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Morphometric Analysis of Parietal Cell Membrane Transformations in Isolated Gastric Glands

A.J. Gibert and S.J. Hersey

Department of Physiology, Emory University, Atlanta, Georgia 30322

Summary. Changes in parietal cell membranous structures that accompany the onset of acid secretion were studied with electron microscopy using isolated gastric glands from rabbit. A stereological analysis was performed to quantitate the morphological changes occurring within 5 min following histamine stimulation. These changes were compared to the changes resulting from osmotic expansion of parietal cell components following addition of 1 mM aminopyrine (AP) to glands incubated in medium containing 108 mM K^+ (high-K^+). Morphometric analyses, together with measurements of glandular water content, indicated that parietal cells swell in high-K^+ medium. Addition of 1 mM AP to glands incubated in high-K^+ medium resulted in massive distention of the secretory canaliculus but no difference was observed in the amount of tubulovesicular membrane or the relative size of these cytoplasmic structures. In the histamine-treated glands the parietal cells displayed a rapid loss of tubulovesicular membrane and a reciprocal increase in canalicular membrane. These morphological changes were complete long before a maximum level of acid formation was achieved. Taken together, these results indicate that; (i) the morphological change accompanying stimulation does not require acid formation *per se*; (ii) the site of acid secretion is the intracellular canaliculus and not the tubulovesicles; (iii) there is no preexisting actual or potential continuity between the tubulovesicular space and the canalicular space; and (iv) the AP-induced expansion of the canaliculus in high-K^+ medium, while yielding some valuable information, is not an appropriate model for studying the normal stimulus-induced morphological transition, despite a superficial similarity of appearance.

Key words gastric glands · acid secretion · morphometry · parietal cells · histamine stimulation · aminopyrine accumulation

Introduction

The dramatic morphological change that gastric parietal cells undergo with the onset of acid secretion has been studied extensively. In the nonsecreting cell the secretory canaliculus is reduced, microvilli are scarce, and the cytoplasm is filled with smooth-membranous elements, the tubulovesicles. With stimulation there is a conspicuous elaboration of canalicular membrane surface in the form of numerous microvilli and a concommitant reduction in cytoplasmic tubulovesicle membrane [7, 8, 10]. Based upon these observations, several investigators have supported the "fusion" model as an explanation for the morphological transition [7, 8]. According to this model, the tubulovesicle membranes fuse with the apical surface membrane to form the extensive secretory canaliculus found in stimulated cells. Recently, Berglindh et al. [1] proposed an alternative explanation for the morphological transition which they term the "expansion" model. This model is based upon an ingenious set of experiments employing isolated gastric glands under special conditions. When gastric glands are incubated in medium containing a high concentration (108 mM) of K^+ without any other stimuli, they apparently form acid as indicated by the accumulation of the weak base, aminopyrine (AP) [2]. Despite the formation of acid, the parietal cells appear morphologically similar to nonstimulated cells; i.e., there is no morphological transition. In an attempt to identify the site of acid formation in the high-K^+ condition, the investigators reasoned that if the AP concentration in the medium was raised sufficiently, i.e., to 1 mM, accumulation of this base at a high ratio in the acid forming compartment would induce osmotic swelling that could be visualized by electron microscopy. Under the conditions of high K^+ and elevated AP concentration, it was found that the parietal cell morphology appeared similar to the stimulated state. Based upon this observation it was argued that osmotic expansion of the tubulovesicles is sufficient to cause a morphological transition. Moreover, it was postulated that the tubulovesicles actually constitute a continuous system of collapsed or condensed elements rather than discrete membranous forms. Therefore, osmotic expansion alone could account for the morphological transition without requiring membrane fusion.

Aside from the controversy over the phenomenological mechanism of the morphological transition, the two models carry different implications regarding

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the role of the morphological change in the acid secretory process. In particular, the expansion model implies that the morphologic changes are secondary to osmotic gradients produced by acid secretion while the fusion model would permit morphological transitions in the absence of actual acid secretion. The possible localization of enzymatic activities related to acid secretion and the mechanism of action of secretory-active agents could differ according to the two models. Therefore, investigation of the morphological transition constitutes a significant approach to understanding the cellular basis of gastric acid secretion. The present work undertakes to determine the time course of the morphological change that accompanies histamine stimulation in isolated gastric glands and to compare it with the morphology at similar time points in high-K^+, high-AP treatment. Our attention was focused on two parameters: first, change in distribution and amount of membranes over time; and second, the site of osmotic swelling in high-AP treatment. In order to make a rigorous comparison, we undertook a morphometric analysis so that we could quantify both parameters. The results of these experiments tend to support a "fusion" model over an "expansion" model for the morphological transition.

Materials and Methods

Gastric Gland Preparation

Gastric glands were prepared from rabbits, as previously described [3]. Briefly, rabbits were anesthetized with Nembutal and the stomach perfused with saline under high pressure. The stomach was removed and the mucosa stripped and minced. The mince was then digested with collagenase (0.1% collagenase) at 37 °C for 30–45 min. The glands were filtered through nylon mesh to remove coarse fragments and rinsed three times in incubation medium. The glands were suspended in medium at a final concentration of 2–3 mg dry wt/ml. The incubation medium contained (in mM): NaCl, 132.4; KCl, 5.4; Na_2HPO_4, 5.0; NaH_2PO_4, 1.0; $MgSO_4$, 1.2; $CaCl_2$, 1.0; phenol red, 10 mg/ml; glucose, 2 mg/ml; and bovine serum albumin, 2 mg/ml (initial pH 7.6). In some experiments, a high-potassium medium was used in which KCl was substituted for NaCl to give a final concentration of 108 mM K. All glands were preincubated for 20 min. in 10^{-5} M cimetidine to ensure that they would be morphologically resting at the beginning of the experimental period.

Measurement of Acid Formation

This was carried out by measuring the accumulation of ^{14}C-labeled aminopyrine (AP) as previously described [1, 9]. The standard medium contained 10^{-6} M aminopyrine of specific activity 84 mCi/mmol. After incubation, the glands were centrifuged, the supernatant was removed, and the glands were dried, weighed, and dissolved in 1 N HNO_3. Samples of the supernatant and glands were counted in a scintillation counter with quench corrections. Extracellular water was determined by the inulin space (*see* below) and after correction for aminopyrine in that space the ratio of AP in intraglandular/AP in extraglandular water was determined.

Measurement of Intraglandular Water

The glandular water content was determined from the total water content, i.e., weight wet minus dry weight, minus the trapped volume of extracellular water. The volume of extracellular water was measured using inulin as a marker. Gland suspensions were incubated for 30 min in the presence of [^3H]-inulin (0.1 mCi/ml), the glands were centrifuged, and the supernatant was removed. Wet and dry weights were determined to calculate total water and the dried glands were dissolved in 1 N HNO_3. Samples of the supernatant and dissolved glands were counted with quench corrections, and the extraglandular volume was calculated from the inulin content of the glands.

Morphometric Analysis

Experiments involving either histamine stimulation or high K^+ treatment were carried out on duplicate aliquots of the same gland preparation to facilitate comparison. For stimulated glands, histamine (1×10^{-4} M) plus 3-isobutyl-1-methylxanthine (IMX; 1×10^{-5} M) was added to the incubation flasks following the 20-min preincubation, and samples were taken for fixation at times thereafter. The high-K^+ treatment involved washing the glands three times in 108 mM K^+ medium, preincubation in this medium, and addition of nonradioactive AP (final concentration, 1×10^{-3} M). Timed samples were taken after the addition of AP. Duplicate aliquots of each treatment were fixed for electron microscopy at 0, 1, 2, 3 and 5 min. Samples were fixed in 2% glutaraldehyde in incubation medium in suspension for 30 min. They were then washed briefly in medium and post fixed for 30 min in 1% OsO_4 in the same medium. Following ethanol dehydration, the samples were embedded in Spurr's low viscosity medium. Centrifugation was not employed at any point. Sections were cut on a Sorval MT2B ultramicrotome, stained with lead citrate and uranylacetate, and viewed with a Philips 400 electron microscope. From each block (which represented one duplicate aliquot), one section was chosen at random to be photographed. From this section, five cells, selected at random, were photographed at 2500×. Each of these cells was then photographed twice (at two different locations in the cell) at 19,500×, making a total of 15 micrographs per aliquot. Morphometric measurements were performed according to the methods of Weibel [12]. The surface density (S_v) of a component was determined by applying a test line overlay to electron micrographs of parietal cells and counting intersections between the test lines and the membrane traces below. Surface density is given by the formula:

$$S_v=\frac{2I}{L_t}$$

where I is the number of intersections and L_t is the total line length. The surface densities were related to a standard unit volume of parietal cell cytoplasm and expressed as m^2/cm^3. Volume fraction (V_v) was estimated by use of a point grid overlay and expressed as percent of total cytoplasmic volume, i.e., excluding nucleus and open canaliculus. The following components were measured: tubulovesicular and canalicular surface density; canalicular, tublovesicular, and mitochondrial volume density. The cross-sectional areas of tubulovesicle profiles were determined using a digitized tablet. The profiles were traced and the surface length converted to area assuming a circular geometry. Examination of tubulovesicle shapes with various treatments showed that most were approximately circular, and therefore the assumption of circular geometry did not introduce substantial error between treatments.

Statistical evaluation was performed using the student t-test or Duncan's new multiple-range test for comparison of means [5].

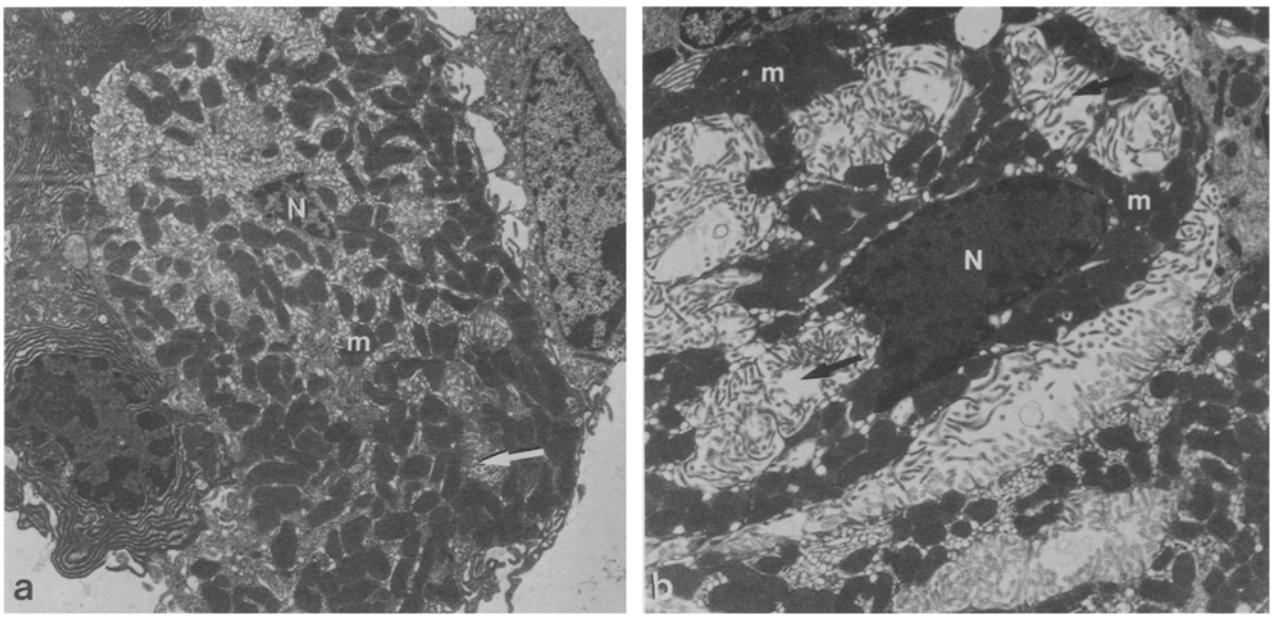

Fig. 1. Electron micrograph of cells in normal medium before (*a*) and after (*b*) histamine stimulation. Canalicular space (arrow) in *a* is just discernable, but in *b* the canaliculus is greatly enlarged. *n*, nucleus; *m*, mitochondria. 5200×

Materials

New Zealand white rabbits weighing 2–3 kg were used. All chemicals were of the highest purity grade available. Collagenase (Type I), aminopyrine, IMX, and histamine hydrochloride were obtained from Sigma Chemical Co. (St. Louis, Mo.). ^{14}C-aminopyrine and ^{3}H-inulin were from New England Nuclear (Boston, Mass.). Cimetidine was a generous gift of Dr. H. Green (Smith, Kline and French, Philadelphia, Pa.).

Results

Histamine Stimulation

Figure 1 shows a comparison between a typical non-stimulated parietal cell (*a*) and a parietal cell after 5 min exposure to histamine plus IMX (*b*). These cells display the typical morphological transition between resting and secreting states described by other workers [7, 8, 10]. Thus, the isolation procedures required to obtain the gastric glands did not appear to damage whatever mechanisms underlie this transition. By the end of a 5-min exposure to histamine plus IMX, almost all of the parietal cells examined displayed a stimulated morphology and longer exposures (up to 60 min) showed no significant further change. Stereological analysis of the parietal cells showed that after 5 min of stimulation the tubulovesicular surface density had declined by about 50% and the canalicular surface density increased about fourfold (Fig. 2). During the same period, the canalicular volume fraction increased about sixfold. Thus, the morphometric data provides quantitative support for

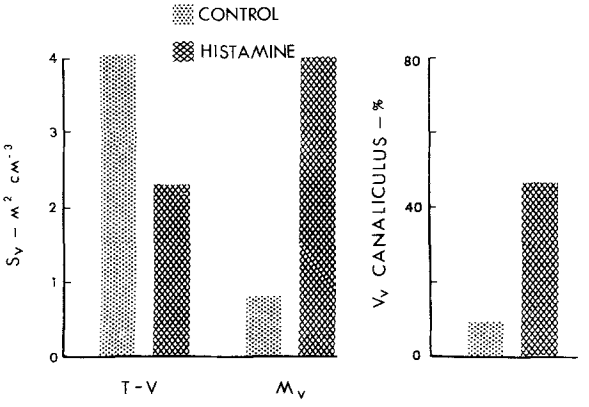

Fig. 2. Morphological changes associated with histamine stimulation. Morphometric analysis of surface densities (S_v) for tubulovesicular ($T-V$) and canalicular (M_v) membranes were performed by counting intersections with a line overlay. Canalicular volume fraction (V_v) was estimated by point counting. Both parameters are normalized for cytoplasmic volume. The bars compare parietal cells exposed to histamine (1×10^{-4} M) plus IMX (1×10^{-5} M) for 5 min with control cells (0 time). All parameters show significant changes with histamine *vs.* control (*see* Table 1)

the impression that isolated gastric glands display a normal morphological transition.

The ability to obtain successive samples of isolated glands at precise intervals permitted us to examine the time course of the transition following histamine addition. The results of this analysis are shown graphically in Fig. 3 and the statistical summary is given in Table 1. Statistically significant changes were found in the canalicular surface density after as little as 1 min of histamine exposure and after only 2 min of exposure, the tubulovesicular surface density was

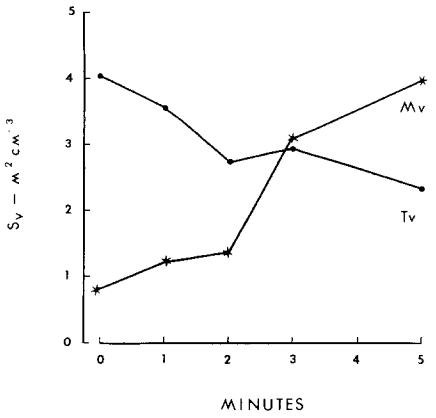

Fig. 3. Time course of morphological change with histamine stimulation. Surface densities (S_v) of tubulovesicular (TV) and canalicular (M_v) membranes were measured during the 5 min period following addition of histamine (1×10^{-4} M) plus IMX (1×10^{-5} M). As tubulovesicular surface density declined there was an approximately parallel increase in canalicular surface density. Values are the mean for n=4 (No. of animals). Statistical analysis is given in Table 1

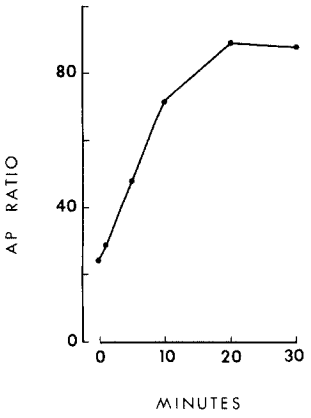

Fig. 4. Time course of AP accumulation with histamine stimulation. AP accumulation is given as the ratio of AP in glandular water to AP in the medium. Histamine (1×10^{-4} M) plus IMX (1×10^{-5} M) was added at zero time and samples taken up to 30 min. Maximum accumulation required about 20 min. The points represent averages of duplicate determination on one preparation of glands. The time course is typical of three other measurements

Table 1. Time course of morphological changes with histamine stimulation

	Time after histamine (min)				
	0	1	2	3	5
$S_v(m^2/cm^{-3})$					
Canaliculus	0.83 ± 0.2^a	1.2 ± 0.3^b	1.6 ± 0.3^b	3.1 ± 0.7^c	4.0 ± 0.3^d
Tubulovesicles	4.00 ± 0.5^a	3.6 ± 0.6^{ab}	2.8 ± 0.4^{cd}	3.0 ± 0.4^{bc}	2.4 ± 0.4^d
$V_v(\%)$					
Canaliculus	7 ± 5^a	11 ± 6^{ab}	23 ± 9^{bc}	34 ± 4^c	48 ± 5^d

Values are mean ±SE for $n=4$ (No. of animals). Means with no superscript in common are significantly different; $P = < 0.05$.

significantly decreased. Changes in surface densities and canalicular volume fraction occurred progressively over the test period and reached a maximum at 5 min. No further changes were seen at 10 min or longer. The very rapid time course of the morphological transition is due in part to the reduced diffusion barrier in isolated glands relative to intact gastric mucosa. However, it also indicates that histamine initiates cellular events almost immediately upon binding to a cell receptor. Parallel experiments measuring the time course of histamine-stimulated acid formation showed that this parameter was slow relative to the morphological change (Fig. 4). While a slight increase in the AP ratio was seen after 1 min of exposure to histamine plus IMX, at 5 min the ratio had reached only about 50% of the maximum. The maximum AP ratio was not achieved until 20 min after stimulation, which was well after completion of the morphological transition. The apparent delay in reaching a maximum AP ratio cannot be ascribed to diffusion delays since AP equilibrates with the glands in less than 1 min (*unpublished observations*). Since the AP ratio represents a steady-state accumulation rather than a rate, the time course may reflect, to some extent, a buildup of hydrogen ion rather than the actual time course of proton pump activation. However, any buildup of hydrogen ion concentration must require a pre-existing volume into which the hydrogen ions can be secreted. Thus, it appears that, whatever factors are involved, the morphological transition precedes the actual accumulation of acid even if it occurs simultaneously with activation of the proton pump.

High K^+ Treatment

Gastric glands incubated in medium containing 108 mM K^+ contain parietal cells which resemble non-stimulated cells in normal medium (Fig. 5*a*). There is some distension of the canaliculus and the mitochondria appear swollen (*see* below), but the appearance is of a resting cell with numerous tubulovesicle

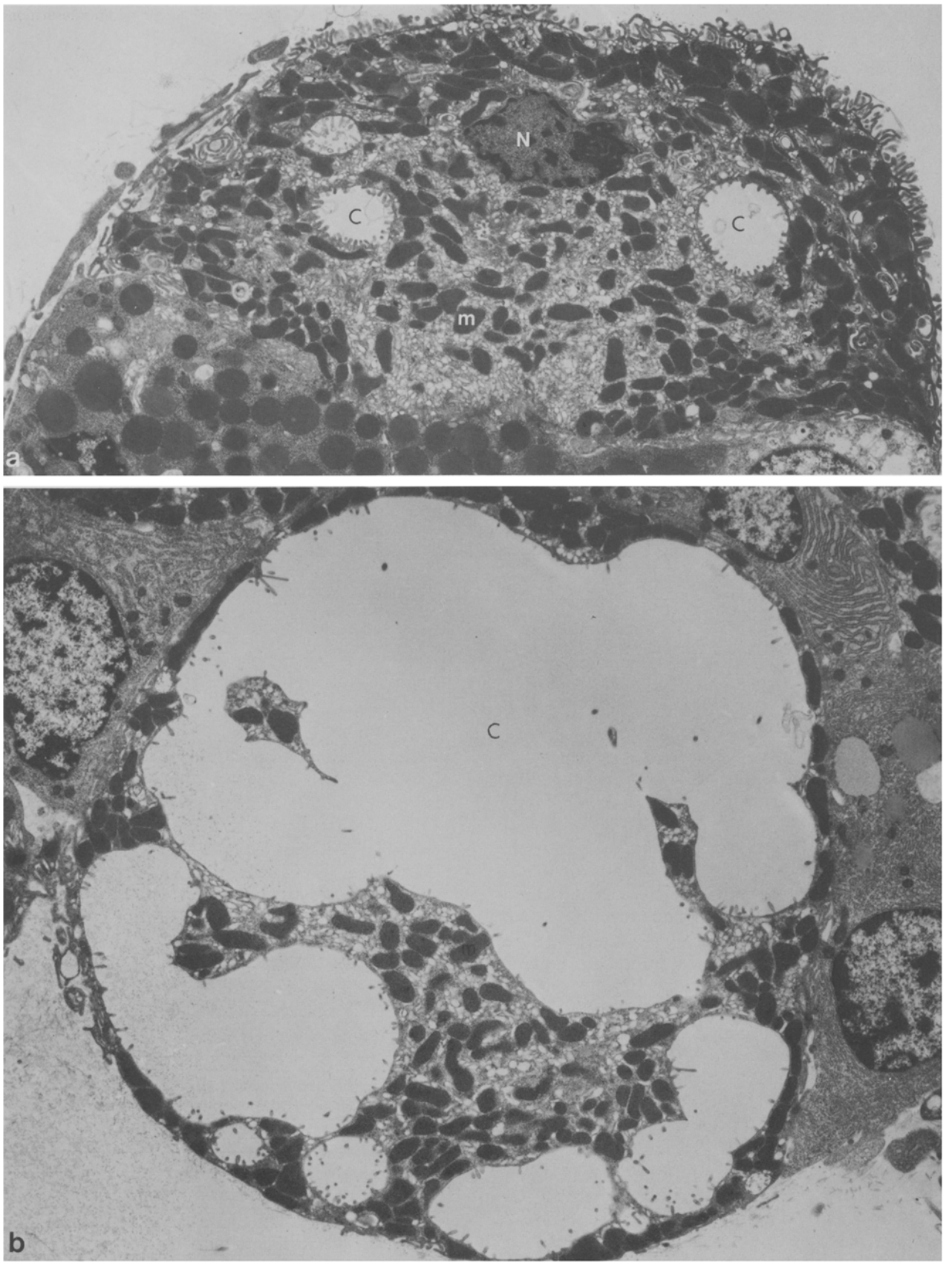

Fig. 5. Electron micrographs of parietal cells exposed to 108 mM K^+ medium. (*a*): A parietal cell exposed to high-K^+ only shows some distention of the canaliculus (*c*) compared to normal-K^+ conditions. (*b*): A parietal cell in high-K^+ medium after 5 min of incubation with 1 mM AP when ballooning of the canaliculus is obvious. Note the persistent appearance of tubulovesicles in *b*. Magnifications are 8800× for *a* and 6400 for *b*

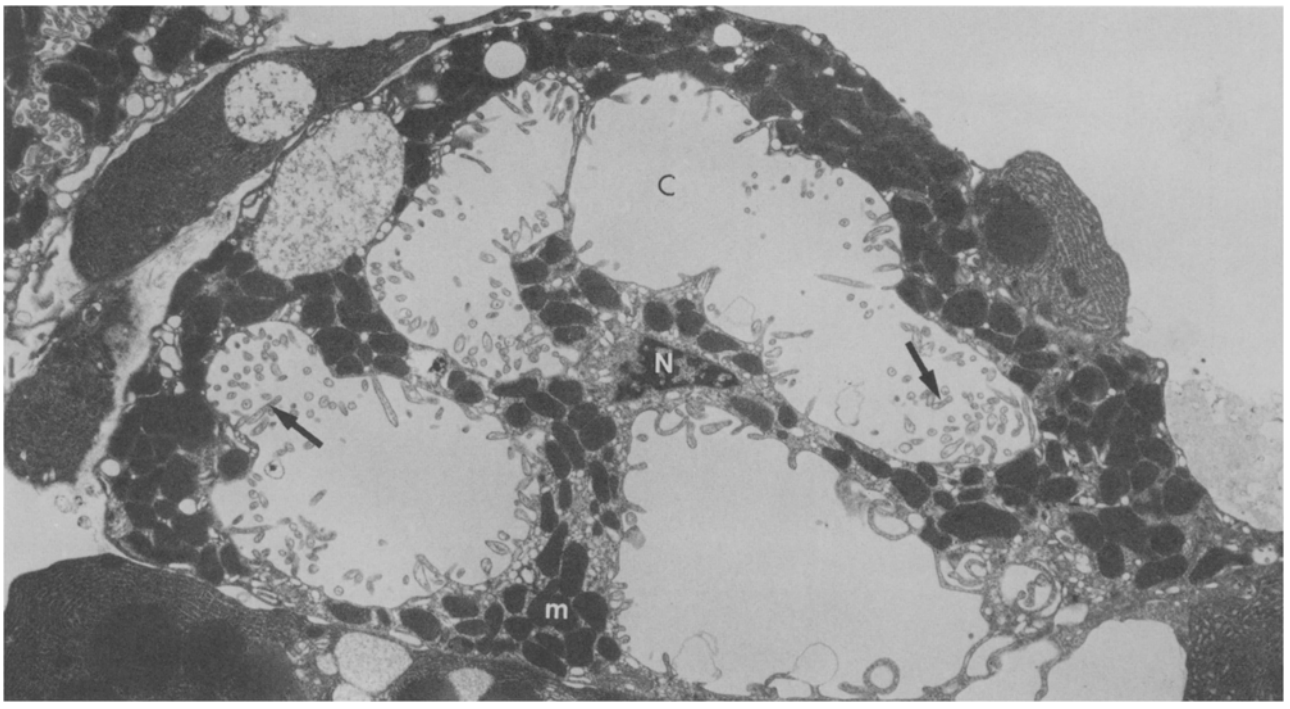

Fig. 6. Electron micrograph of a parietal cell in high-K^+ medium after 3 min of incubation with 10^{-3} M aminopyrine. Microvilli (arrows) are numerous. The canaliculus (*c*) is moderately dilated. 6450×

profiles and little canaliculus. However, unlike resting cells in normal-K^+ medium, cells in high-K^+ medium accumulate AP with AP ratios which can approach those found with histamine stimulation [1, 9]. Since these cells do not contain extensive secretory canaliculi, the exact site of acid, and thus AP, accumulation has not been identified. In analogy with the experiments of Berglindh et al. [1], we have attempted to identify the site of AP accumulation by raising the AP concentration to a level that would cause significant osmotic swelling of those structures that accumulate the weak base. Major differences in this study are that we have performed quantitative measurements and also have examined several time points following AP addition. In agreement with the previous work [1], we found that addition of 1 mM AP to glands incubated in high-K^+ medium resulted in dramatic changes in the appearance of parietal cells (Fig. 5*b*). As in the case of histamine stimulation, the changes were rapid and complete within 5 min of the AP addition. At 5 min the most conspicuous change was the massive distension of the secretory canaliculus, which appeared almost devoid of microvilli. At earlier times the canaliculus appeared distended but microvilli were more numerous (Fig. 6). One might envisage the change as involving a distension of the canaliculus with progressive flattening and disappearance of the microvilli. Superficially, except for the reduction in microvilli, the cells exposed to high-K^+ and 1 mM AP resembled the histamine-stimulated cells. To this extent, our results agree with the observations of Berglindh et al. [1].

Morphometric analysis of the high-K^+, high-AP condition revealed some distinct differences between this treatment and histamine stimulation. A comparison of the changes in canalicular volume fractions with the two treatments (Fig. 7) showed that the increase with histamine stimulation was substantially greater. This difference was due in part to a larger initial canalicular volume fraction with high-K^+ treatment and in part to a larger final volume with histamine stimulation compared to the 1 mM AP addition. A more striking difference between the two treatments was found in the analysis of surface densities. As shown in Fig. 8 and Table 2, addition of 1 mM AP resulted in only a small increase in canalicular membrane surface density and no significant change in tubulovesicular surface density. The increase in canalicular membrane surface density was less than half that found with histamine stimulation and appeared to be transient, reaching a maximum at 2 min and then declining. The tubulovesicular membrane surface density remained constant over the treatment period. However, it was noted that the value at zero time was substantially less than that found in normal medium at zero time (Table 1) and similar to the surface density seen after 5 min of histamine stimulation. A partial explanation for this difference may lie in the

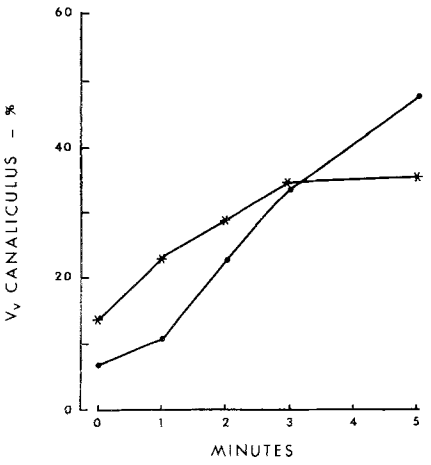

Fig. 7. Time course of changes in canalicular volume following addition of histamine or AP. Volume fraction (V_v) of canaliculus is expressed as percent of parietal cell cytoplasm. Histamine (1×10^{-4} M) plus IMX (1×10^{-5} M) was added at zero time to glands incubated in 5.4 mM K^+ medium (·——·). AP (1×10^{-3} M) was added to glands incubated in 108 mM K^+ medium (*——*). Time courses are similar for the two treatments, but histamine-stimulation results in a larger change

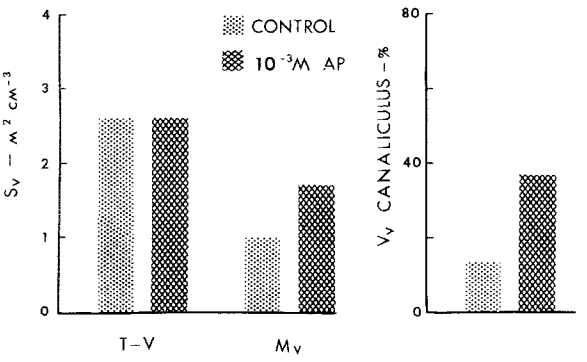

Fig. 8. Morphometric analysis of parietal cells exposed to high-K^+ medium and 1 mM AP. Surface densities (S_v) for tubulovesicular ($T-V$) and canalicular (M_v) membranes and volume fraction (V_v) of canaliculus were estimated in parietal cells exposed to 108 mM K^+ (control) and cells after addition of 1 mM AP. Significant differences were found for canalicular surface density and volume fraction but not for tubulovesicular surface density (*see* Table 2)

Table 2. Time course of morphological changes after addition of 1 mM AP in high-K^+ medium

	Time after AP addition (min)				
	0	1	2	3	5
$S_v(m^2/cm^{-3})$					
Canaliculus	1.0 ± 0.2^a	1.6 ± 0.5^b	2.1 ± 0.7^c	1.6 ± 0.1^b	1.7 ± 0.1^b
Tubulovesicles	2.6 ± 0.4^a	$25.\pm0.4^a$	2.5 ± 0.4^a	2.6 ± 0.5^a	2.6 ± 0.3^a
$V_v(\%)$					
Canaliculus	$14\ \pm7^a$	$23\ \pm9^{ab}$	$29\ \pm10^b$	$35\ \pm6^b$	$36\ \pm10^b$

Values are mean ±SE for $n=4$ (No. of animals). Means with no superscript in common are significantly different; $P=<0.05$.

cellular swelling produced by the exposure to high-K^+ concentrations (*see* below). In any case, the morphometric analysis demonstrates that the initial impression of similarity between histamine stimulation and treatment with high-K^+ plus high-AP is not quantitatively accurate. Not only the time course but also the values of canalicular volume fractions, canalicular surface densities, and tubulovesicular surface densities are different for the two treatments. The key observation was that tubulovesicular surface density did not change with high-AP treatment, since this change is a fundamental feature of the morphologic transition observed with histamine stimulation.

Additional differences were noted between histamine-stimulation and high-K^+ treatment. These differences are related to the apparent swelling of cells during exposure to high K^+ concentrations. A comparison of the ultrastructural appearance of the cytoplasm in the two conditions illustrates these differences (Fig. 9). In normal medium, the nonstimulated cell (Fig. 9*a*) was characterized by tightly packed tubulovesicles and moderately dense mitochondria. The free cytoplasm contained numerous ribosomes and microtubules. Microfilaments were found in a regular arrangement within the microvilli and in the cytoplasm adjacent to the surface membrane. Histamine-stimulation (Fig. 9*b*) resulted in the appearance of a reduction in free cytoplasm and tubulovesicles with dense mitochondria occupying most of the cellular space. Cells exposed to 108 mM K^+ (Fig. 9*c*) showed two obvious differences from those in normal medium. First, the cytoplasm appeared more amorphous and the tubulovesicles were less densely packed. Secondly, the mitochondria showed obvious signs of swelling, including separation of cristae. This general appearance was retained following addition of 1 mM AP (Fig. 9*d*) where the major change was expansion of the canaliculus. Swelling with the high-K^+ treatment would be of some significance and we, therefore, sought to provide quantitative verification. A direct,

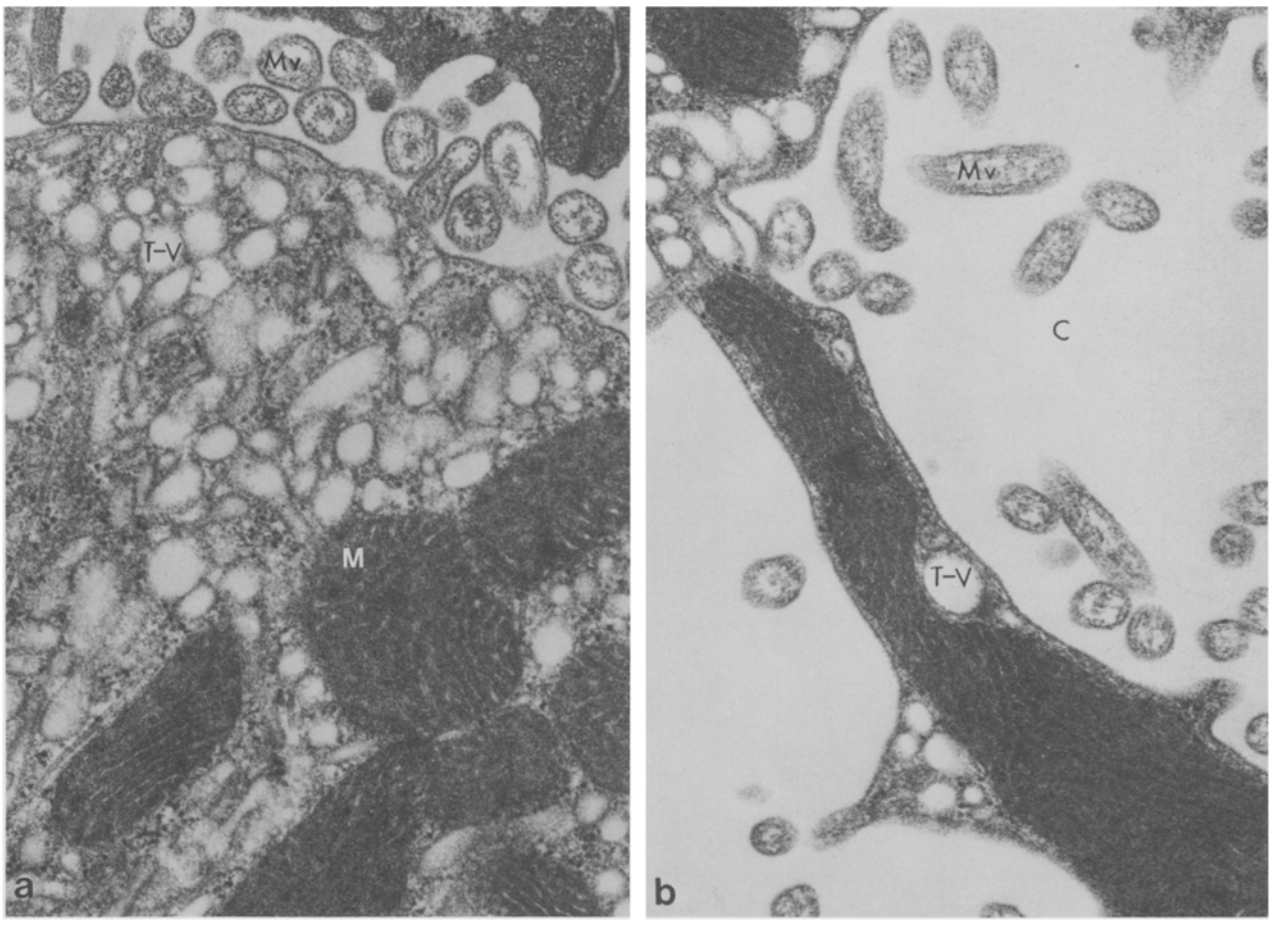

Fig. 9 (*a* and *b*). Electron micrographs showing a comparison of the appearance of parietal cell cytoplasmic structures with different treatments. (*a*): Cytoplasm of an unstimulated cell in normal medium. Numerous tubulovesicles (*tv*) are present. The mitochondria (*M*) appear compact. Microvilli (*Mv*) can be seen in cross-section at the luminal surface of the cell. (*b*): Cell after 5 min histamine stimulation.

though not very sensitive, estimate was obtained by measuring intraglandular water content. As shown in Table 3, high-K^+ treatment resulted in a small ($\sim$15%) but significantly greater glandular water content. The parietal cell water content may be elevated even more than the glandular water content since there was no microscopically observable swelling of chief cells. Morphometric analyses (Table 3) provided support for the notion that parietal cells swell in high-K^+ medium and indicated that the swelling was relatively uniform among the cellular organelles. Comparison of cross-sectional areas, of tubulovesicular profiles in normal-K and high-K media showed that the average value was significantly greater with high-K^+ treatment, indicating that these structures became enlarged. The observation that there was no difference in tubulovesicular volume fraction between normal and high-K^+ media suggests that swelling of these structures was proportional to total cell swelling. The small changes in volume fraction of mitochondria and free cytoplasm suggest that these cellular compartments did change somewhat, relative to the cell volume. Thus, the smaller mitochondrial volume fraction in high-K^+ medium indicates that these organelles are able to volume regulate better than the cell as a whole. Since electron micrographs show the mitochondria to be visibly swollen in high-K^+ medium, the lack of an increase in their volume fraction provides additional evidence that the entire cell is swollen.

Microscopic examination of cells treated with high-K^+ plus 1 mM AP showed expansion of the canaliculus, thereby identifying those structures as a site of acid accumulation. However, the question of whether acid also accumulates in the tubulovesicles could not be answered by simply inspecting micrographs since there was no obvious expansion of structures which could be identified as tubulovesicles. In order to determine if some swelling did occur, even transiently, following AP addition, the cross-sectional areas of tubulovesicle profiles were measured at various times after AP addition. Figure 10 shows the

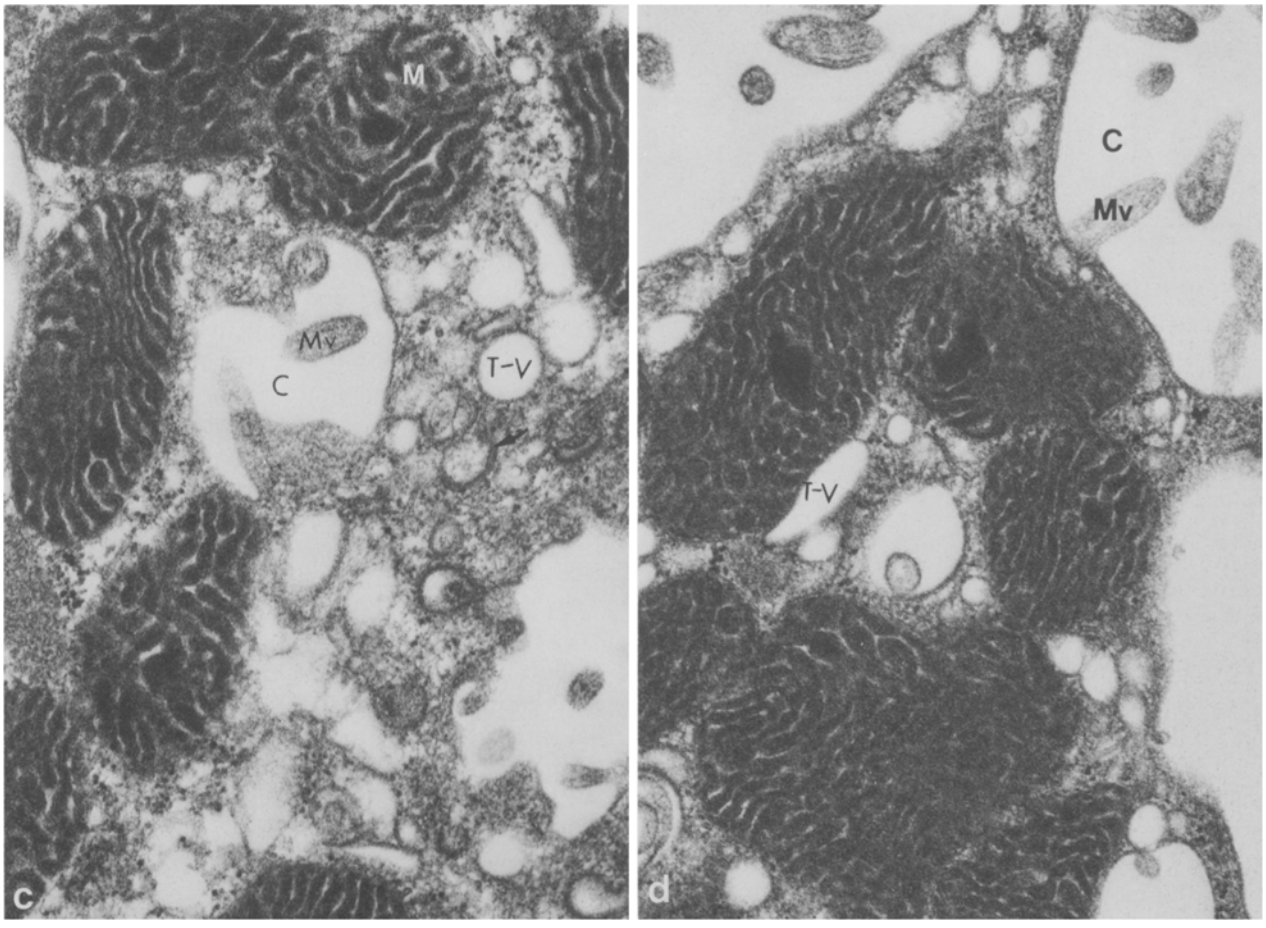

Fig. 9 (*c* and *d*). Microvilli and canaliculus (*C*) are increased, tubulovesicles reduced. (*c*): Cell incubated in high-K^+ medium. Mitochondria appear swollen. Several pentilaminar structures (arrows) can be seen. Tubulovesicles appear less numerous than in *a*, and some canalicular space (*C*) is present. (*d*): Cell incubated in high-K^+ medium 5 min after the addition of 10^{-3} M aminopyrine. The appearance of the cytoplasm is similar to that in *c*, but the canalicular space is increased. 76,000 ×

Table 3. Comparison between 5.4 mM K^+ and 108 mM K^+ media

	Intraglandular water content ($\mu l\ mg^{-1}$)	Tubulovesicular cross-section area (μm^2)	Volume fraction (%)		
			Tubulovesicle	Mitochondria	Cytoplasm
5.4 mM K^+	1.2±0.2	0.012±0.0005	15.3±1.9	43.7±2.5	41.0±2.5
108 mM K^+	1.5±0.2[b]	0.019±0.0007[a]	14.3±2.2	36.3±3.3[a]	49.5±2.4[a]

Values are mean ±SE for $n=4$ (No. of animals).
[a] $P<0.05$ by unpaired *t*-test; [b] $P<0.05$ by paired *t*-test.

distribution of profile areas and gives the mean value for each time point. There was no significant difference in mean values nor did there appear to be a shift in the population as a function of time.

Discussion

Isolated gastric glands are a relatively new preparation, which appears to offer significant advantages for studying the cellular mechanism of gastric acid secretion. The present study demonstrates that gastric glands are highly responsive and quite suitable for investigating the morphological transition that accompanies the onset of acid formation. Pretreatment with low doses of histamine H_2-receptor antagonist, cimetidine, resulted in a uniformly resting appearance of the parietal cells, and upon addition of histamine, virtually 100% of the parietal cells showed a transition to the stimulated state. The observed increase

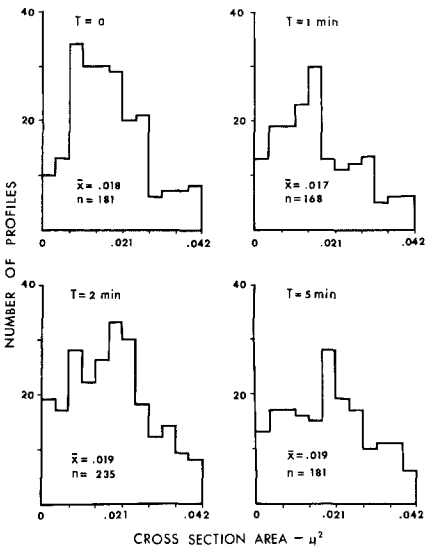

Fig. 10. Distribution of tubulovesicular profile areas. Glands were incubated in high-K^+ medium and 1 mM AP added at zero time. The cross-sectional area of tubulovesicles was estimated from high magnification ($\sim 60{,}000\times$) electron micrographs. The number (n) of profiles measured and the mean value ($\bar{x}$) is given for each time point. There is no significant difference between any of the means

in canalicular surface and decrease in tubulovesicular surface indicates that the transition is similar, if not identical, to that observed in more intact preparations [7, 8, 10]. The mechanism underlying this rearrangement is not understood fully, but additional insights were obtained in the present study.

The ability to obtain multiple samples at precisely timed intervals is a distinct advantage of the gland preparation and allowed us to establish a time course for the morphological changes. Statistically significant changes in membrane surface densities were detected within 1 min of histamine addition and the transition was essentially complete within 5 min. Allowing for some diffusion delay, it is clear that histamine initiates cellular events very quickly after binding to its cellular receptor. The observation that the morphological transition is complete before AP accumulation reaches its maximal value indicates that the morphological change is independent of acid accumulation. This is consistent with previous observations where a dissociation between acid formation and morphological appearance has been demonstrated [4, 6, 9, 14]. Thus, it would appear that the expansion of the secretory canaliculus is not due simply to the accumulation of isosmotic hydrochloric acid. Instead, another force, either mechanical or osmotic, must account for the rapid morphological transition.

Currently, two models have been proposed to account for the morphological transition, the "fusion" model and the "expansion" model. As illustrated in Fig. 11, the "fusion" model [7, 8] postulates that the tubulovesicles are discrete membrane-bound structures which are numerous in the nonstimulated state. Upon stimulation, elements of the tubulovesicles are incorporated into or fused with the canalicular membrane. Thus, in the "fusion" model reciprocal changes in tubulovesicular and canalicular membrane are predicted. Indeed, the observation of such a reciprocal relationship between these membrane components is the strongest evidence for the "fusion" model. It should be noted that the model does not depend upon a particular mechanism for the incorporation of tubulovesicular membrane into the canalicular membrane. Specific models which postulate an exocytotic-type fusion of entire vesicles with the surface [7, 8] have little evidence to support them [10]. In the present study, examination of over 1800 micrographs obtained at various time points during the morphological transition failed to show any definite examples of fused vesicles. One would expect to see many fusion profiles, particularly at the early times, if the transition involved fusion of entire tubulovesicles with each other or with the canalicular surface. Failure to observe fusion profiles suggests that incorporation of membrane into the canaliculus does not involve a classical exocytotic mechanism. Regardless of the exact mechanism for fusion, the incorporation of tubulovesicles into the secretory canaliculus could account for the morphological transition without requiring actual secretion of hydrochloric acid.

The "expansion" model, as proposed by Berglindh et al. [1] postulates that the tubulovesicles constitute a collapsed system of tubules in the resting state (Fig. 11). These tubules are postulated to be continuous with one another and to connect with the pre-existing canalicular space. The connections with the canaliculus are thought to be restricted and few in number since continuity between tubules and canaliculus has not been demonstrated [7, 10]. Transition to the stimulated state, according to this model, is due to osmotic expansion of the tubulovesicle system with a concomitant opening of the connections between the tubules and the pre-existing canaliculus. In addition to the continuity of the tubular system, the "expansion" model also proposes that the initial event in the morphological transition involves an increased flux of KCl from the cytoplasm into the tubular lumen. Volume flow associated with the KCl flux would result in tubular expansion without requiring HCl secretion [1]. Subsequent exchange of K^+ for H^+ would result both in maintaining the tubular expansion and in acid rather than KCl secretion. While the proposed tubular continuity is a fundamental requirement of the "expansion" model, the postulated increase in KCl flux is compatible with either the

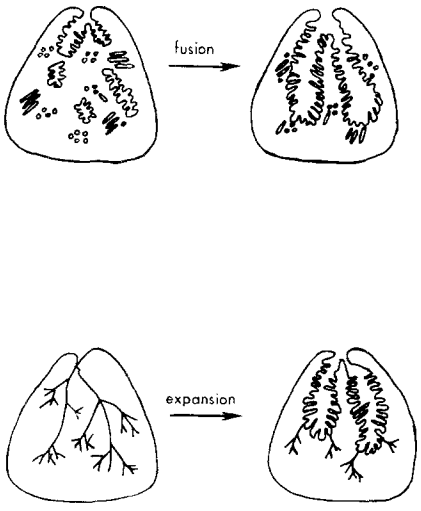

Fig. 11. Schematic representation of current models for the morphological transition. The fusion model (top) postulates that tubulovesicles are discreet elements which become incorporated into the secretory canaliculus. The expansion model (bottom) postulates a continuous membrane system which undergoes osmotic expansion (*see* Ref. [1])

"expansion" or "fusion" model and thus, is not fundamental to either one.

Primary support for the "expansion" model rests on the observation that osmotic swelling due to accumulation of AP in acid-forming spaces results in an appearance similar to the stimulated state [1]. However, no direct connection of the tubules with the canaliculus has been demonstrated nor has the tubule system been shown to be continuous. In the present study, the critical observations that; (i) the tubulovesicular surface density does not decrease following addition of 1 mM AP in the presence of high-K^+; and (ii) the tubulovesicles do not undergo expansion, would seem to argue against the "expansion" model for the morphological transition. The lack of change in surface density of the tubulovesicular membrane indicates that these structures are not being incorporated into the canalicular surface either by expanding or fusing under these conditions. Thus, the high-K^+, high-AP treatment does not appear to result in a normal morphological transition at all. Rather, the appearance of the stimulated state, as illustrated in Fig. 12, results from an osmotic expansion of pre-existing canaliculus. The enlarged canaliculus dominates the appearance of the cell giving the impression that tubulovesicular membrane is decreased when, in fact, it has not changed. The obvious expansion of the canaliculus should result in expansion of all of those elements which are continuous with the canaliculus. Failure to observe expansion of the tubulovesicles provides evidence that these structures are closed to the canaliculus. The morphometric measurements, therefore, provide evidence against the concept that

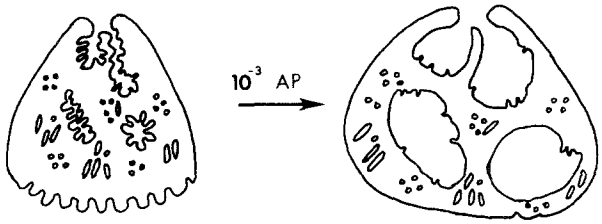

Fig. 12. Schematic representation of changes following AP addition to cells in high-K^+ medium. Exposure to high-K^+ results in cell swelling while addition of 1 mM AP results in ballooning of the pre-existing canaliculus. The amount and size of tubulovesicular elements are not altered by exposure to 1 mM AP

the tubulovesicles maintain a connection with the canaliculus or that such a connection can be induced by osmotic swelling of the canaliculus. Thus, the fundamental premise of the "expansion" model, i.e., tubular continuity, does not seem to hold. Furthermore, the experimental observations leading to the "expansion" model appear to have been misinterpreted.

The observed swelling of the canaliculus induced by AP under high K^+ conditions identifies these structures as a site of acid formation. As pointed out by Berglindh et al. [1], the accumulation of AP to an osmotically significant level requires, not only the presence of acid, but that hydrogen ions that are neutralized by the weak base must be continuously replaced, i.e., secretion of acid must be actually occurring. Since the addition of 1 mM AP in normal (5.4 mM) K^+ medium does not lead to canalicular expansion, the presence of high-K^+ must somehow activate hydrogen ion secretion in the canalicular membrane. The mechanism of this activation is unknown at present but may be due the high-K^+, resulting in achievement of a critical concentration of K^+ at some site in the canalicular membrane. This hypothesis would be consistent with observations on vesicles isolated from gastric mucosa where the rate of hydrogen ion transport is limited by the availability of K^+ at the interior surface of the vesicle membrane [11], i.e., the luminal surface of the canaliculus. In high-K^+ medium, increased K^+ availability at the luminal surface of the canaliculus could be due to an increased flux from the cytosol or to direct access from the gland lumen. The observation that ouabain, which reduces cytosolic K^+, inhibits AP accumulation in high-K^+ medium [1] suggests that direct access from the gland lumen is not a sufficient explanation of the high-K^+ activation and some influence of cytosolic K^+ must be required.

The failure to observe swelling of the tubulovesicles upon treatment with 1 mM AP demonstrates that continuous acid secretion does not occur in these structures. Since the tubulovesicles do not form acid in either normal or high-K^+ medium, it is reasonable

to speculate that these membranes lack some factor which is present in canalicular membranes. Wolosin and Forte [13] have suggested that this factor may relate to a K^+ permeability of the membrane. However, the present study suggests that tubulovesicles normally are permeable to K^+ and that some additional factor is responsible for the lack of acid formation. Thus, morphometric studies showed that in high-K^+medium the tubulovesicles swell in proportion to the cellular swelling. The simplest explanation for this swelling is that the K^+ content of the tubulovesicles has increased in proportion to the increase of cytoplasmic K^+ content. Such an increase in K^+ should lead to activation of the hydrogen ion pump in these structures if K^+ availability is a sufficient condition. The interpretation of these findings must be made with caution for at least two reasons. First, the swelling of tubulovesicles in high-K^+ medium does not prove that these structures are permeable to K^+, and second, treatment with high-K^+ medium may not reflect a physiologically normal state. Clearly, the stimulated appearance of cells exposed to high-K^+ plus 1 mM AP is not an adequate model for studying the normal morphological transition. In addition, the observation that acid formation induced by exposure to high-K^+ medium is not associated with increased oxygen consumption [9] suggests that the mechanism of acid formation by the secretory canaliculus may differ from that which occurs during secretagogue stimulation. Thus, while treatment of glands with high-K^+ may yield useful information, the relationship between this treatment and normal physiological states remains uncertain.

The morphometric analyses presented in this study provide challenging arguments against the "expansion" model as proposed by Berglindh et al. [1]. However, the results do not provide direct evidence in favor of the "fusion" model. While most of the data are consistent with the hypothesis that tubulovesicular membrane is incorporated into the canalicular surface, this incorporation must be accompanied by some fundamental change in the properties of the membrane so as to initiate acid secretory activities. A simple fusion of tubulovesicles with the surface appears to be insufficient to account for the functional transformation. Definitive evidence favoring the "fusion" model must go beyond disproving alternative models. Ideally, one would like to demonstrate the enzymatic basis for membrane incorporation and identify the functional changes associated with this event. Such evidence would support a detailed model for the morphological transition and provide a clearer understanding of the role of the transition in regulation of hydrogen ion transport by the parietal cell.

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