ULTRASTRUCTURE OF BLEBBING AND PHAGOCYTOSIS OF BLEBS BY HYPERPLASTIC THYROID EPITHELIAL CELLS IN VIVO

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ABSTRACT

In addition to pseudopods, somewhat pleomorphic blebs were consistently found protruding from the apical surfaces of hyperplastic rat thyroid epithelial cells into the follicular lumens in vivo. Many blebs were knobby, roughly hemispherical protrusions, with a diameter of 2–3 μm. Such blebs had a densely packed microfilamentous core and contained numerous apparent ribosomes. They were morphologically similar to blebs that have been observed in a variety of cultured cells. Other blebs were larger, more elongate, and less knobby, but had a similar ultrastructural organization. Blebs of all sizes appeared to be phagocytosed on some occasions by nearby epithelial cells. The phagocytic process involved partial engulfment of the bleb by a typical epithelial pseudopod, followed by an apparent pinching-off process, presumably resulting in the separation of the bleb from its cell of origin. The pinching-off process was associated with a band of approx. 6-nm diameter microfilaments that developed within the pseudopod cytoplasm surrounding the base of the bleb and is postulated to function as a contractile ring.

The finding of blebbing is an intact tissue in vivo indicates that this phenomenon is not restricted to cultured cells, and thus tends to extend the significance of in vitro observations of the process. In relation to their occurrence in the hyperplastic thyroid gland in vivo, possible interconversions are considered between different types of blebs, and between blebs and pseudopods.

Cellular blebs, or zeiotic processes, are rounded, knobby structures, which protrude from the cell surface and then retract. Such structures have been observed for many years (3, 5, 25, 28, 29), largely in cultured cells, where they have been described in connection with cellular locomotion (6, 17, 36) and with the mitotic cycle (3, 21). In addition, blebbing in vitro has been induced with a variety of agents (1, 7, 8, 10, 14, 16, 18, 19, 21, 24, 27, 30).

The extent to which cellular blebbing occurs in vivo is unknown at the present time. The small number of reports dealing with blebbing in vivo include light microscope findings of blebs on embryonic cells in mitosis (3), and an ultrastructural description of a large cellular protrusion, possibly related to blebs, also from an embryonic cell (17).

The present report describes the ultrastructure of blebs which occur in hyperplastic thyroid epithelial cells of adult rats in vivo. Blebs, similar to those in vitro, are depicted projecting into follicular lumens. Phagocytosis of these blebs by nearby epithelial cells appears to take place under the same conditions and is also described.

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MATERIALS AND METHODS

Thyroid hyperplasia was induced in 30 male Fischer rats (250–350 g) by feeding the Remington low iodine diet (approx. 0.02 μg iodine/g diet, Teklad Test Diets, The Mogul Corp., Madison, Wisconsin) containing 0.25% thiouracil for 4–7 days. Rats were then anesthetized with Nembutal (Abbott Laboratories, North Chicago, Ill.) and perfused with fixative through an 18-gauge needle inserted into the left ventricle. Perfusion was carried out for 3 min at 37°C and at a pressure of 95 mm Hg. Drainage was afforded by a large right atrial incision. The fixative solution contained 2% formaldehyde (freshly generated from paraformaldehyde), 2.5% glutaraldehyde (Ladd Research Industries, Inc., Burlington, Vt.), and 1.5-3% dextran (mol wt 35,000–50,000, ICN Nutritional Biochemicals Div., International Chemical & Nuclear Corp., Cleveland, Ohio) (2) in 0.04 M sodium cacodylate, pH 7.35.

After perfusion fixation, thyroid glands were excised, cut into 1 mm cubes, and immersed in the same fixative for an additional 1-3 h at room temperature. Tissue cubes were then postfixed for 1 h with cacodylate-buffered 1% osmium tetroxide, rinsed with water, and stained *en bloc* for 1 h with 1.5% aqueous uranyl acetate. They were then dehydrated with a graded series of ethanols and propylene oxide, and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate and examined in an AEI EM 6B or a Philips 201 electron microscope.

RESULTS

Occurrence of Pseudopods and Blebs

Various profiles of epithelial pseudopods projecting into follicular lumens were frequently observed in the hyperplastic thyroid tissue examined. These pseudopods were electron-transparent structures, often having 0.1–0.2 μm thick, flaplike distal extensions. They were ultrastructurally similar to pseudopods which have been associated with the macropinocytosis of luminal colloid (34, 37), and contained a loosely packed array of microfilamentous material and occasional dense particles that appeared to be ribosomes. Cellular organelles were essentially excluded from the pseudopod cytoplasm.

In addition to pseudopods, another type of protrusion from the apical surface of epithelial cells was consistently observed under the present conditions, and is referred to herein as a bleb (Figs. 1–6). Blebs were characteristically more electron dense than pseudopods in overall appearance (Fig. 7), and showed more compaction of their structural elements. Also, in contrast to pseudopods, blebs were generally rounded or knobby in outline. Like pseudopods, blebs were observed exclusively as projections from the apical surface of follicular epithelial cells into the follicular lumen, and were never found in association with either the basal or lateral surfaces of these cells. Typically, one to four blebs were recognizable in a given 0.5-mm^2 thin section, which contains about 50 follicular profiles. In a few instances, structures were observed which were not clearly either blebs or pseudopods but which were ultrastructurally intermediate between the two forms.

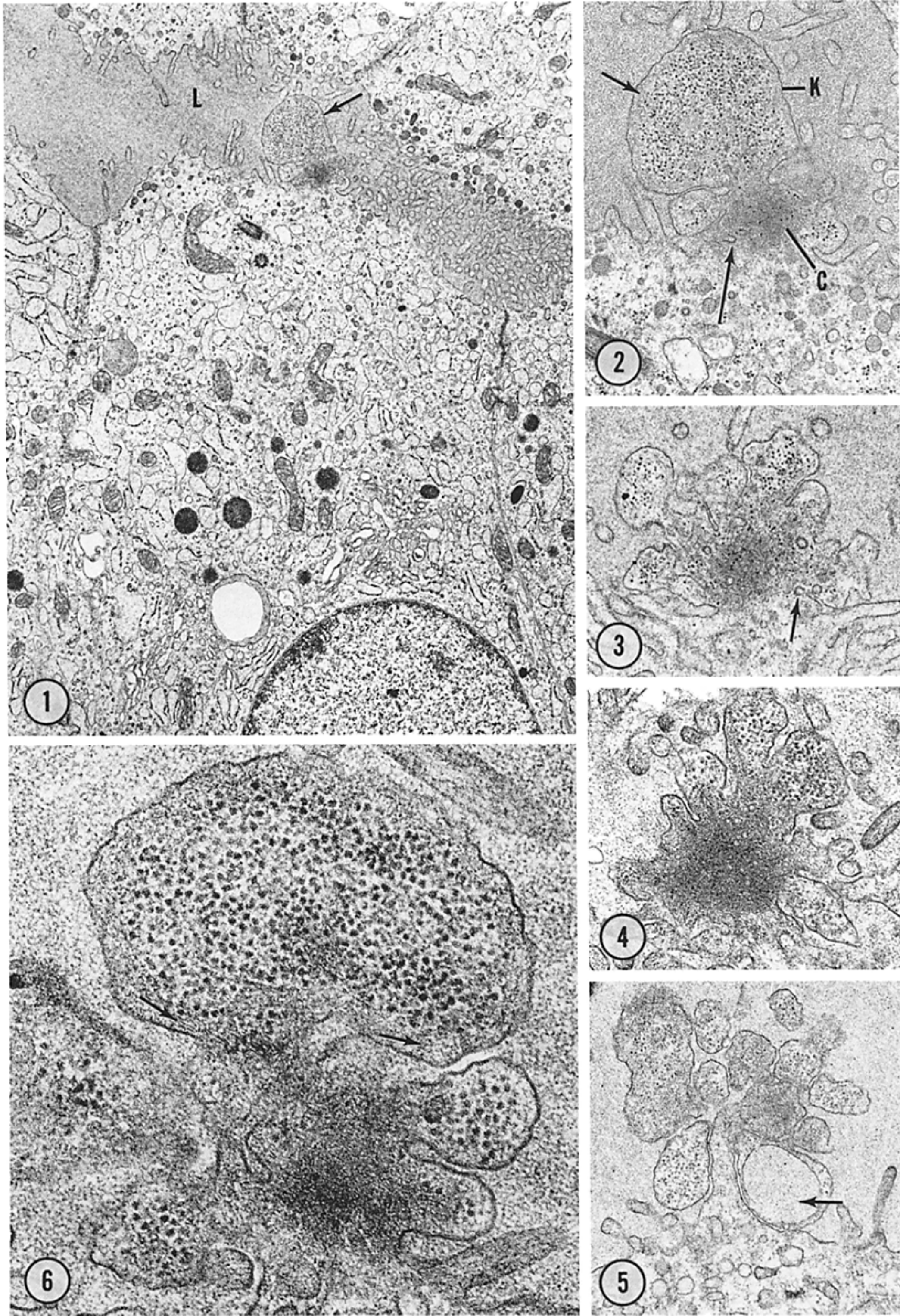
The particular epithelial cells which were associated with blebs did not themselves show any unusual ultrastructural specializations (Fig. 1). Although an association between blebbing and mitosis has frequently been reported (3, 21), none of the bleb-bearing cells observed in the present study appeared to be undergoing mitosis. Except for the presence of apical blebs, these cells appeared morphologically equivalent to neighboring cells on which blebs were not observed.

Ultrastructure of Blebs

Blebs were somewhat variable in size and shape. Many were knobby, roughly hemispherical protrusions with a diam of 2–3 μm, and were connected with the apical cytoplasm by a short, thick stalk (Figs. 2–5). Other blebs were elongate structures up to 8 μm in length and lacked the small knobs associated with shorter blebs (Fig. 9).

The organelle content and ultrastructural organization of many blebs were rather uniform. At the base of the bleb, an electron-dense core containing a tangled feltwork of microfilamentous material was often apparent (Figs. 2–4 and 6–9). In some blebs this material appeared to be continuous with approx. 6-nm diam microfilaments that extended distally into the cortical regions of the bleb (Fig. 6). These microfilaments ran in a layer of up to about 80 nm in thickness, just subjacent to the limiting membrane of the bleb. Such layers were observed in many, but not all, bleb profiles, and were frequently associated with either larger blebs (Fig. 9) or larger knobs of short blebs (Figs. 2 and 6). A cortical microfilament layer was often lacking in the small knobs of short blebs (Figs. 3–5).

Another characteristic of blebs was the presence of quantities of approx. 25-nm diam electron-dense particles which, by size, shape, and electron density appeared to be ribosomes. The number of these ribosomes observed varied from bleb to bleb, but at least a moderate number were always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evident (Figs. 2–9). The highest concentration of ribosomes was found in the distal portions of blebs, especially in small knobs. Here, ribosomes were excluded from the cortical microfilament layer, where present, but were often tightly packed throughout the remainder of the distal bleb cytoplasm (Figs. 2, 4, and 6). In lower concentrations, ribosomes were usually also visible within the core of the bleb, amongst the strands of the microfilament feltwork (Figs. 2–4 and 7–9). Most of these ribosomes were present as individually isolated particles, and clear evidence of polysome formation was not observed.

Another structure, observed in many blebs, was the coated vesicle. These 50–80-nm diam structures were most often associated with short, knobby blebs, and were frequently located in the core of such blebs or at the base of the small knobs (Figs. 2–4). Partially formed coated vesicles (coated pits) were sometimes observed in apparent continuity with the limiting membranes of knobs, suggesting the possibility of vesicle origin by micropinocytosis at the base of these knobs (Figs. 2 and 3). However, since similar coated vesicles were also visible in the apical epithelial cytoplasm, the possibility that these coated pits were involved in a membrane-expanding exocytotic process could not be excluded.

An additional structure which was observed in some blebs appeared identical to the typical colloid droplet that is associated with the macropinocytic uptake of luminal material. Such colloid droplets were frequently found in large blebs (Fig. 9) and were occasionally noted in the knobs of short blebs (Fig. 5).

Except for microfilaments, ribosomes, coated vesicles, and colloid droplets, recognizable cellular elements appeared to be essentially excluded from the bleb cytoplasm.

Phagocytosis of Blebs

OCCURRENCE: Images suggesting the phagocytic uptake of blebs by nearby follicular epithelial cells were consistently found in sections of hyperplastic thyroid glands. Small knobby blebs appeared to undergo phagocytosis only occasionally, while the vast majority of larger blebs which were observed had been at least partially phagocytosed. The phagocytic process seemed to be generally similar for blebs of various sizes. It appeared to involve engulfment of the bleb by a typical pseudopod, pinching-off of the bleb from its cell of origin, and retraction of the pseudopod and bleb-containing phagosome into the apical epithelial cytoplasm.

FIGURE 1 From the apical surface of a stimulated thyroid epithelial cell, a small bleb (arrow) protrudes into the flattened follicular lumen (*L*). The remainder of the cell shows no unusual ultrastructural features. × 8,500.

FIGURE 2 Detail of the bleb in Fig. 1 is shown. This small bleb has one prominent knob (*K*), which is largely filled with apparent ribosomes, except in a thin cortical layer (short arrow). At the base of the bleb, a dense core (*C*) is apparent. In this region a coated vesicle may be in the process of formation (long arrow). × 19,600.

FIGURE 3 A longitudinal section of a small, knobby bleb is depicted. Ribosomes are relatively sparse in this bleb, but coated vesicles are numerous. One coated vesicle may be in the process of formation (arrow) at the base of a knobby projection. × 18,200.

FIGURE 4 A cross section (nearly parallel to the apical epithelial surface) of the core region of a knobby bleb is shown. Knobs have an appearance, in this plane, similar to that found in longitudinal section (Fig. 3), indicating their polypoid character. Several small vesicles are apparent within the markedly electron-dense core. × 22,600.

FIGURE 5 An apparent colloid droplet (arrow) is evident within one of the small knobs of this bleb. The narrowness of the stalk and the appearance of the knobs indicate that this section passes through a lateral portion of the bleb. × 16,100.

FIGURE 6 Detail of a portion of a bleb is shown. The dense core at the base of the bleb appears to contain a feltwork of microfilaments, some of which (arrows) appear to enter the large knob at the top. In this knob, densely packed ribosomes are evident but appear to be excluded from the cortical microfilament layer. × 51,200.

ENGULFMENT PROCESS: The process of phagocytic engulfment appeared to differ somewhat for different blebs. Small, knobby blebs were observed to be engulfed by a loosely apposed pseudopod, such that a considerable quantity of luminal colloid was included in the phagosome (Fig. 8). Larger blebs, or blebs which lacked small knobs, were generally surrounded by a closely apposed pseudopod, and luminal colloid was largely excluded from the region between pseudopod and bleb membranes (Figs. 9–12). These membranes were separated by a 0–30-nm irregular space, which generally appeared empty and may have been a shrinkage artifact (Figs. 10 and 11).

The pseudopods involved in the engulfment of blebs (Figs. 7–12) were ultrastructurally similar to those employed in the macropinocytosis of luminal colloid and described above. The distal portion of a pseudopod which had engulfed a bleb had a characteristic thickness of about 0.1 μm. In some cases (especially in very narrow follicular lumens) blebs were observed to be surrounded by two or more layers of pseudopods (Fig. 14). These pseudopods were occasionally traceable to different epithelial cells, so that it appeared possible for a bleb, which had been engulfed by one pseudopod, to then become at least partially enveloped by a second. On some occasions an apparently engulfed bleb was observed to be surrounded by a thin, membrane-enclosed channel (Fig. 15), raising the possibility that a pseudopod layer had been taken up along with the bleb.

Many blebs appeared to undergo only modest ultrastructural alterations during the engulfment process (Figs. 8 and 16). One structure which did appear to be characteristically associated with engulfed blebs was a crystalloid-like accumulation of rather straight, oriented microfilaments, each having a diam of about 6 nm (Figs. 12–14).

PINCHING-OFF PROCESS: To complete engulfment, a bleb had to be separated from its cell of origin. This appeared to be accomplished at least in part by a type of pinching-off process. In

FIGURE 7 A typical pseudopod (*P*), presumably projecting from a nearby epithelial cell, has come into contact with a small bleb. Compared to the bleb, the pseudopod cytoplasm is less electron dense but contains similar ultrastructural elements. × 14,100.

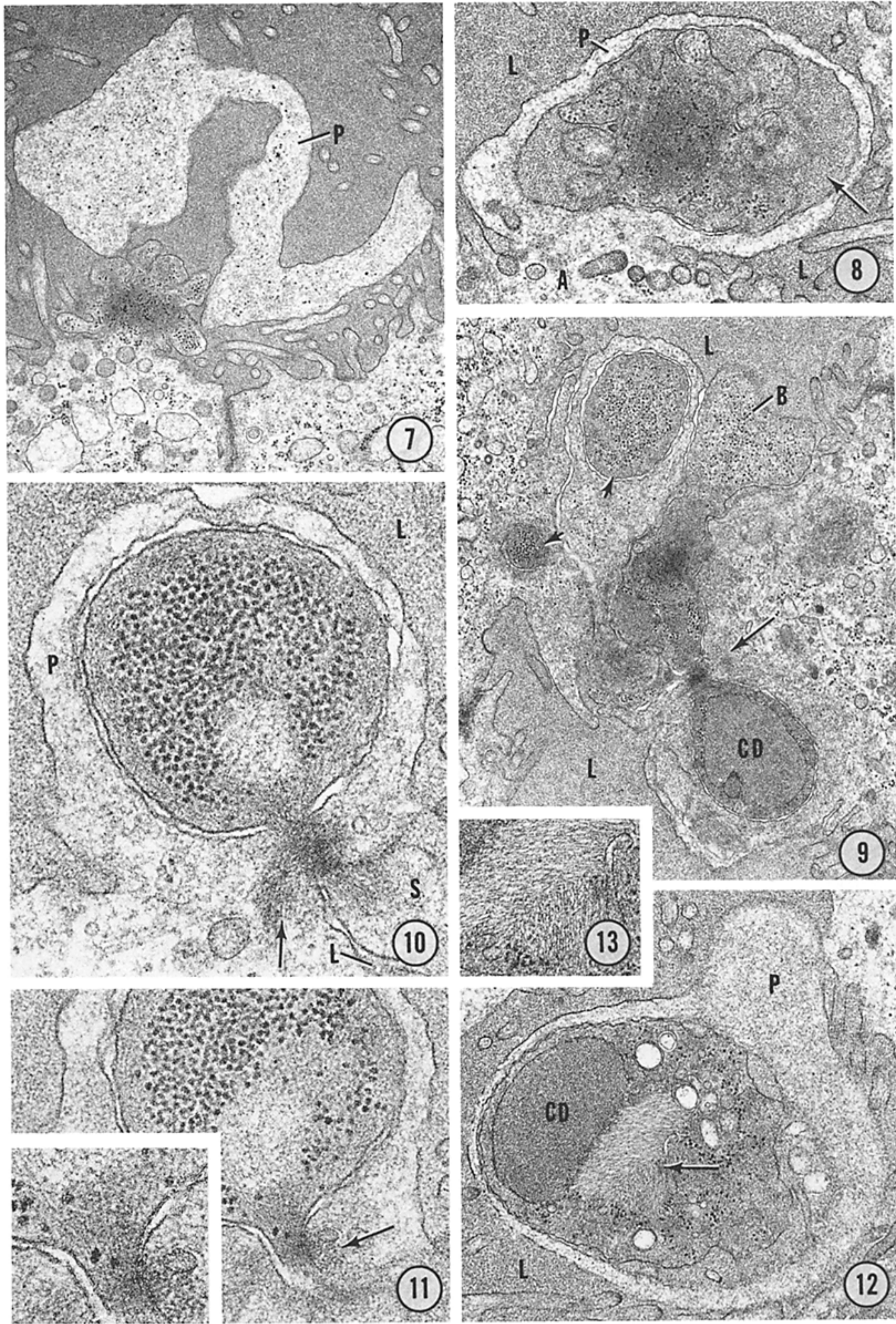
FIGURE 8 A small, knobby bleb appears to have been engulfed by a loosely apposed pseudopod (*P*) which projects from the apical surface (*A*). Considerable material (arrow) which resembles luminal colloid (*L*) appears to have been taken up along with the bleb. × 21,900.

FIGURE 9 A large, elongate bleb (*B*) is shown. A portion of this bleb projects into the follicular lumen (*L*), while another portion, containing an apparent colloid droplet (*CD*), appears to be partially phagocytosed. At one point, the bleb appears constricted and an increased density in the surrounding pseudopod cytoplasm is evident (long arrow). This suggests that the bleb may be undergoing pseudopod-induced segmentation before its ingestion. Other portions of ingested blebs are also visible (short arrows). × 15,200.

FIGURES 10 and 11 Nonconsecutive serial sections of a small, rounded bleb undergoing an apparent pinching-off process are shown. The bleb projects into the follicular lumen (*L*) on a stalk (*S*), which was observed in other sections to lead to a nearby epithelial cell. The bleb is surrounded by a closely apposed pseudopod (*P*). At the base of the bleb, a thin neck is apparent and contains longitudinally oriented microfilaments. These microfilaments splay out into the body of the bleb, imparting a constricted appearance to the neck (Fig. 11, *inset*). In the pseudopod cytoplasm surrounding the neck, microfilaments are also apparent. In sections which pass just tangential to the neck these microfilaments appear in longitudinal orientation (Fig. 10, arrow), whereas in sections passing directly through the neck they appear in (presumed) cross section (Fig. 11, arrow), suggesting that they at least partially encircle the neck. Figs. 10 and 11, × 45,000; *Inset,* × 70,000.

FIGURE 12 A large bleb is surrounded by a closely apposed pseudopod (*P*) which projects into the follicular lumen (*L*). The bleb contains an apparent colloid droplet (*CD*) and a crystalloid-like accumulation of oriented microfilaments (arrow, see Fig. 13). × 21,600.

FIGURE 13 Detail of the accumulation of oriented microfilaments depicted in Fig. 12 is shown. × 36,600.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association with this process, the formation of a thin neck in between the bleb and the rest of the cell cytoplasm was observed (Fig. 11). This neck characteristically contained a number of tightly packed, longitudinally oriented microfilaments (Fig. 11, *inset*). The orientation and distribution of these microfilaments suggested that the neck was being constricted.

About the periphery of a neck region, a closely apposed, somewhat thickened portion of pseudopod cytoplasm was generally found. This cytoplasm characteristically contained a bundle of approx. 6-nm diam microfilaments. This bundle measured 0.1–0.2 μm in width. Its microfilaments were seen in longitudinal orientation when the section was just tangential to the neck region (Fig. 10) and in (presumed) cross section when the neck was longitudinally cut (Fig. 11), suggesting that the microfilaments at least partially encircled the neck. The location and orientation of these microfilaments raised the possibility of their functioning as a contractile ring during the pinching-off process.

Another pinching-off process, morphologically similar to that just described, was also observed, occasionally, in association with the apparent division of large blebs into segments, before their ingestion (Fig. 9). In this case the apparent division(s) occurred in the mid portion of the bleb rather than at its point of attachment to the apical cytoplasm.

RETRACTION PROCESS: After engulfment by a pseudopod, the bleb appeared to be retracted into the apical cytoplasm of the phagocytosing cell (Figs. 16–18). In association with the retraction process, an accumulation of microfilamentous material immediately around the phagosome periphery was generally observed (Figs. 16–18). This material formed a 0.1–0.2-μm layer composed of approx. 6-nm diam microfilaments which were oriented around the phagosome surface. This periphagosomal microfilament layer was not observed to extend far into pseudopods, but was associated with that portion of a partially retracted phagosome which had entered the apical cytoplasm. It was not possible to be certain that a given bleb had been completely pinched-off before the retraction process, and in a single instance a retracted bleb was observed that was not completely separated from its cell of origin (Fig. 17). All other profiles of retracted blebs which were observed resembled that shown in Fig. 18. In a few instances the retracted bleb had moved somewhat toward the central region of the cell (leaving the apical cytoplasm) and lacked a periphagosomal microfilament accumulation. Although these observations tended to suggest that pinching-off had been completed, even in such instances the possibility that some (or all) blebs might remain attached to their cells or origin (by a thin neck) could not be entirely ruled out.

DISCUSSION

The present study reports that typical blebs can

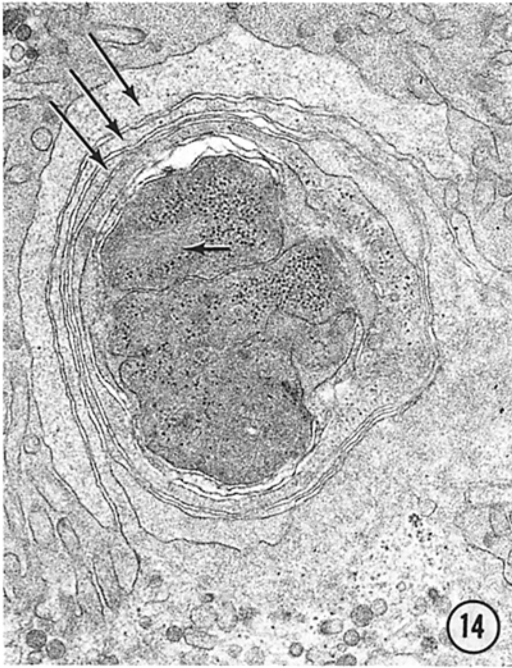
FIGURE 14 An engulfed bleb is surrounded by multiple layers of pseudopods (long arrows). An accumulation of oriented microfilaments similar to those in Fig. 12 is apparent within the bleb (short arrow). × 19,700.

FIGURE 15 A bleb appears to have been engulfed by a pseudopod. In between the bleb and the pseudopod is a thin, membrane-bounded channel (arrows), raising the possibility that a pseudopod layer (like those shown in Fig. 14) has been engulfed along with the bleb. × 15,900.

FIGURE 16 This phagosome, containing a small, knobby bleb, has been retracted into the apical cytoplasm. Inside the phagosome, considerable luminal colloid appears to have been taken up (long arrow). Around the phagosome, an accumulation of microfilamentous material is apparent (short arrows). L = Follicular lumen. × 10,600.

FIGURE 17 A large bleb appears to have been phagocytosed by a cell on the opposite side of the follicular lumen (L), and appears to have been retracted into the apical cytoplasm of this cell. This bleb, however, has not been completely pinched-off from its cell of origin, and remains attached by a narrow stalk. Inside the bleb, several colloid droplets (*) are apparent. Immediately surrounding the bleb, a periphagosomal microfilament accumulation is visible (arrows). × 14,100.

FIGURE 18 A retracted phagosome containing a bleb is shown. Periphagosomal microfilaments, oriented around the phagosome periphery, are evident (arrow). L = Follicular lumen. × 35,700.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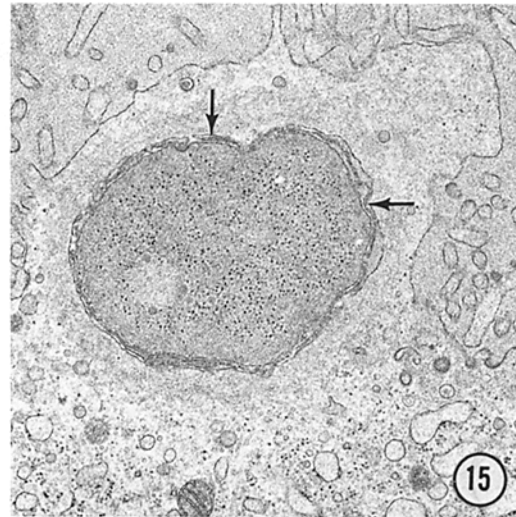
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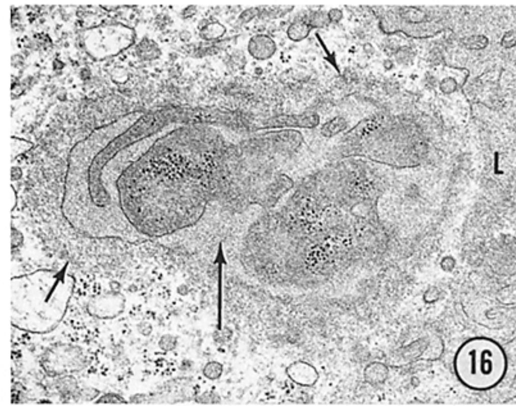
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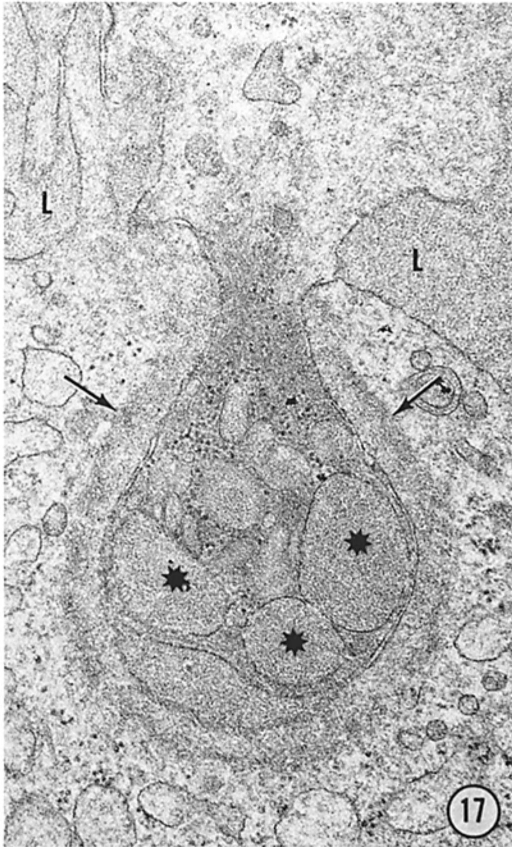
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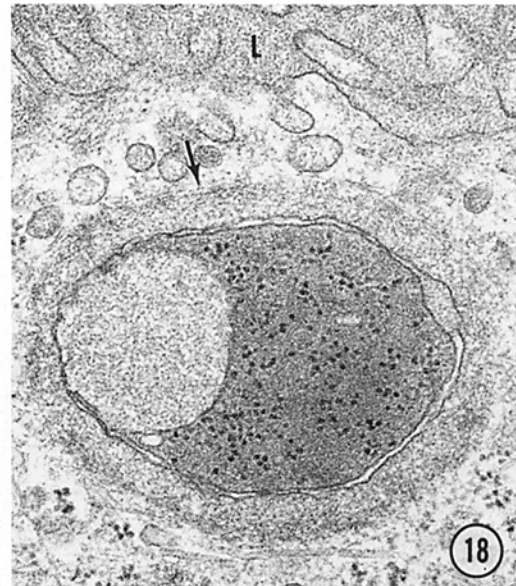
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occur in vivo in the hyperplastic thyroid gland and implies that blebs are not restricted to isolated cells. It adds to accumulating evidence (13, 22, 23, 36) against the contention that blebbing is an unhealthy cellular process related to environmental abnormalities, and thus tends to extend the significance of in vitro studies of the phenomenon.

Several circumstances which have been associated with the occurrence of blebbing in vitro also appear to prevail in hyperplastic thyroid epithelial cells in vivo. First, epithelial cell types have themselves often been reported to undergo blebbing in vitro (6, 10, 25). Second, an association of blebbing with free (unattached) cell surfaces in vitro has been repeatedly noted (4, 6, 31). The restriction of blebbing to the apical surface of thyroid epithelial cells would appear to coincide with this pattern. Third, blebbing in vitro has on several occasions been associated with pseudopods and cell movement (6, 17, 36). This would correlate with the high degree of pseudopod activity typical of stimulated thyroid glands.

The relationships between the various forms of blebs observed during the present study remain uncertain. Some heterogeneity may arise as a result of observing blebs at different stages in the blebbing process. Harris has reported light microscope observations of cellular protrusions up to 10 μm in length, which appear to wrinkle as they retract (13). It thus seems possible that at least some of the large blebs observed in the present study may normally be transformed into small knobby structures during retraction.

Although it is conceivable that cytoplasmic colloid droplets might pass into blebs, the frequent association of such droplets with large blebs raises the additional possibility that at least some blebs may originate from pseudopods. Although apparently less compact than blebs, pseudopods contain virtually the same ultrastructural elements. It is thus conceivable that pseudopods, after engulfing luminal colloid, might on certain (uncommon) occasions increase in density, become more rounded, and undergo transformation into blebs. Such a transformation of pseudopods (and ruffles) into blebs has in fact been reported, based upon light microscope observations (6, 13, 36). A difficulty in distinguishing some blebs from pseudopods during the present study also supports this possibility.

A further consideration regarding the possible transformation of pseudopods into large blebs is the high frequency with which large blebs were observed in the process of being phagocytosed. This relationship with phagocytosis raises the possibility that transformation of a pseudopod into a bleb may be induced by a second pseudopod. We have previously noted the frequent interactions of pseudopods in the hyperplastic thyroid gland, including the apparent attempts of some pseudopods to engulf others (39). In such instances it may be that the pseudopod being phagocytosed is sometimes transformed into a large bleb.

The phagocytic uptake of blebs has not to our knowledge been previously reported. In fact it might be considered surprising that epithelial cells, which are in contact with one another along their lateral surfaces, are capable of a phagocytic response toward portions of one another. A phagocytic response toward adjacent dying cells has, however, been reported for several cell types (15), and in special instances cells have been reported to routinely ingest portions of adjacent living cells as a physiological function (26, 35, 38). In the present instance an explanation for the apparent cellular cannibalism which was observed may be related to the nature of the apical surface of thyroid epithelial cells. This apical surface does not normally come into contact with other cells, and appears to be specialized for the production of pseudopods, which are initiated by thyroid-stimulating hormone (TSH), and which engulf luminal colloid. Moreover, in the hyperplastic thyroid gland, follicular lumens become markedly narrowed (Fig. 1), so that such pseudopods might on occasion encounter (Fig. 7), and subsequently engulf, a bleb projecting from the opposite side of the lumen.

In addition to the possibility of engulfment of blebs by TSH-initiated pseudopods, it is also possible that blebs may themselves be capable of initiating pseudopods. In the presence of narrowed follicular lumens, blebs may come directly into contact with the apical surface of opposing epithelial cells and (by contact) initiate the formation of pseudopods. This kind of pseudopod initiation apparently can occur when a pseudopod makes contact with an opposing epithelial cell; a second pseudopod often appears to be initiated at the site of contact (39). A sequence of such pseudopod initiations may explain the multiple layers of pseudopods observed around some blebs in the present study.

Two types of engulfed blebs were observed in this investigation at various stages of ingestion, those which were surrounded by a closely apposed

pseudopod (Figs. 10, 12, 15, 17, and 18) and those which appeared to have been engulfed along with a considerable portion of luminal colloid (Figs. 8 and 16). These latter blebs were small, knobby structures, suggesting the possibility that the engulfing pseudopod had been unable to completely follow their irregular contours. Alternatively, such blebs may have been engulfed by macropinocytic mechanisms, as opposed to phagocytic mechanisms, and might therefore lack the close apposition of pseudopod membrane which appears to be characteristic of phagocytosed material (40), and which may be essential for phagocytic engulfment to proceed (11, 12).

Of particular interest in the phagocytosis of blebs is the pinching-off process, by which blebs appear to be separated from their cell of origin. This process appears to involve constriction of the bleb base by pseudopod cytoplasm, which contains a band of 6-nm microfilaments that appears to at least partially encircle the bleb base. A similar band of microfilaments, the mitotic contractile ring, is believed to be responsible for cytokinesis (33), and has been shown to contain actin (20, 32) and possibly myosin (9). In pseudopods of hyperplastic thyroid epithelial cells, a similar contractile ring is also believed to be responsible for an erythrocyte fission process (40). The microfilaments observed in the present study thus appear to constitute a second example of contractile ring formation in thyroid epithelial pseudopods.

After the pinching-off process, phagosomes containing blebs appeared to be retracted into the apical cytoplasm. This retraction process was associated with a periphagosomal accumulation of microfilaments. Similar microfilaments have not been reported around macropinocytosed colloid droplets, but have been observed around phagocytosed erythrocytes in thyroid epithelial cells, and have been associated with the retraction stage of phagocytosis in several systems (40).

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