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CELL POLARITY AND WATER TRANSPORT IN THYROID EPITHELIAL CELLS IN SEPARATED FOLLICLES IN SUSPENSION CULTURE

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Abstract

Separated thyroid follicles maintained in suspension culture can be used to study the properties of thyroid epithelium in the virtual absence of other cell types and to study the effect of extracellular materials on the follicles. They can be prepared by enzymatic separation of thyroid into single cells followed by reaggregation of the epithelial cells and also by collagenase treatment of thyroids to release follicles and sheets of epithelia that can be separated from other materials by differential filtration. The follicles can exist with normal orientation or inverted (inside out). The follicles are inverted in the presence of high serum concentrations (5%) but can have normal orientation when embedded in a type I collagen gel, even at high serum concentrations. When normally oriented follicles invert, the polarity of the epithelial cells reverses while they are connected to neighbors. During inversion, bipolar cells are observed having microvilli-bearing surfaces at both lumen and medium. Inverted follicles can revert to normal orientation when embedded in collagen gel. Various functional properties of normally oriented follicles are similar to those of follicles in vitro. However, inverted follicles do not concentrate iodide, although they synthesize thyroglobulin and secrete it into the medium. Mutants are available in established cell lines. They have functional and organizational properties that differ from those of normal cells and demonstrate a lack of coupling between functional properties and organization. Inverted follicles transport water from medium into the lumen, although at rates somewhat less than MDCK cells.

KEY WORDS: Thyroid Epithelium, Cell Polarity, Suspension Culture, Thyroid Cell Lines, Thyroglobulin, Iodination, Iodide Concentration, Water Transport, Thyroid Follicles, Follicle Inversion, Thyroid Function, Follicular Lumen Formation, Stabilization of Follicles.

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Introduction

Suspension cultures of thyroid follicular cells derived from adult mammals have been obtained in many laboratories in the last decade [7, 15, 17, 20, 21, 24, 26, 29]. The suspension culture approach, introduced by Mauchamp and Fayet [23], has turned out to be very useful, since the cells do not attach to the plastic dish, freely interact with each other, and do not undergo drastic shape changes due to spreading on a substratum that may modify their functional properties. In this culture system, several functional properties of the thyroid gland can be conveniently studied, especially those that are only expressed when epithelial cells are organized in three-dimensional structures with lumens, essentially in the absence of other cell types. Reorganized follicles can also be embedded in a suitable matrix in the form of a gel, thus allowing the reconstitution of a system in which cell/matrix interactions can be investigated [12]. Several properties of this model system have been already reviewed [18, 19, 37]. We will focus here on some data on the correlation between structural and functional polarity, on water transport in thyroid epithelium, and on the properties of established thyroid cell lines cultured in suspension in the form of aggregates.

Materials and Methods

Preparation of thyroid follicle cultures

There are two principal ways in which follicles have been prepared.

Aggregation of separated cells. A suspension of isolated follicular cells is first obtained by conventional tryptic digestion of the thyroid tissue [20, 24]. The epithelial cells, perhaps representing the majority in a mixed population of cells, spontaneously reaggregate and reorganize into follicles when cultured in suspension [24].

Isolation of separated follicles and sheets of epithelial cells [7, 16, 21, 29]. Thyroids are excised, washed, and minced in Hank's salt solution, then incubated for 3 to 4 hr at 37°C in complete tissue culture medium containing 1-1.5 mg/ml of collagenase (Worthington, CLS II). Tissue is then mechanically disrupted by pipetting. Follicles and sheets of epithelial cells are then

obtained that can be separated from isolated cells and from large undigested tissue, either by centrifugation [7] or by filtration through fine mesh [16, 26]. We have used 5- to 6-wk-old Fischer rats and have separated the follicles and clusters of epithelial cells by a double filtration through a 60-μm and 15-μm Nitex mesh [29], which allows the separation of a population of follicle fragments of a discrete size range from undissociated tissue (which is retained on the 60-μm filter) and from individual cells, including most of the mesenchymal cells (which pass through 15-μm filter). The absence of major cell contaminants in this preparation is assessed by electron microscopy [29]. After several hours of suspension culture in agarose-coated dishes in Coon's modified Ham's F12 medium containing either 0.5% or 5% calf serum, the follicles reseal.

Agarose-coated dishes. An aqueous solution of 0.5% or 1% agarose (Sigma, Type II) is sterilized by boiling and then poured, while still hot, into regular tissue culture dishes to cover their bottoms. The agarose solution is aspirated within 1 min, and the dishes are left at room temperature until the remaining thin layer of agarose solidifies [29].

Collagen gel. Purified acid-soluble rat tendon collagen or calf skin collagen is dissolved in cold 0.5 M acetic acid overnight. The solutions are dialysed in the cold for 6-8 h against tissue culture medium, and immediately thereafter, small drops of the dialysed solution are added to the cultures to a final concentration of 50-100 μg/ml to test for stabilization against inversion of follicles [12].

Light and electron microscopy

Follicles or cell clusters are collected by centrifugation, fixed for 15 min in 2.5% glutaraldehyde in 0.1 M cacodylate buffer for 20 min, and rinsed in H_2O. Samples are then stained en bloc for 1 h with 1% aqueous uranyl acetate, dehydrated with a graded series of ethanols, and embedded in Epon 812. Thick sections are stained with toluidine blue for light microscopy; thin sections are stained with uranyl acetate and lead citrate and examined with a Philips 201 C electron microscope [29].

Iodide trapping and organification

Follicles are incubated in a shaking bath at 37° C in 0.5 ml of complete culture medium containing 10 mM HEPES, 3 mM methimazole, 10^{-6} M $Na^{127}I$ and 5 x 10^5 cpm of ^{125}I. At the end of the incubation, the follicles are centrifuged, the supernatant is carefully removed, and the radioactivity in the pellet is counted. To evaluate the amount of organified iodine, the same procedure is used, but methimazole and $Na^{127}I$ are omitted from the incubation medium and TCA-precipitable counts are determined both in the follicles and in the culture media [34].

Metabolic labeling of thyroglobulin

Follicles are starved for 4 hr in methionine-free medium and incubated for 24 hr in fresh medium containing 150 μCi/ml of [^{35}S]methionine (sp. act. 1050 Ci/mmol; Radiochemical Co., Amersham, UK) and 90 μg/l of cold L-methionine. Alternatively, follicles are incubated for 4 hr in medium containing 1 x 10^6 cpm of ^{125}I. After either labeling procedure, follicles are collected by centrifugation and lysed in a 0.01 M Tris-HCl buffer, pH 7.2, containing 0.1 M NaCl, 0.001 M EDTA, and Nonidet-P40. Both the supernatant of lysed follicles and the culture medium are immunoprecipitated with affinity-purified anti-thyroglobulin antibodies. Samples are finally loaded onto a 6.5% polyacrylamide slab gel electrophoresis [34].

Microcinematography

Episodes of shrinkage and dilation of follicles cultured in suspension are observed using darkfield illumination [32]. Recordings were made using time-lapse video-enhanced image intensification taking one frame every 8 sec. During recording, the follicles in the dish were in a chamber at 37°C and were exposed to an atmosphere of air containing 10% CO_2. Measurements of follicular lumens are made on a video screen that is calibrated by projection of a recording of the grid from a hemocytometer plate. The diameter of the lumen (d) can be taken to be the square root of the product of the major and minor axes. The surface area of the lumen is taken to be πd^2.

Results and Discussion

Characteristics of follicle preparations

It was clear from the very beginning of the culturing in suspension of thyroid epithelium that the follicles obtained can exist in two different configurations depending mostly on culture conditions: normally oriented follicles, in which the polarity of the cells is similar to those follicles in vivo, i.e., with the microvilli-bearing surface facing the lumen (Fig. 1), and inverted follicles, in which the cells have an opposite polarity, with the microvilli-bearing surface in contact with the culture medium (Fig. 2). Normal follicles have been obtained by culturing collagenase-digested sheets of rat thyroid follicle cells in medium containing low calf serum (0.5%) concentrations [29]. It should be pointed out that in these preparations the cells surrounding the lumen were not always organized in a single layer, that some cells were occasionally found outside the follicle wall with their microvilli-bearing surface in contact with the culture medium (Fig. 1), and that a significant number of cell clusters (spheroids) were obtained that had multiple microlumens, indicating rearrangements of cells within a cluster [29]. Normal follicles have also been obtained from pig thyroid cells cultured either in the presence of low serum concentrations [22] or in the presence of agents capable of elevating the cAMP concentration in the cells [24]. Most authors agree that, when freshly dissociated thyroid cells are cultured in a medium containing high serum concentrations (>5%), the follicles invert and form a rather uniform population of inverted follicles (17, 24, 31). Such inverted follicles also develop from spheroids present in a culture of normally oriented follicles [20] in a medium containing 5% calf serum. Finally, the population of inverted follicles is not completely homogeneous but contains a small fraction of inverted follicles, called mixed follicles, that have a normally oriented follicle completely inside the lumen but attached to the lumen wall [14]. It has been claimed that by using a particularly mild dissociation technique it is possible to obtain pig follicles that retain their

normal orientation even in the presence of high serum concentrations [15]. This was possibly due to the fact that some extracellular matrix, not removed by enzymatic digestion, was still surrounding the follicles and preventing inversion from occurring [15].

Mechanism of inversion of follicles

Our observations suggest that follicle inversion occurs by polarity reversal of the individual cells in the wall of the follicle while they are connected to neighbors by tight junctions. This hypothesis is based on the presence of cells in intermediate stages of polarity reversal, e.g., cells that have microvilli and tight junctions on both the luminal and medium sides (Figs. 3 and 4) and in which the intracellular organelles do not appear to have the stratified distribution of normally polarized cells [31]. That tight junctions are probably always present is evidenced by the presence of colloid in the follicle lumen until the follicle is completely inverted. We do not have evidence that this is the only mechanism of inversion.

Stabilization of cell polarity

Since follicles have normal orientation in vivo, it is possible that the polarity of their epithelial cells is stabilized by substances present in their neighborhood. When embedded in a collagen gel, follicles have a normal orientation even in the presence of high serum concentrations [4]. We have tested a number of substances to determine which would stabilize the orientation of normal follicles [12]. We found that no substance tested in solution stabilized follicle orientation; to be able to stabilize orientation, molecules had to be in the form of gels adhering to the basal pole of follicle cells (Table 1). Among the gels tested, only type I collagen and plasma clot [12] were effective.

Reorganization of inverted follicles embedded in a collagen gel

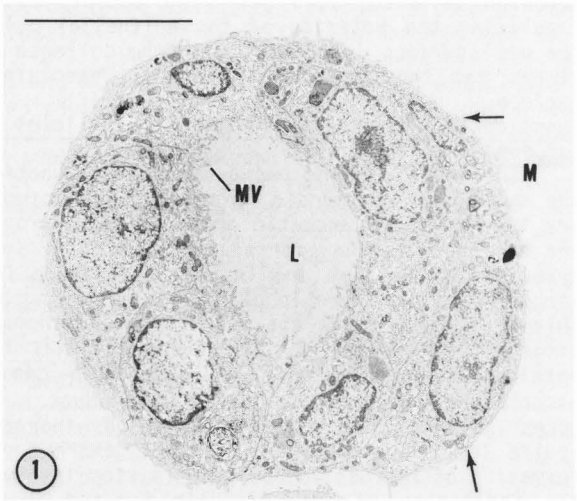
Although inverted follicles can be stable and persist in suspension culture for months, they undergo extensive reorganization when embedded in a collagen gel. The nature of the reorganization appears to differ depending on the species.

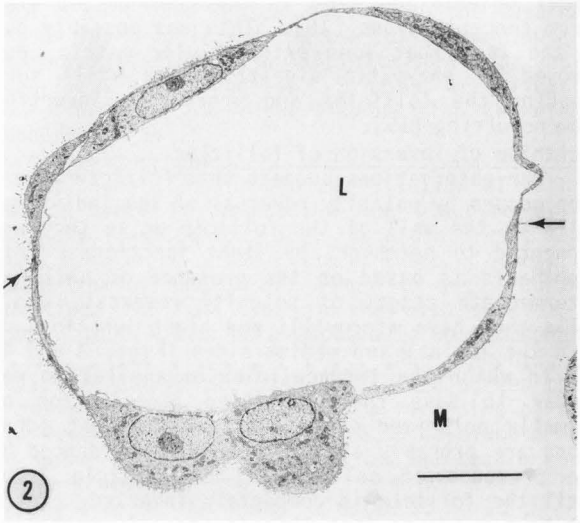
Inverted pig thyroid follicles apparently revert to normal follicles bounded by a monolayer of epithelial cells [5], possibly by direct reversal of cell polarity (a direct reversal of the process of inversion). On the other hand, inverted rat thyroid follicles have a stage, possibly an intermediate stage, in which they form spheroids with multiple microlumens (Fig. 5). Intermediate stages in the reorganization of rat follicles were investigated by electron microscopy [13]. The first change observed was shrinkage of the lumen associated with a shape change of the cells from attenuated to cuboidal or columnar. A loss of tight junctions between some cells was likely to be responsible for the shrinkage [13]. This was followed among other things by shape changes of some cells suggestive of migratory activity. At the end of the reorganization, there were spheroids in which the cell surface in contact with the collagen had a loss of microvilli. The reorganization did not appear to occur merely by direct polarity reversal of the epithelium of the inverted follicle wall. Contact with and adhesion to a collagen gel is a potent feature regulating the polarity of the epithelial cells. The cell surface in contact with the collagen gel always has the characteristics of a basolateral surface.

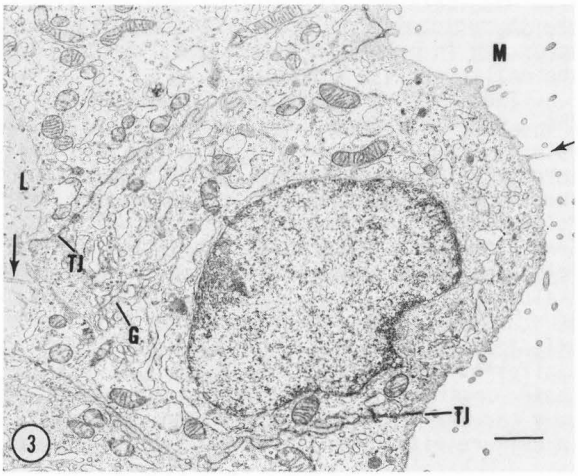
Functional properties of thyroid follicles in suspension culture

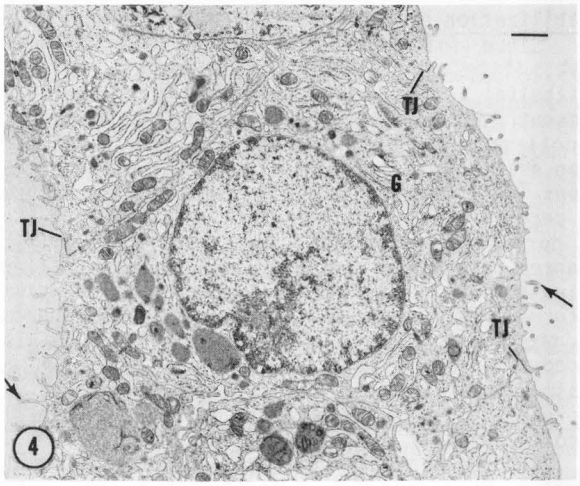
The thyroid gland has an important function: the synthesis of thyroid hormones. The hormones are two iodinated aromatic amino acids, thyroxine and triiodothyronine, formed by coupling of iodotyrosines while they are in peptide linkage in a large protein, thyroglobulin. In carrying out this synthesis, there are a number of biochemical steps within the cell or at a characteristic site outside. The occurrence of each step can be ascertained by detection of its product. The steps include the concentration of inorganic iodide located in the basal plasma membrane, the formation of luminal iodoprotein (a step involving the iodination of thyroglobulin located in the follicular lumen at the microvilli-bearing plasma membrane), coupling of iodotyrosines while in peptide linkage in thyroglobulin to form thyroxine and triiodothyronine, uptake of the thyroglobulin into the cell by endocytosis, and hydrolysis of the thyroglobulin to release the thyroid hormones occurring in colloid droplets and lysosomes within the cells.

The results described below indicate that the steps that occur or, more precisely, their rates depend on whether the follicle is normal or inverted. There have been several reports on the functional properties of normal and inverted follicles in suspension culture [7, 17, 21, 34]. Some reports on functional properties of thyroid follicles in suspension [21] cannot be properly evaluated since it is not clear what percentage of follicles in those preparations were normally oriented as opposed to inverted. Normal follicles manifested most functional properties present in their counterparts in vivo in experiments that were carried out on follicles that were maintained in culture either for a few hours [7] or for a few days [34]. In particular, they were capable of iodide trapping, thyroglobulin synthesis and secretion, thyroglobulin iodination, and synthesis and secretion of thyroid hormone [34]. There is also a response to acute [29] and chronic [30] thyrotropin stimulation. Inverted follicles, instead, did not show the ability to concentrate iodide; iodination of thyroglobulin was detected in pig thyroid follicles [18] but not rat, and secretion of thyroid hormone [34] was not detected in rat. Thyroglobulin was synthesized but was secreted by inverted follicles into the culture medium [17, 34] instead of being accumulated in the lumen. Lack of iodide trapping can be explained assuming that the iodide pumps are located on the basolateral side of the cells [6] (i.e., in contact with the lumen of the inverted follicle) and therefore not in contact with the medium where iodide is present. Other functional properties, such as iodination [8] or diiodotyrosine coupling to form thyroxine, may not be detected in inverted follicles in the rat simply because the concentration of thyroglobulin in the medium is so low instead of being 10-20% as in the lumen of the normal follicle. Lack of some extracellu-









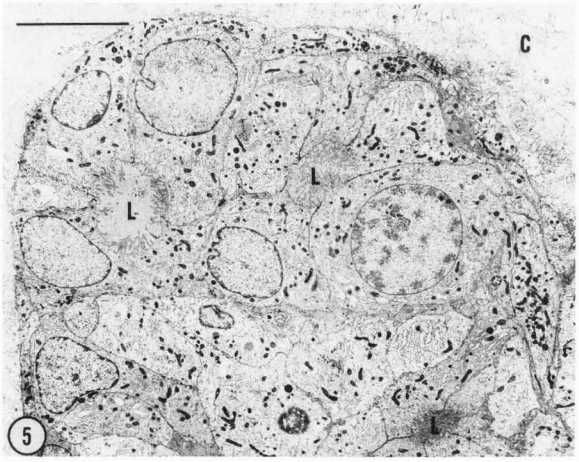


Table 1. Stabilization of cell polarity: Normal thyroid follicle [12]

Substance	Concentration	Physical state	Stabilization
Gelatin	20, 30 mg/ml	Sol	-
Cellular fibronectin	50 μg/ml	Sol	-
Plasma fibronectin	50 μg/ml	Sol	-
Laminin	1.7-17 μg/ml	Sol	-
Methyl-cellulose	14.4 mg/ml	Sol	-
Agarose	50 μg/ml	Sol	-
Agarose	100-500 μg/ml	Gel	-
Calf skin collagen	50, 100 μg/ml	Gel	+
Rat tendon collagen	50, 100 μg/ml	Gel	+
Heat denatured collagen	50-400 μg/ml	Sol	-
α2(I) chains	100, 400 μg/ml	Sol	-
CB7 fragments	200, 400 μg/ml	Sol	-
Plasma clot	0.5 or 4%	Gel	+

Figure 1. Normal follicle in suspension culture. Most of the lumen (L) containing an electron-dense material is bounded by a cell monolayer with microvilli (MV) extending into the lumen. However, there are two additional cells (arrows) on the medium (M) side of the follicle wall. Bar = 10 μm.

Figure 2. Inverted follicle in suspension culture. The electron-lucent lumen (L) is bounded by a cell monolayer with cell microvilli extending into the medium (M). Most of the cells are attenuated, those at the right of the figure with few microvilli (arrows), whereas those at the left with many. Bar = 10 μm.

Figure 3. An intermediate stage of polarity reversal. Bipolar cell with microvilli (arrows) and tight junctions (TJ) at surfaces in contact with lumen (L) and medium (M). Note that the Golgi area (G) is on the luminal side of the nucleus. Bar = 1.0 μm.

Figure 4. Another bipolar cell as in Figure 3, except that it is at a later stage in the inversion process in which the Golgi area is on the side of the nucleus near the medium. Bar = 1.0 μm.

Figure 5. A spheroid formed within 48 h after an inverted follicle was embedded in a collagen gel (C). Note the presence of several microlumens (L). Bar = 10 μm.

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lar post-translational modification could also explain the fact that the thyroglobulin immunoprecipitated from the culture medium of inverted rat follicles migrates as a single band on SDS-PAGE and not as two bands as in normal rat follicles either in vitro [34] or in vivo [33]. The response to acute thyrotropin stimulation has not been clearly assessed, although there appeared to be a response to chronic stimulation [17].

Lumen formation and function in established cell lines in suspension culture

Several rat thyroid cell lines (T78, FRTL5, PC, and FRT) have been established and grown in monolayer culture. The cells differ from cells from the normal thyroid gland in both functional and organizational properties when put into suspension culture. To obtain data on these points, monolayer cultures were trypsinized, and the cells were allowed to aggregate in suspension culture [27]. In some cases, only aggregates of selected sizes, obtained by double filtration, were further cultured in suspension [11]. The T78 cells manifested the ability to aggregate and to form lumens but were much less sensitive to serum factors that promote inversion [27]. No true follicles with a lumen bounded by a cell monolayer were observed [27]. Moreover, the cells did not form lumens any longer after a large number of generations (>50), although they still expressed other thyroid functional properties, such as iodide trapping and thyroglobulin synthesis and secretion [27]. Two other cell lines, FRTL5 [2] and PC [10], both cultured in monolayer for a large number of generations, did not form lumens in suspension but expressed other thyroid-specific differentiated properties (Nitsch and Garbi, unpublished). On the other hand, the FRT epithelial cell line, which was also derived from rat thyroid [1] but did not express functional properties of the thyroid epithelium any longer, was shown to be polarized in monolayer culture [28] and formed hollow cysts resembling the inverted follicles in suspension culture, independent of the serum concentration in the culture medium [11]. In FRT cells that were kept in monolayer culture for several days after confluency, we have observed that hollow cysts, having a structure similar to the inverted follicles, formed directly from domes. Some domes became much enlarged until they appeared to be like cysts connected to the rest of the monolayer through a neck that progressively narrowed until the cyst floated into the culture medium (Nitsch, unpublished). It therefore appears that function and lumen formation are not strongly coupled characteristics, and lumens are neither necessary nor sufficient for function. Furthermore, it may be that the ability of FRT to form cysts does not reflect a specific morphogenetic property of the thyroid cells since other polarized epithelial cell lines have been shown to be able to form similar cysts in suspension culture [9, 35, 36].

Finally, cells of established lines that form inverted follicles, like FRT, also reorganize and form normal follicles when embedded in a collagen gel. In this case, however, other non-physiologic substrata, such as agarose gels, also induce aggregate reorganization and reversal of cell polarity [11]. This may be due to the ability of the FRT cells to adhere to and grow within agarose gels and raises the question of whether collagen can be considered to act through receptors or whether cell polarity can be regulated by any substance that adheres to the cell.

Estimation of rates of water flux through thyroid epithelium

The properties of inverted thyroid follicles in suspension culture suggest that there is resultant water transfer into the lumen from the medium. Most inverted follicles in suspension culture, including the FRT cysts [11], have large electron-lucent lumens surrounded by flat attenuated cells [14, 17, 26]. This appears to be due

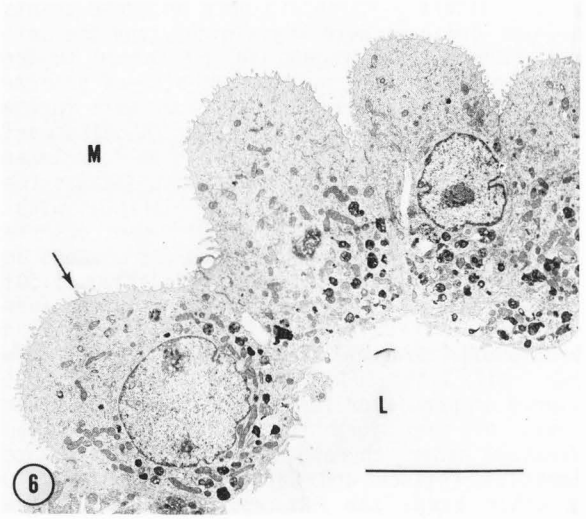

Figure 6. Portion of the wall of an inverted follicle fixed seconds after puncture of the wall while the lumen (L) was dilated. The lumen bounded by attenuated cells shrank promptly, yielding columnar cells coated with an abundance of microvilli (arrow) on the surface in contact with medium (M). Bar = 10 μm.

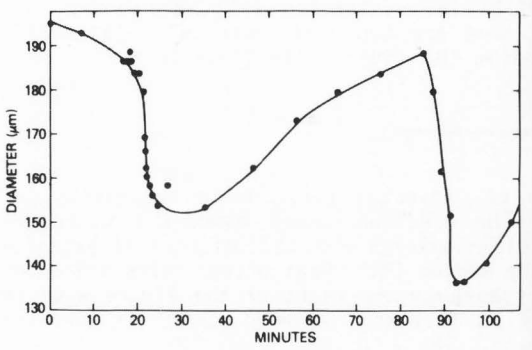

Figure 7. Graph showing variation with time of diameter of lumen of inverted follicle in suspension culture.

to fluid accumulation in the lumen that is distended while the cells are stretched in the plane of the follicle surface. Consistent with this hypothesis is the observation [14] that, if punctured, an inverted follicle collapses within seconds and the cells acquire a columnar or cuboidal shape (Fig. 6). Further studies show that the follicle wall is not only stretched but that inverted thyroid follicles undergo episodes of shrinkage and dilation [32]. Preparations of inverted follicles were observed by dark-field illumination and time-lapse video-enhanced image intensification. The shrinkage (Fig. 7) was rather variable from follicle to follicle. It can be very fast, almost entirely occurring within an 8-sec interval, or slow and progressive, or in several fast steps separated by short periods during which no shrinkage is observed. Shrinkage rates for several follicles are shown in Table 2. Shrinkage was followed after 10 min or so by a dilation that was always slow and progressive, the increase in diameter being almost linear with time. Rates of fluid influx were of the order of 0.5 $\mu m^3/\mu m^2$ of luminal surface per minute (Table 2). The phenomenon can be interpreted as follows. Shrinkage of the inverted follicles may be due to the opening of a tight junction or even to the rupture of a cell. Cells of inverted follicles may be very stretched and as thin as 0.2 μm or less in some places [14]. After closure of the hole, there is a flux of water through the cell into the lumen (from the microvilli-bearing surface through the surface in contact with the lumen) possibly coupled to the transport of some ion(s) [5]. Similar transport of fluid was demonstrated to occur in MDCK cells on filters [3, 25]. In that case the net water flux was almost three times higher than in the thyroid cells. The function of such water transport in the normal thyroid might be to keep concentrated the thyroglobulin stored in the lumen so that it can be metabolized efficiently as described above.

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Table 2. Inverted follicles: Characteristics of shrinkage and dilation [32]

Follicle	Diameter (μm)	Extent shrinkage (%)	Shrinkage rate ($\mu m^3/\mu m^2$/min)	Dilation rate ($\mu m^3/\mu m^2$/min)	Time to reseal (min)	Interval between shrinkages (min)
1	184	20	7.8	0.44	12	70
		30	5.6			
2	193	23	2.9	0.65	<6	45
		16	3.9	1.2	10	-
3	184	15	7.8	0.93	<6	19[a]
		17	3.5			
4	190	20	9.3	ND	-	-
5	90	34	11.4	-	10	-[b]
		28	2.0	-		
6	187	12	0.93	0.35	6	90
		27	8.7			
7	152	35	1.9	-	-	-
8	165	26	0.36	0.26	<10	-

[a] Shrinkage followed by a 9-min pause and then additional shrinkage.
[b] Two nonconsecutive shrinkages separated by a 3-hr interval.
ND, no dilation during period of observation; -, not observed or could not measure accurately.

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Discussion with Reviewers

V. Herzog: The authors mention that inverted follicles are unable to iodinate or that they lack some other post-translational modifications. It has been shown, however, that thyroglobulin is secreted by inverted follicles as an iodinated protein when cultured in the presence of NaI-125 (ref. 18). Other reports indicate that inside-out follicles also release sulfated (35-SO_4) or phosphorylated (32-PO_4) thyroglobulin. Indeed, such inverted follicles are employed in these laboratories for the preparation of biosynthetically iodinated, sulfated, or phosphorylated thyroglobulin.

L. Nitsch: Dr. Herzog tells me that his studies on the iodination of thyroglobulin as well as post-translational modification of thyroglobulin were done in dense cultures of inverted follicles of the hog, whereas our studies of the rat follicle used low culture densities that were the same for normally oriented follicles and inverted follicles. We must therefore conclude that the difference in results between normal and inverted follicles may only be quantitative and not qualitative. Of course, we do not know whether there is a species difference, since our studies were done in rat whereas Dr. Herzog's were in hog. Finally, one possible additional complication in the interpretation of Dr. Herzog's paper: we do not know if his dense cultures were shaken during incubation or to what extent his large and fragile follicles were intact or fragmented and open during incubation.

V. Herzog: The mechanisms of inversion of normally oriented follicles is still unclear. The authors had shown previously (ref. 31) that tight junctions can remain intact throughout the inversion process. In follicle preparations of other species it has been shown, however, that simply the rupture of follicles and mechanical inversion of the entire epithelial layer may occur (or is regularly observed).
Authors: It seems unlikely that fragmentation of a normally oriented follicle into a sheet followed by closure to form an inverted follicle occurs to any extent in the rat follicles we have studied. These follicles are very small, with one or more microlumens 6-12 μm in diameter bounded by cuboidal epithelial cells with a lot of contact between their lateral surfaces. The follicles differ markedly in size from the hog thyroid follicles that others have studied. These are very large and are reported to be very fragile (see ref. 19). If such large follicles ruptured, and we do not know what proportion do (we do not even know if the cultures were shaken), it is probable that some would reseal to form inverted follicles as Dr. Herzog reports.

G. N. Barrow: It would have been interesting to measure specific apical membrane enzymes during the inversion process.
Authors: We have no information on the amount or location of apical membrane-specific enzymes on the plasma membranes of the inverted follicle or during the invasion process. It would be of much interest to know.

S. R. Hilfer: Do inverted follicles form a basal lamina at the luminal surface?
Authors: We do not recall a single example of a definite basal lamina on the luminal side of an inverted follicle. On the other hand, we have seen what appeared to be material resembling basal lamina between cells in an inverted follicle (see Figs. 15-17 in ref. 14).

S. R. Hilfer: Has anyone attempted to present immobilized laminin or fibronectin (as on beads) to the basal cell surfaces?
Authors: We do not know of such experiments.