, the changes that occur during polarity reversal are clearly different from those that occur during mitosis and probably are not a reflection of the decrease in cytoplasmic microtubules outside of the spindle which has been suggested as the origin of the changes during mitosis. What does appear probable is that the changes are regulated. In another case of polarity reversal that was described by Trelstad (6), involving the change of position of the Golgi apparatus in the corneal epithelium during embryonic development, the polarity change is also carefully regulated. It is reversible and the Golgi apparatus migrates to the side of the cell on which collagen is being deposited.

The inversion of follicles has polarity reversal of the follicular epithelial cells as one of its elementary processes. We do not mean to imply, however, that polarity reversal is the only change occurring during the formation of inverted follicles in

our suspension cultures. Initially, the clusters of epithelial cells in our suspension cultures have various structures. Some are simple follicles but others contain several small lumens (4). After inversion each cluster ends up as a single vesicle with an electron-lucent lumen. The process of inversion, therefore, requires cell migration and fusion of lumens in some clusters and other differences in detail, depending on the precise structure of the original cluster.

The difference in the organelle complement in cells that have reversed their polarity is striking. The loss of rough endoplasmic reticulum in many of the attenuated cells resembles that in thyroid epithelial cells spread in monolayer culture (4). It raises the question again of the extent to which this change in organelle complement is regulated by cell shape. Further studies will be needed to answer this question.

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