TEM Morphometry Reveals Membrane Deficits in Parietal Cells Lacking Specific Ion Transporters

Miller ML, Gawenis LR, Andringa A, Shull GE
University of Cincinnati, College of Medicine
millermn@email.uc.edu

Morphometry

The late 1940s marked the appearance of the first publications using transmission electron microscopy (TEM) and by 1950, with improved embedding and sectioning methodology, reports began to include mammalian tissues (PubMed). However, 15 years lapsed before morphometry and stereology were integrated into TEM studies (Loud 1965; Elias 1971; Weibel 1969). The utility of a quantitative approach to electron microscopy was obvious, but its popularity has waxed and waned considerably over the last half century; its staunch supporters, however, have stayed the course (Weibel 2001; Diaspro 2004). Early computers, though slow and unreliable, brought some degree of automation to morphometry in the 1980s, but did little to ease tedium or increase accuracy (Caruntu 2002). Even now, neither free, nor high-priced software tangibly increases efficacy in discriminating complex images over the original manual methods, at least when applied on a limited scale. In addition, dedicated image analysis software has been somewhat inflexible, especially since each new tissue and experimental design requires some adaptation of the methodology. High-end graphics programs and embedded scripting language offer greater potential for accurate automated image analysis than typical image analysis programs (Okunade et al, 2004).

Despite these factors, classic 'region-of-interest' morphometry, which defines finite anatomical objects in a selected space, continues to be incredibly valuable for quantifying subtle ultrastructural events, as exemplified in the study of parietal cells of transgenic mice in which individual ion transport genes have been targeted for deletion (Shull, et al., 2000, Spicer et al., 2000; Gawenis et al., 2004a, Schultheis et al., 1998a, Schultheis et al., 1998b, Bell et al., 1999). The numeric data derived can be compared with other quantitative data on blots, microarrays, gels, *etc.*, to produce integrated information about the effects of the loss of a single gene *in vivo*. Morphometry provides invaluable contributions to histological phenotyping, diagnosis, and prognostication in research and clinical situations (Gil et al., 2002: Miller et al., 2002; Diaspro 2004).

One approach is to use grids for point-counting which can be applied to micrographs using a printer, or digital images can be overlain with a virtual grid in PhotoShop. The test grids should produce the best compromise between the ease of counting and the frequency and size of the objects being analyzed, and can be derived from statistical analysis of a sample data set. Images and data can be comfortably managed using individual dedicated software programs such as PhotoShop (image preparation), SigmaScan (digitizing and measuring), Excel (data management and sorting), SAS (statistical analysis), SigmaPlot (graphing and plotting), Corel Draw (figure production with vector and raster and exporting for publication) and PowerPoint (presentation). When used in this order, these programs offer considerable inter-program compatibility, and have superior flexibility when compared to programs that try to handle all aspects of image quantification in the same program.

Parietal cells

Parietal cells are stimulated to secrete acid into the gastric lumen in response to vagus nerve stimulation, and the release of gastrin and histamine. Secretion is a highly organized process, requiring numerous transporters for the import and export of ions (especially H^+, K^+, Na^+, Cl^- and $HCO3^-$), which are all strategically located in the apical, basolateral or cytoplasmic membranes. Balanced ion exchange is essential for maintaining secretory function, structural integrity, and cell viability (Shull et al., 2000; Gawenis et al., 2004b).

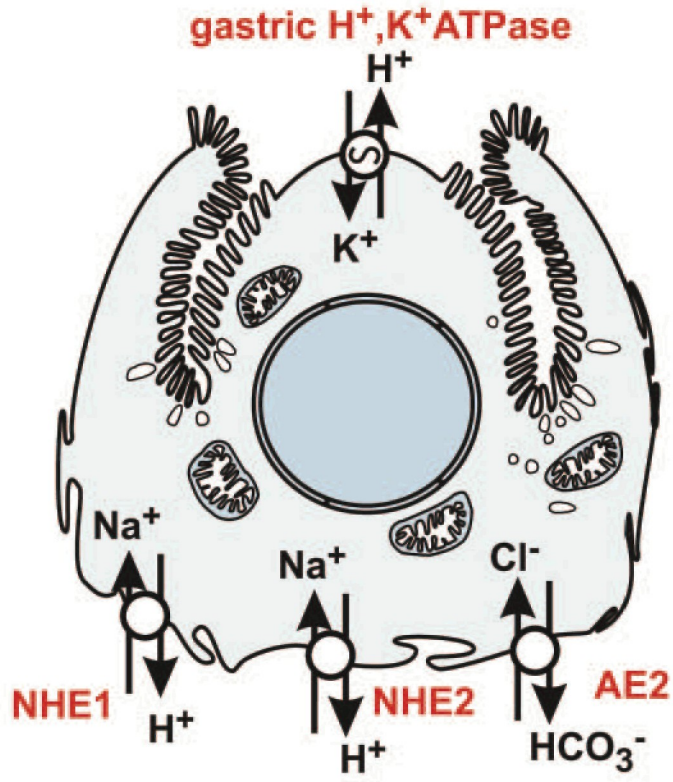

Figure 1. A model parietal cell shows the membrane locations of the gastric H+,K+ATPase -/-, NHE1, NHE2, and AE2.

Standard morphometric principles have been applied to electron microscopic profiles of parietal cells from at least a dozen different knockout (KO), double KO, and transgenic mice (a few of which are shown in Table 1). Because of the involvement of all membrane compartments with some aspect of acid secretory activity or potential, all were quantified: membranes directly involved in secreting acid, such as the tubulovesicles, canaliculi and apical plasmalemma, as well as those involved in supporting acid secretion such as the basloateral membranes, round vesicles, Golgi, and the rough endoplasmic reticulum. The loss of

Table 1. Knockout Mouse Models

Gene	Function	Membrane compartments
gastric H^+,K^+ATPase - *Atp4a(-/-)*	ATPase, H^+,K^+ exchanger alpha polypeptide	secretory apical, canalicular and tubulovesicular
AE2 - *Slc4a2(-/-)*	Cl^-/$HCO3^-$ exchanger	basolateral
NHE1 - *Slc9a1(-/-)* NHE2 - *Slc9a2(-/-)* NHE3 - *Slc9a3(-/-)**	Na^+/H^+ exchanger	basolateral basolateral apical
Atp4a(-/-)/Slc9a2(-/-) - (double KO)	H^+/K^+ exchanger plus Na^+/H^+ exchanger 2	as above

**not expressed in parietal cells of the mouse, but this KO is used as an internal control for the effect of expression of the selectable marker (neomycin resistance gene) on the measurement of parietal cell membranes and mitochondria in the KO mice.*

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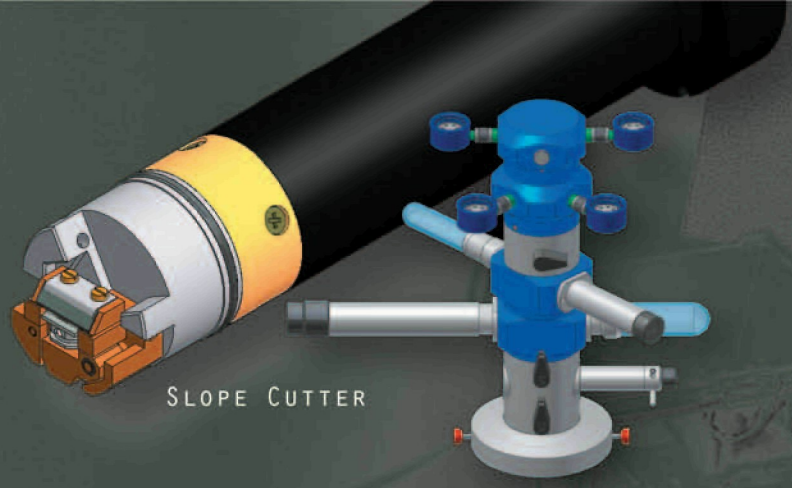

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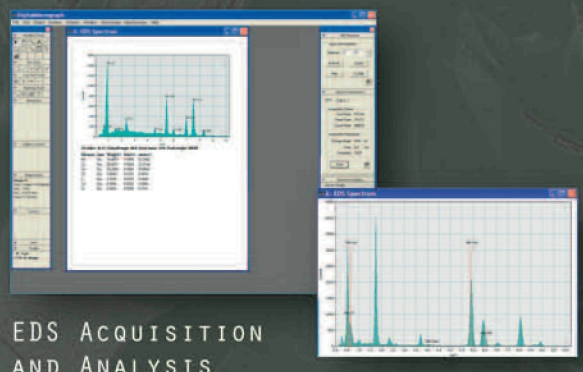

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individual transporters listed in Table 1 elicited quantifiable parietal cell changes specific for that transporter. Re-quantification of the micrographs was necessitated on numerous occasions by the identification of novel variations in the parietal cell structures that emerged with each new KO mouse model.

The gastric H^+,K^+ATPase (composed of α and β subunits) is located in the microvilli of apical and canalicular membranes and also in tubulovesicles; it is absolutely required for acid secretion (Spicer et al., 2000). The other exchangers and transporters affect acid secretion in a less direct manner but critically, nonetheless. AE2 provides for the uptake of chloride and extrusion of bicarbonate at the basolateral membrane to balance proton extrusion and provide Cl^- for secretion across the apical membrane (Figure 1). On the basolateral membrane, two of the nine known coupled Na^+/H^+ exchangers (NHE1 and NHE2; Bell et al., 1999 and Shull et al., 2000) are present, and NHE2 is critical for parietal cell homeostasis. NHE3 is an apical membrane transporter that is not expressed in mouse parietal cells (but is expressed in rat) and caused no significant morphometric changes from wild type parietal cells. The double KO (gastric H^+,K^+ATPase-/- and NHE2-/-) mice showed the extreme phenotype of the gastric H^+,K^+ATPase KO alone.

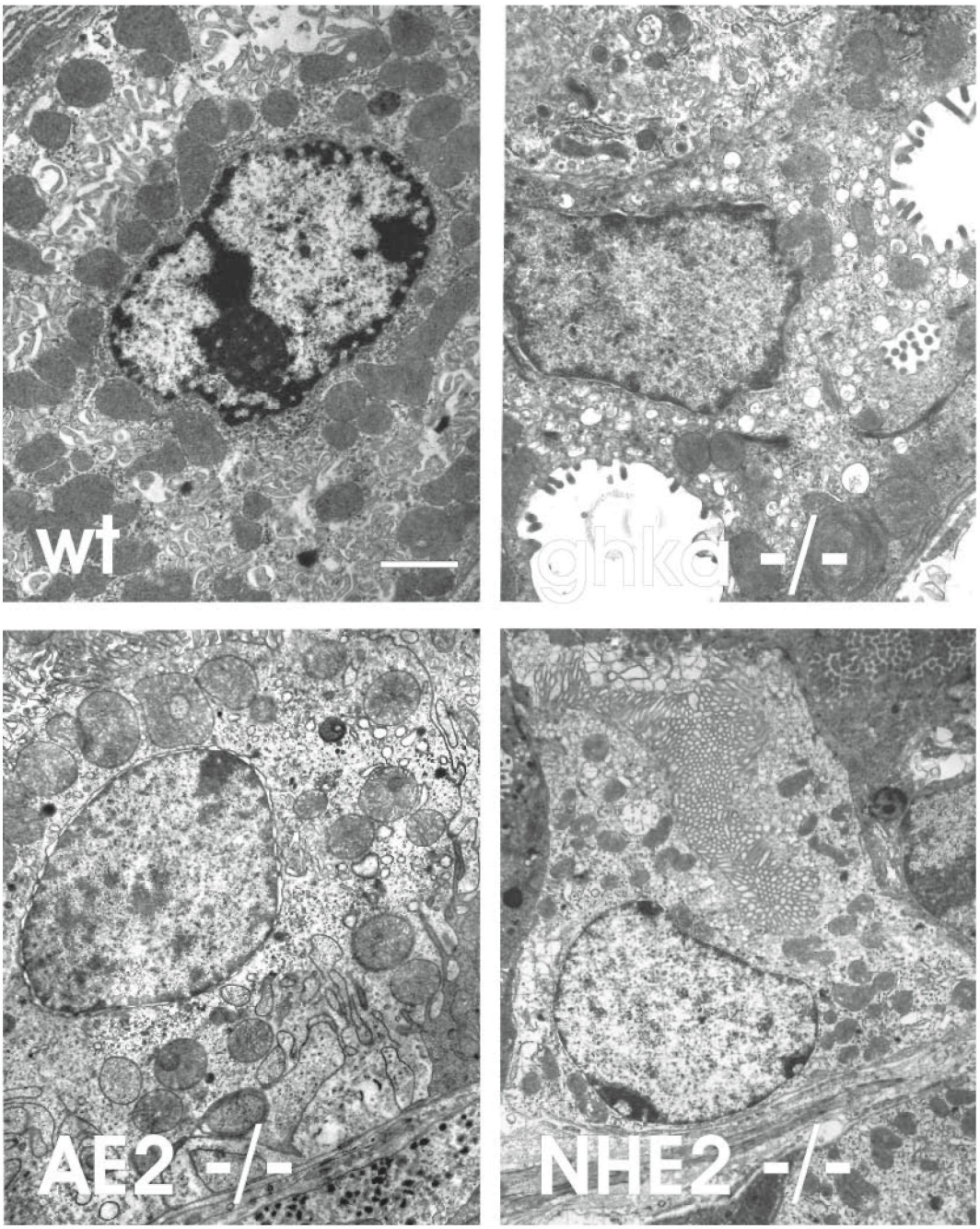

Figure 2. Parietal cells from a wild type mouse (wt), shows typical apical and basolateral membranes and a normal mitochondrial Vd. A parietal cell from a mouse lacking the gastric H+,K+ATPase -/- (gHKa-/-), has distinctly shortened microvilli on the apical membrane, as well as within the canaliculi. Vd of mitochondria is decreased also. A mouse lacking a basolateral chloride bicarbonate exchanger (AE2 -/-) tended to be hypoactive, showing fewer secretory membranes and decreased Vd of mitochondria. A parietal cell from a mouse null for a critical basolateral sodium/hydrogen exchanger (NHE2-/-) showed significantly greater ratio of canalicular to tubulovesicular membranes, long microvilli, and increased Vd of basolateral membrane. Bar=2 μm.

Canalicular and apical membranes composed of long undulating microvilli were more abundant during active acid secretion, while the volume density (Vd) of tubulovesicles was strongly increased during resting states. With the complete inability to secrete acid (Table 2, Figure 2), as in the case with the gastric H^+,K^+ATPase null mice, no typical secretory membranes were found. Instead, non acid-secretory canalicular and apical membranes composed of short microvilli replaced the typically long fluid parietal cell microvilli of wild type mice (Table 2). The former were virtually identical to those on adjacent (non-acid producing) mucous and zymogen cells. The gastric H^+,K^+ATPase constitutes a large percent of the secretory membrane's protein and its failure to be incorporated into membranes was evidenced as microvillar shortness, inflexibility and loss of attachment to structural proteins such as actin (Miller et al., 2002). Parietal cell microvilli in other KO mice were not so dramatically affected. In NHE2 null mice they tended to be longer than in wild type mice, though this was not statistically significant ($p<0.1$), it did parallel the apparent state of active acid secretion (low Vd of tubulovesicles). The Vd of canalicular and apical membranes of the NHE2 (-/-) mice were not significantly different from wild type mice, but because of their inability to balance ion transport these cells were highly vulnerable to a progressive dilation of the canaliculi, loss of homeostasis and death.

The Vd of round vesicular membranes increased when acid secretion or balance of transporters was compromised. The Vd of

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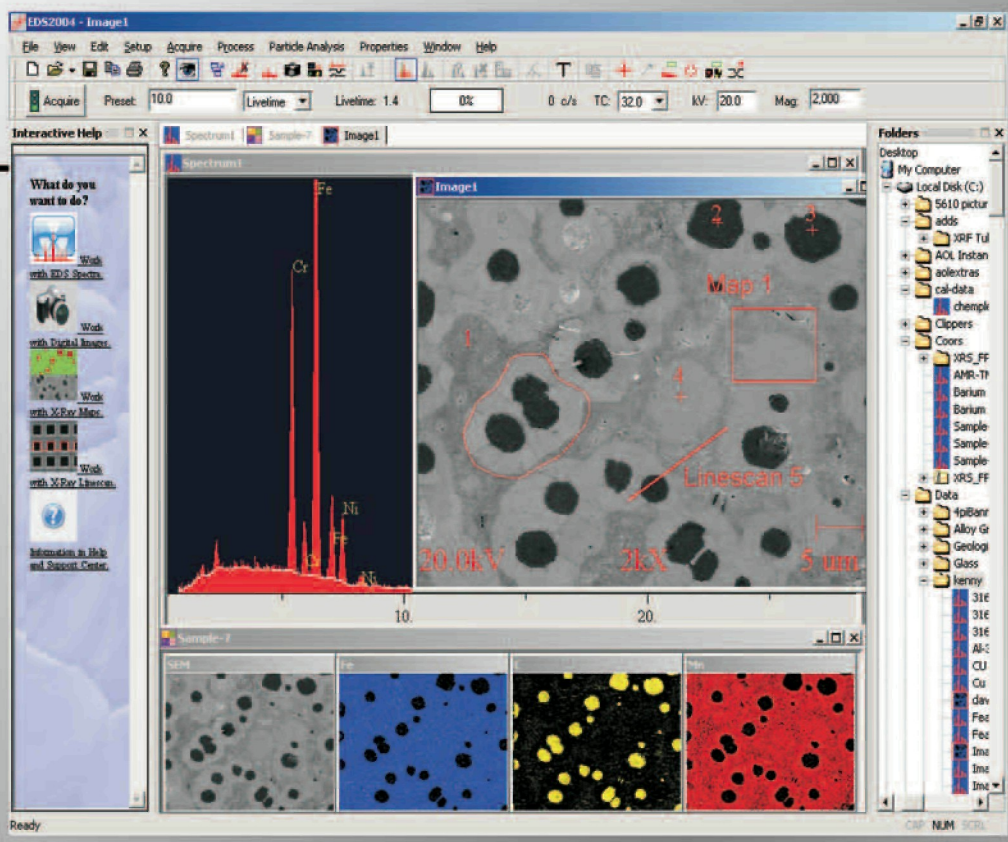

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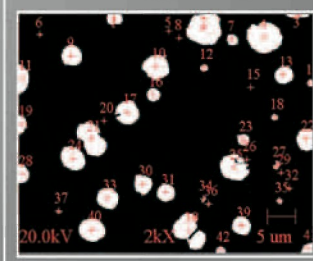

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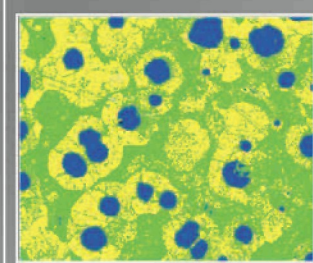

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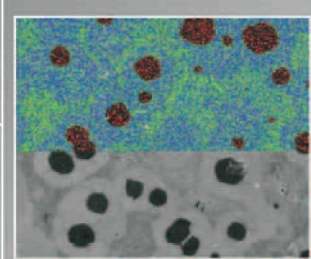

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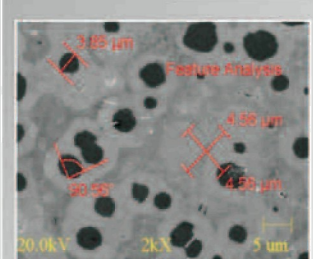

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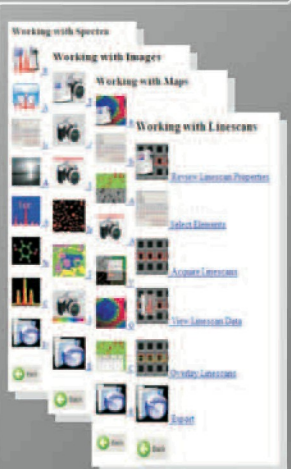

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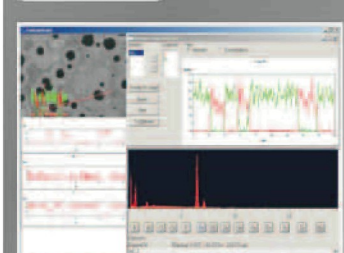

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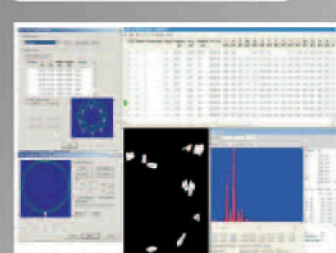

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Table 2. Secretory Membrane and Mitochondrial Volume Densities (Vd), and Microvillar Profile Length and Width (Mm) In Five Ko Mice And Their Wild Type Pairs

	Wild type n=29	NHE3 n=3	AE2 n=3	NHE1 n=2	NHE2 n=6	Gastric H^+,K^+ ATPase n=11
Acid Secreting Membranes (Vd)						
Canalicular and apical membranes	16+1	20+1	18+5	19+2.4	14+1	0.2+0.2*
Tubulovesicles	7.9+1.5	6.6+1.1	3.2+1	10+0.1	2.2+0.75*	0.4+0.2*
Total secretory membrane	30+1.6	27+1.08	22+4.4	30+2.39	17+1.1*	5.4+0.40*
Canalicular / tubulovesicular membrane^	5.7+4.4	3.2+0.4	8.9+5.6	1.9+0.25	15.3+8.1*	0.9+0.8+
Non-Acid Secreting Membranes (Vd)						
Non-secretory apical membrane	0.36+0.3	0	0.42+0.4	0	0	3.9+0.4*
Basolateral membrane	3+0.4	3+0.2	4.6+1	4+0.3	4.5+0.4*	5+0.7*
Round vesicles	0.15+.04	0.2+0.1	0.5+0.4	0.14+0.04	0.8+0.3	1.4+0.2*
Mitochondria (Vd)						
Mitochondria	30.2+1.1	34+11	23+0.2+	30+2.5	24+3*	21.9+1.8*
Microvilli (μm)						
Microvillar length	1.2+.034	1.12+.1	1.1+0.1	1.3+0.7	1.4+0.02+	0.5+0.04*
Microvillar width	0.27+0.1	0.31+.01	0.18+.01	0.27+.01	0.27+.01	0.16+0.0*

** Significantly different than wild type group p<0 .05: wild type group=age and gender matched pairs from each of the 5 KO groups*

+ Marginally significantly different than wild type animals p<0.09

^ The ratio of canalicular to tubulovesicular membranes is one indicator of secretory activity

the basolateral membrane, normally just a modest percent of the total membrane compartment, tended to increase during ion transport disturbances

While parietal cells in NHE1-/- mice had normal membrane and mitochondrial volume densities, and the profiles of microvilli were not statistically different from the wild type mice, the gastric mucosa overall was atrophic, and the adjacent lamina propria was significantly thickened.

The Vd of mitochondria mirrored the level of secretory activity of parietal cells and was reduced in the parietal cells producing less acid and where ion homeostasis was compromised (AE2, NHE2 or the gastric H^+,K^+ATPase -/-) (Table 2). No significant changes in Vd of membranes, mitochondria or in microvillar measurements were observed in parietal cells from NHE3 (-/-) mice, which supports the efficacy of these techniques.

Summary

The main function of the parietal cell, acid secretion, requires a balanced import and export of ions. Membranes involved in this process show both subtle and dramatic ultrastructural changes with the loss of specific ion transporters. The data reveal that unbalanced or compromised acid secretion, in general, significantly alters the Vd of the total secretory membrane pool, accompanied by a slight increase in Vd of the basolateral membrane compartment and a decrease in mitochondrial Vd. Depending upon whether the transporter is apically or basolaterally located had further ramifications for membrane alterations. Examining the histological phenotypes arising from gene targeting strategies significantly advanced the understanding of the functions of these transport proteins in parietal cells *in vivo* and has contributed to a more accurate understanding of how separate membrane compartments participate in the process of acid secretion. ■

Acknowledgements

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