ULTRASTRUCTURAL TOPOGRAPHY OF PARIETAL CELLS OF THE GASTRIC MUCOSA (MORPHOMETRIC INVESTIGATION)

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A new method is suggested for studying the ultrastructure of the parietal cells of the gastric mucosa: After an electron-microscopic study of the submicroscopic organization of cells at the three main levels of the principal glands (generative zone, zone of the most active cells, and fundus of the gland) a morphometric analysis of the results is made. Considerable differences in the ultrastructural morphometric indices of the parietal cells at different levels of the glands were revealed by the use of this method. It is suggested that despite its somewhat laborious nature, this approach can also be used to study other cells of the gastric mucosa and also many other tissues with a constant rapid rate of cell removal.

KEY WORDS: *Ultrastructural morphometry; parietal cells of the stomach; biopsy of the human stomach.*

The fact has now been repeatedly confirmed that cells arising during mitosis in the foveolae of the gastric glandular epithelium migrate either upward, when they are converted into surface cells, or downward, in which case they are transformed into chief and parietal cells. According to Lipkin [6] the life span of human parietal cells varies from 1 to several years. In rodents the entire population of parietal cells is replaced much more rapidly (3 months) [11] but, nevertheless, several times slower than renewal of the surface epithelium. Lawn [5] first pointed out that the ultrastructure of the parietal cells close to the generative zone differs considerably from the structure of the cells in the fundus of the gland. This was later confirmed by other workers [2, 3, 9]. However, despite its undoubted importance, more recent investigations of the ultrastructure of the parietal cells in different functional and pathological states have disregarded this fact. To assess the response of the parietal cells to various factors correctly the submicroscopic changes must be studied not only in the cells of the body of the gland (as most investigators have in fact done), but also in the parietal cells of the generative zone and fundus.

However, before the reaction of the parietal cells is studied in different functional and pathological states, it is necessary to know how the submicroscopic organization of these cells in the normal gastric mucosa varies during their migration from the generative zone into the body and from thence into the fundus of the glands. This problem was the subject of the investigation described below.

EXPERIMENTAL METHOD

The test material consisted of 30 biopsy specimens of unchanged gastric mucosa from people whose hydrochloric acid secretion was within normal limits. Pieces of tissue were fixed with a 4% solution of paraformaldehyde in phosphate or cacodylate buffer, postfixed with 1% OsO_4 solution, dehydrated, and embedded in Epon 812.

Sections from the various layers were cut by a specially developed method on the LKB ultramicrotome. The stomach tissue was so arranged in the block that the glands lay in the

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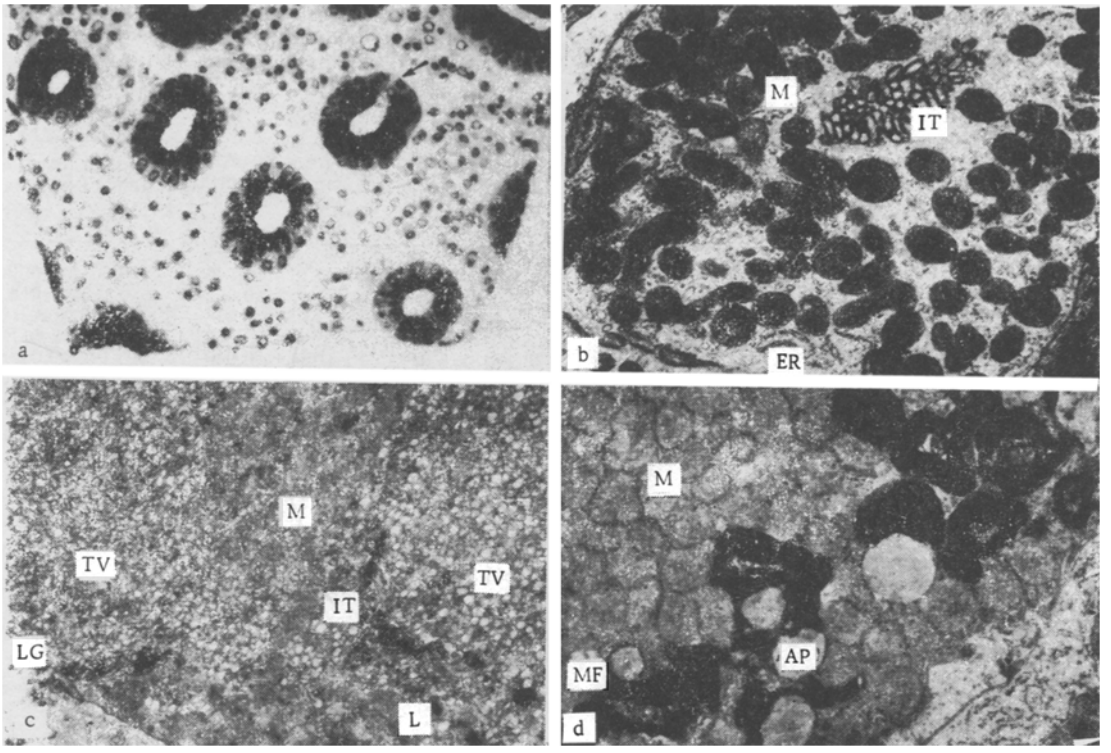

Fig. 1. Structure of parietal cells at different levels of principal glands of the stomach: a) semithin section 1 μ thick, arrow indicates appearance of first parietal cells at level of 100 μ from surface of mucose (toluidine blue; 100×); b) ultrastructure of immature parietal cell from generative zone of principal glands (8000×); c) ultrastructure of parietal cell from zone of most active (level 400-500 μ from surface of mucosa, 5000×); d) ultrastructure of parietal cell from fundus of gland; large number of autophagosomes and myelin figures in cytoplasm (10,000×). M) Mitochondria; ER) endoplasmic reticulum; IT) intracellular tubule; TV) tubulovesicles; L) lysosomes; AP) autophagosomes; MF) myelin figures (residual bodies); LG) lumen of gland.

transverse direction in the plane of the section. After a semithin section 1 μ thick had been cut and stained with toluidine blue, five or six glands were chosen, a pyramid trimmed above them, and a series of ultrathin sections cut and stained with uranyl acetate and lead citrate. A layer of material 20 μ thick was then cut off the block and a semithin section (Fig. 1a) and ultrathin sections were again prepared. The whole piece of tissue was cut into sections on the ultratome in this way down to the fundus of the glands (about 40 levels), with continuous observation of the semithin sections along the course of the same five or six glands.

After the material had been studied in the ÉVM-100L and IEM-7A electron microscopes the results were subjected to morphometric analysis by the method suggested by Yagubov and Kats [1], modified for our own purposes. Morphometry was carried out on electron micrographs under a magnification of 50,000 times, using a grid with a 10-mm step. To count the test points a specially made electromechanical probe connected to a pulse counter was used, and it greatly simplified the procedure of obtaining the primary morphometric indices. The following primary indices were obtained in this way: P) the number of test points on the grid; P_M) the number of test points falling on the profile of the mitochondria; P_L^M) the number of times the profiles of the mitochondria crossed the horizontal lines; P_L^{CR}) the number of times membranes of the cristae crossed the horizontal lines; N_{CR}) the number of cristae; P_E) the number of test points falling on the profile of the endoplasmic reticulum; P_L^E) the number of times the membranes of the endoplasmic reticulum crossed the horizontal lines; N_L) the number of lysosomes; P_L) the number of test points falling on the profile of the lysosomes; P_{TV}) the number of test points falling on the profile of the tubulovesicles; P_S^{TV}) the

TABLE 1. Changes in Ultrastructural Indices of Parietal Cells Depending on Their Position in Principal Glands of Human Stomach (M ± m)

Level of glands in cell, μ	Mitochondria				Secretory membranes
	area of mitochondria, S_M	surface area of cristae, S^{CR}	coefficient of fragmentation of cristae, K_F^{CR}	degree of swelling of mitochondria, V_V^M/S_V^M	level of development of microvilli, K_{MV}
100—150	27,17±0,44	0,893±0,096	0,645±0,080	1,073±0,046	0,061±0,001
200—300	38,43±0,429	0,929±0,07	0,610±0,038	1,183±0,055	0,195±0,018
400—500	43,03±0,328	1,131±0,073	0,503±0,057	1,256±0,046	0,208±0,021
600—700	36,48±0,377	0,895±0,081	0,611±0,034	1,056±0,048	0,164±0,017
750—800	32,40±0,322	0,849±0,059	0,613±0,047	0,934±0,049	0,102±0,013

Level of glands in cell, μ	Secretory membranes	Endoplasmic reticulum		Lysosomes	
	volume coefficient of tubulovesicles, K_{TV}	surface area of membranes of endoplasmic reticulum, S_V^E	degree of dilatation of cisterns of endoplasmic reticulum, V_V^E/S_V^E	number of lysosomes, N_L	area of lysosomes, S_V^L
100—150	0,153±0,036	0,390±0,10	0,345±0,021	0,9±0,10	6,32±0,253
200—300	3,73±0,127	0,168±0,059	0,253±0,028	2,033±0,225	17,49±0,467
400—500	16,29±0,254	0,057±0,08	0,257±0,029	3,2±0,163	27,53±0,573
600—700	9,38±0,174	0,046±0,027	0,210±0,026	5,16±0,211	49,02±0,885
750—800	4,59±0,139	0,039±0,029	0,157±0,028	6,3±0,272	93,99±1,61

number of test points falling on the area occupied by tubulovesicles; P_L^{MV}) the number of times the membranes of the microvilli of the intracellular tubules crossed the horizontal lines.

After primary parameters had been obtained from at least 30 cells the secondary indices were calculated as quantitative characteristics of the organelles of the parietal cells: $(P_M \cdot 100)/P = S_M$, the area of the mitochondria (in % of the total area of the cell); $P_M/P_L^M = V_V^M/S_V^M$, the degree of swelling of the mitochondria; $P_L^{CR}/P = S^{CR}$, the surface area of the mitochondrial cristae; $N_{CR}/P_L^{CR} = K_F^{CR}$, the coefficient of fragmentation of the cristae; $(P_L^E \cdot 10)/P = S_V^E$, the surface area of the membranes of the endoplasmic reticulum; $P_E/P_L^E = V_V^E/S_V^E$, the degree of dilatation of the cisterns of the ergastoplasm; $P_L^{MV}/P = K_{MV}$, the level of development of the microvilli; $(P_{TV} \cdot P_S^{TV})/P = K_{TV}$, the volume coefficient of the tubulovesicles.

The results of morphometric analysis of the parietal cells at 40 levels of the gland were pooled and reduced to five levels (zones): 100-150, 200-300, 400-500, 600-700, 750-800 μ from the surface of the gastric mucosa.

EXPERIMENTAL RESULTS

Immature parietal cells, the differentiation of which is not yet complete, were found in the generative zone of the neck of the principal glands, as the workers mentioned above described previously. They were characterized by weak development of the system of intracellular tubules, by the small number (or almost total absence) of tubulovesicles containing chlorides [4], by solitary lysosomes, and by a relatively well developed endoplasmic reticulum (Fig. 1b).

During migration from the generative zone into the depth of the glands the parietal cells acquired (quite quickly) features of maturity and increased functional activity. This can be judged from the increase in area of the membranes of the intracellular tubules and the increase in number of tubulovesicles. The number of lysosomes (both primary and secondary — dense bodies) increased a little, but the number of cisterns of the endoplasmic reticulum decreased.

At the level of 400-500 μ cells with morphological evidence of the highest functional activity were found (Fig. 1c), i.e., the intracellular tubules in them reached their maximal development, and tubulovesicles occupied nearly the whole space of the cell free from mitochondria. The structure of the mitochondria did not differ visually from that in the

cells of the generative zone. During migration deep into the glands from this level the area of the membranes of the intracellular tubules and the number of tubules both diminished, although it was difficult to reach this conclusion visually. The number of lysosomes continued to rise but the number of cisterns of the endoplasmic reticulum fell.

In cells located in the fundus of the glands (Fig. 1d) not only was the number of lysosomes increased, but they showed qualitative changes also. Besides small myelin-like figures, large autophagosomes containing lipofuscin, myelin lamellae, and drops of neutral lipids also were found, i.e., the picture of initial degenerative changes described by Shiao-Fu Chiao [10] and characteristic of cells completing their life cycle appears. However, as the electron micrograph shows, the mitochondrial structure was not significantly changed.

The results of morphometric analysis (Table 1) indicate that the most active cells (as regards the level of development of the microvilli and the volume coefficient of the tubulovesicles) lay at the level of 400-500 μ. No visual differences were found between the mitochondria of cells at the various levels. However, morphometric analysis shows that the most active cells at the level of 400-500 μ corresponded to mitochondria with evidence of the highest functional activity. They occupied the largest area in the cell. They contained cristae with the largest surface area and the lowest coefficient of fragmentation.

The state of the lysosomal system is a particularly important factor for the interpretation of the pathological changes in the parietal cells. As the cells migrate from the generative zone (where there are solitary lysosomes) the number of lysosomes increased progressively and reached a maximum in the cells of the fundus of the glands. Not only their absolute area increased under these circumstances. The relative size of the single lysosome was doubled on account of the appearance of large cytosegresomes.

After examination of these results it was concluded that the study of parietal cells must (and, evidently, can adequately) be carried out at the three main levels of the glands: the generative zone (100-150 μ), the zone of cells in the most active state (400-500 μ), and the fundus of the glands (750-800 μ). All other levels are intermediate in nature.

Parietal cells located at different levels thus differ considerably from each other in their morphometric features. Knowing the detailed characteristics of parietal cell ultrastructure at different levels of the glands it is possible to assess deviations in their submicroscopic organization objectively in different functional states. The method of descriptive electron microscopy, although suitable for the study of extreme pathological states, cannot be used to investigate different functional states of the gastric mucosa and, in particular, those very slight changes that can take place under the influence of certain types of unbalanced feeding. The approach now suggested, namely electron-microscopic investigation of parietal cells at the three main levels of the principal glands followed by morphometric analysis of the results, seems to be completely justified. Despite its rather laborious nature, such an approach is evidently applicable also to the study of other cells of the gastric mucosa and also of many tissues with a rapid and constant rate of cell renewal.

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