to cryostat sections seemed more suitable for the demonstration of the AChE reaction in cells such as glomus cells, which also exhibit catecholamine fluorescence.

The presence of AChE reaction in the fluorescent glomus cells is in agreement with the concept that these cells belong to the APUD cells[6]. The present observations are also in agreement with the electron-microscopical findings of Papka[7] on the localization of the AChE in the glomus bodies of rabbit heart. In his study the AChE was localized in the glomus cell perinuclear cisternae, glomus cell endoplasmic reticulum, glomus cell membrane, at the axolemma of unmyelinated axons and around the synaptic terminals to the glomus cells. The presence of AChE in catecholamine-containing glomus cells can be explained in many ways[7]. However, this finding and the observation of Ballard and Jones[8] on the presence of choline acetyl transferase in glomus cells of cat carotid body and the finding of Fidone et al.[9] on the uptake of $^3$H-choline by the glomus cells support the presence of active acetylcholine metabolism in the glomus cells. Acetylcholine might be synthetized and stored in the glomus cells and discharged from the cells, to have an excitatory action on the chemonsensory nerve ending. However, although acetylcholine does excite the chemonsensory nerve ending, the chemonsensory nerve ending itself most is probably not cholinergic because impulses produced with physiological stimuli are not abolished by the action of cholinergic blocking agents[10]. The carotid body contains large quantities of dopamine and noradrenaline[11]. The effect of catecholamines, especially that of dopamine, on the chemonsensory drive is inhibitive in nature[12].

The definite presence of both catecholamines and acetylcholinesterase activity in the same glomus (type I) cells of carotid body, as demonstrated in this study, leads us to suggest that the glomus cells might store both excitatory and inhibitory (modulating) transmitters which do influence the chemosensory nerve ending.


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## Particle aggregates in plasma and intracellular membranes of toad bladder (granular cell)[1]


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*Summary.* Freeze-fracture of granular cells of toad urinary bladder (*Bufo marinus*) reveals the presence, hitherto undescribed, of intramembranous particle aggregates in intracytoplasmic structures (tubules, vacuoles and vesicles) both in resting and vasopressin-stimulated epithelia.


Amphibian bladders have long been used as privileged models for studying water permeability in tight epithelia and its modulation by neurohypophyseal hormones. Convergent evidence from transmission[4] and scanning electron microscopy[5] indicates that there is cellular specificity for the hydrosmotic effect of vasopressin, the apical membrane of the granular cells being the primary target of the hormone. Moreover, cell organelles such as lysosomal granules and their attendant enzymes[6], microtubules and microfilaments[7–10] and phenomena such as exocytosis[11], pinocytosis[12] and changes in membrane fluidity[13] appear to be associated with the increase in permeability to water.

Recently, characteristic organizational patterns of membrane particles in different cell types of toad bladder were revealed by the freeze-fracture technique[14–16]. In addition, conspicuous aggregates of particles were seen in frog[17,18] and toad bladders[19–22] challenged with oxytocin or vasopressin. So far, these morphological alterations have only been found in the P and E fracture faces[23] of the apical


1 This work was supported by grants No. 3.553.75, 3.120.77 and 3.1300.73 from the Swiss National Science Foundation.

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3 The authors are indebted to Mrs Marthe Sidler-Ansermet, Miss Jacqueline Rial and Mr Michel Bernard for valuable technical assistance.



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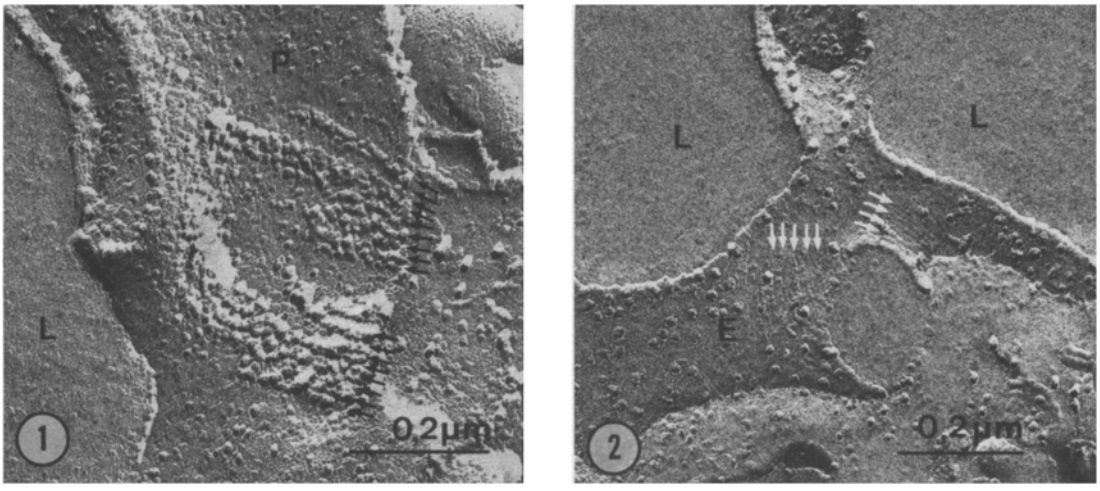
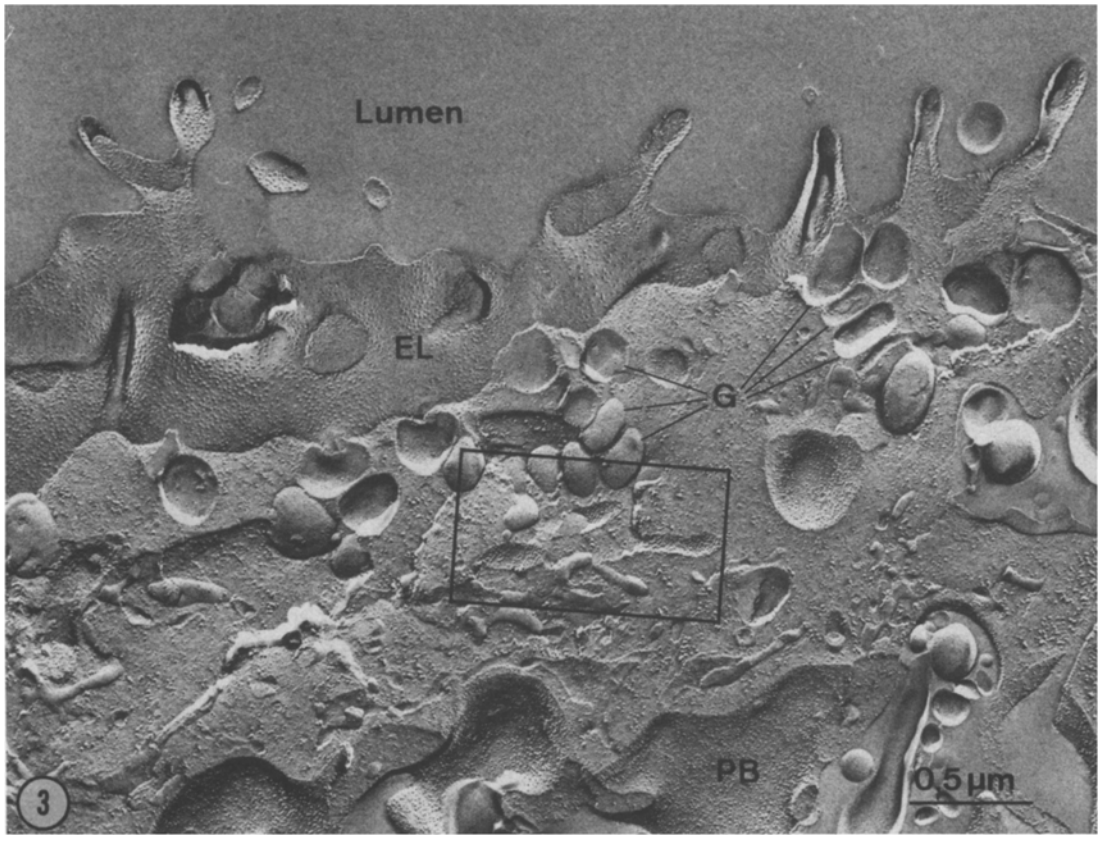
membrane of the granular cell. We report here that granular cells of both resting and vasopressin-stimulated toad bladders contain a population of membrane-bounded compartments (tubules, vacuoles and vesicles), the limiting membranes of which disclose the same pattern of intramembranous particle aggregates previously described in the lumenal plasma membrane of bladders exposed to vasopressin.

*Materials and methods.* Toads (*Bufo marinus*) were obtained from Mogul-Ed Co., Oshkosh, Wisconsin, USA, and kept in a terrarium at 22 ± 2 °C, with free access to water. The excised urinary bladders were mounted as sacs or as diaphragms between glass hemi-chambers. The mounting procedure, the composition of the Ringer solutions and the method used for recording water flow have been described elsewhere [5, 14].




Figures 1 and 2. Freeze-fracture preparations of the lumenal plasma membrane of vasopressin-stimulated granular cells. Fig. 1. The protoplasmic or P face (P) contains 2 large aggregates composed of parallel arrays of tightly packed intramembranous particles (arrows). L, Lumen of the bladder. ×92,000. Fig. 2. The exoplasmic or E-face (E) discloses a pattern of linear depressions (arrows). L, Lumen of the bladder. ×82,000.




Figures 3–5. Freeze-fracture replicas of vasopressin-stimulated toad bladders.

Fig. 3. The fracture plane has exposed the plasma membrane and the cytoplasm of a granular cell. In the cytoplasm, an area containing 3 tubular profiles is outlined by a rectangle and shown at higher magnification in figure 4. EL, Exoplasmic face of the lumenal membrane. PB, Protoplasmic face of the basal membrane. G, Granules. ×32,000.

Tissues were fixed with a 2% glutaraldehyde solution containing 0.1 M phosphate or cacodylate buffer. Small pieces of epithelium were soaked in a phosphate – or cacodylate – buffered 30% glycerol solution, then freeze-fractured in a Balzers BAF 301 unit[24]. Freeze-fracture replicas deposited on coated copper grids were examined in a Phillips EM 300 electron microscope.

*Results and discussion.* In freeze-fracture replicas of control toad urinary bladders, the lumenal membrane of unstimulated granular cells exhibits the distinctive organization previously described[14,15], i.e. a high density of large intramembranous particles on the E face, whilst the P face has fewer and smaller particles. In vasopressin-stimulated granular cells, the lumenal membrane is decorated with aggregates of linearly packed intramembranous particles on the P face (figure 1), matched by parallel linear depressions on the E face (figure 2). These latter findings confirm earlier reports on oxytocin-[17,18] or vasopressin-induced[19–22] structural changes in amphibian bladder lumenal membrane. To assure that bladders exposed to vasopressin were indeed responsive to the hormone, the transepithelial hydrosmotic flow was continuously monitored with an optical technique[5] prior to fixation of the tissue.

Careful examination of the fractured cytoplasm of granular cells in both control and vasopressin-stimulated toad bladders, revealed the presence of similar sites of particle aggregation in the limiting membrane of intracytoplasmic compartments: tubules, vacuoles and vesicles. As shown in figure 3, the fracture process reveals at least 3 main types of intracytoplasmic membranes: a) membrane showing the usual pattern of randomly distributed particles; b) membranes of the dense granules, carrying only rare intramembranous particles; c) membranes displaying a distinctive organizational pattern in arrays. The protoplasmic or P face[23] of these latter membranes is charac-

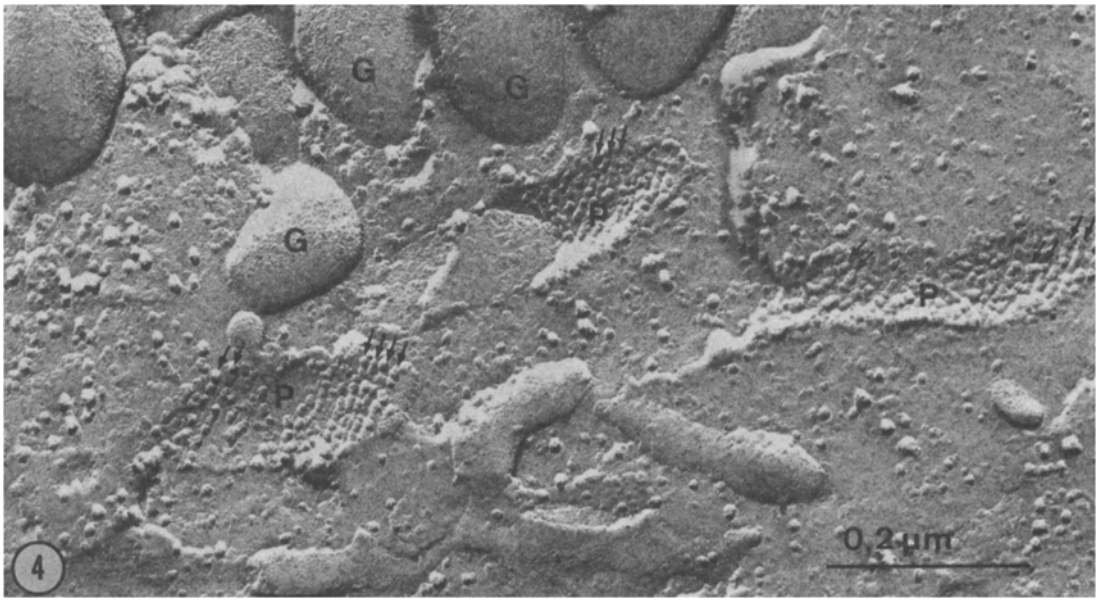



Fig. 4. The protoplasmic membrane face (P) of 3 tubular profiles exhibits a distinctive organization which consists in the parallel arrangement of linear aggregates of closely packed intramembranous particles (arrows). G, Granules. ×134,000.

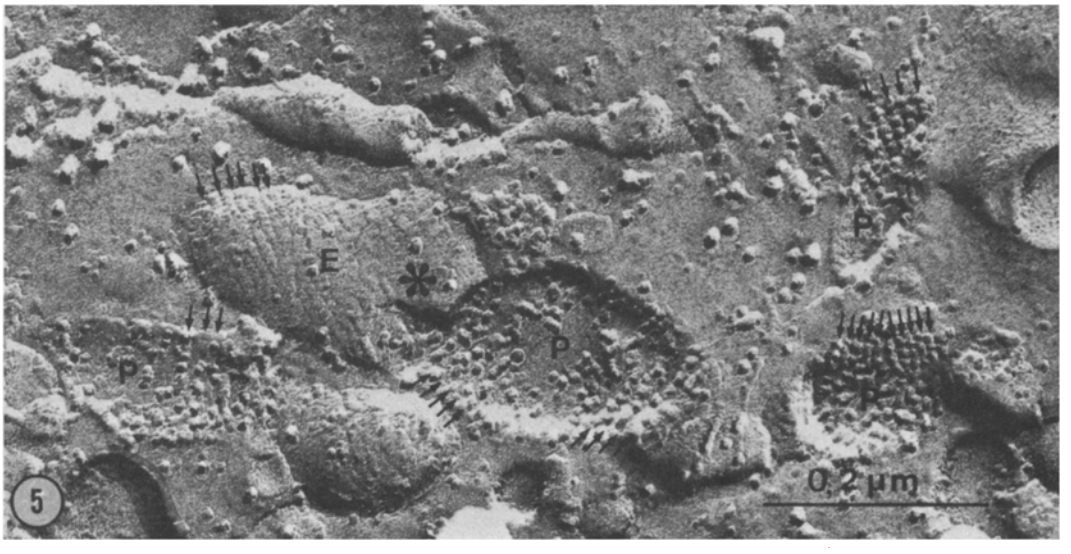



Fig. 5. Both the P and E faces of an elongated vacuole or tubule (asterisk) have been exposed by the fracture process. Linear arrays of particles (arrows) on the P face (P) and corresponding parallel grooves (arrows) on the E face (E) are seen. Neighbouring vesicular or tubular structures also show linearly organized aggregates (arrows) on their P face (P). ×147,000.

terized by the parallel arrangement of linear arrays of tightly packed intramembranous particles (figures 3 and 4), while its exoplasmic or E face is furrowed by parallel linear depressions (figures 5 and 6). Most membranes exhibiting these unique structural features appear to belong to tubules or elongated saccules and, less frequently, to vesicles or larger vacuoles, distributed through the entire granular cell cytoplasm. Although they are often encountered in proximity to the apical surface (figure 3), a basal location is not uncommonly seen (figure 6). This observation seems to rule out the possibility that all tubular and vesicular profiles represent invaginations of the lumenal membrane, unless the basal location is explained by cytoplasmic streaming of these structures. Finally, the pattern of particle aggregates described here somewhat resembles the particle distribution characteristic of the P face of gap junctions. However, the intracytoplasmic location of these aggregates and the presence of complementary grooves in the E fracture face, unequivocally distinguish them from gap junctions, which, surprisingly enough, have not been observed so far in fracture membranes of granular cells.

The relative frequency of membrane-bounded compartments carrying linear aggregates in vasopressin-stimulated and in control granular cells, cannot be established at present, since precise morphometric evaluation is needed. The similarity between the intracellular aggregates and those found at the lumenal surface suggests the existence of a dynamic interrelationship between the 2 topographically distinct membrane systems. However, the nature of such a relationship remains unknown at this point and we can only suggest some possibilities.

The population of particle aggregate-containing tubules and/or elongated vacuoles may represent a pool of intracellular membranes, the controlled insertion of which in the plasma membrane could provide a mechanism for hormonal modulation of particle aggregate density at the lumenal surface. As an alternate possibility, tubules and vacuoles could represent internalized portions of the lumenal plasma membrane. In this respect, it may be appropriate to recall that neurohypophyseal hormones have been reported to induce pinocytotic uptake of peroxidase from the mucosal solution into small tubules and vesicles[12]. The latter, as judged from the published electron micrographs[12], may correspond to the structures carrying linear aggregates of intramembranous particles in our freeze-fracture preparations. Again, it is conceivable that selective internalization of aggregate-containing membrane patches might be involved in modulating structural and functional responses of the lumenal membrane of the granular cell to vasopressin.

In any event, the existence of a pool of membrane-bounded compartments having the same organizational pattern as previously found only in the lumenal membrane of vasopressin-stimulated cells, must now be taken into account in studies concerning the mechanism of vasopressin-induced permeability changes in amphibian urinary bladder. The biological relevance of these findings for the understanding of the structure-function relationship in the mammalian kidney must await further investigation. However, it is of interest to point out that, in the collecting duct, clusters of particles are also seen in the apical membrane of light cells[25] and that gap junctions are also strikingly absent in this tight epithelium[26, 27].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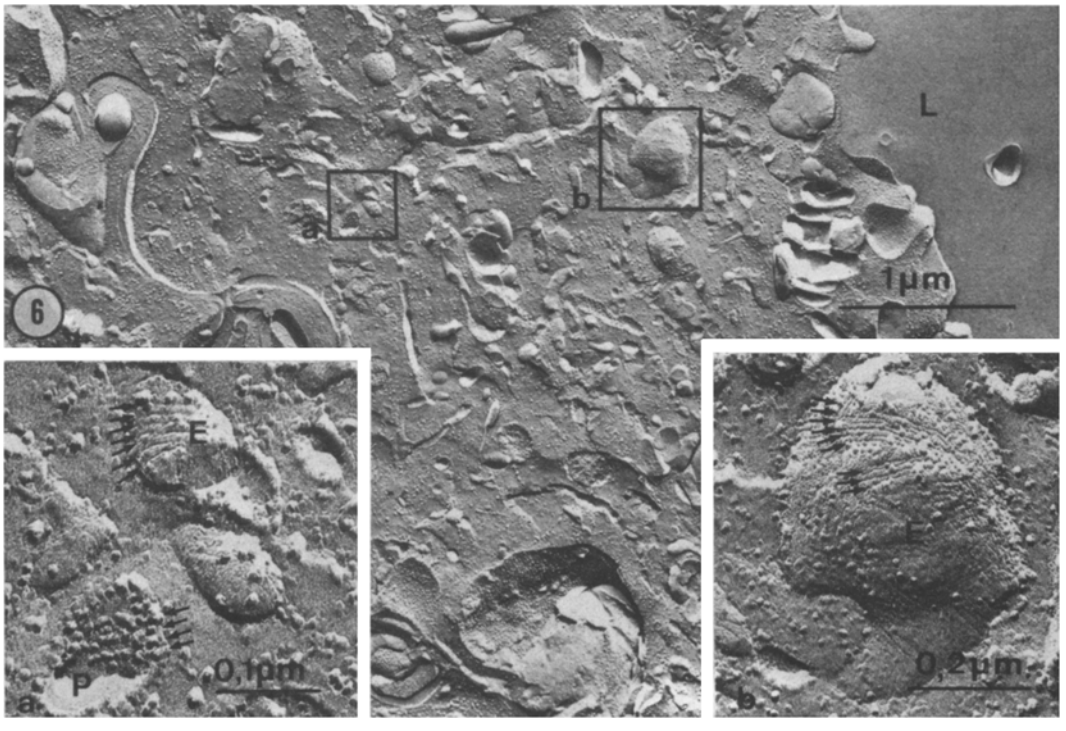


Fig. 6. Freeze-fracture replica of a control granular cell. 2 areas from the basal and the apical cytoplasm respectively are enclosed in black squares and shown at higher magnification in the insets. *a* 2 vesicles show the characteristic linear aggregates on the P face (P) and parallel grooves on the E face (E). *b* The exoplasmic face (E) of an irregularly shaped apical vacuole discloses a pattern of linear depressions (arrows). ×23,000. Inset a ×134,000; inset b ×79,000.