

FENESTRAE IN THE ROUGH ENDOPLASMIC RETICULUM OF THE EXOCRINE PANCREATIC CELLS

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INTRODUCTION

Since its original description, the endoplasmic reticulum (ER) has been considered to be a system of flattened cisternae interconnected with canalicular bridges or tubules (1). This three-dimensional model of the ER was inferred mainly from images obtained on spread cells *in toto* where the ER appears as a continuous branching network. Subsequently, Palade (2) reported that the ER cisternae were not only branched and connected with bridges but also fenestrated. However, since thin sections for electron microscopy give only a limited sampling of an entire ER sheet, the number and the distribution of the fenestrae could not be accurately assessed.

By exposing large areas of cellular membranes, the technique of freeze etching retrieves the three-dimensional image lost by sectioning, and this communication will present data from freeze-etched preparations showing clear images of fenestrae in the ER cisternae of the exocrine pancreatic cells of several species.

MATERIALS AND METHODS

Pancreata from spiny mice (*Acomys cahirinus*), rat, dog, and human were fixed in 2% glutaraldehyde buffered with phosphate (4), then soaked for 1 or 2 hr in a 20% solution of glycerol also buffered with phosphate. The tissue fragments were frozen in Freon 22, cooled with liquid nitrogen, then freeze etched according to Moor et al. (5) in a Balzers freeze-etching device, (Balzers AG, Balzers, Liechtenstein). The fracturing temperature was -100°C and the etching time 1 min. Platinum-carbon replicas were cleaned with sodium hypochlorite and distilled water, recovered on 200 mesh copper grids, and examined in a Philips EM 300 electron microscope. Conventional EM thin sections were obtained from glutaraldehyde-fixed, Epon-embedded tissue.

RESULTS AND DISCUSSION

A portion of a pancreatic exocrine cell after freeze etching is presented in Fig. 1. The cell organelles such as the nucleus and zymogen granules are

easily recognizable. The appearance of the ER component depends upon whether the plane of fracture splits the membranes longitudinally or breaks them transversely. When fractured transversely, the ER cisternae appear as successive ridges (see also Figs. 2 and 4). When split longitudinally, ER profiles are seen as sheets of smooth or granular membrane faces containing small circular depressions. At higher magnification (Fig. 2), the transversely fractured cisternae have the conventional appearance of ER profiles seen in sectioned material, i.e., a succession of elongated vesicular structures connected one with the other by narrow bridges. With this orientation of the fracture plane, the three-dimensional extension of the ER network cannot be estimated with more precision than with sectioned material. In contrast, when the fracture plane splits the ER parallel to the longitudinal orientation of the cisternae, these appear as large sheets of membrane faces. Most of the membranes were split (6) so as to reveal the so-called B-face, which shows few particles (7, 8). These membrane faces contain multiple circular depressions of constant size, about 700 Å in diameter. In this area of ER exposed, the number of depressions per square micron is approximately 17. In addition, areas are seen in which the ER membranes resemble a network of tubules (see Fig. 2) probably representing the branching arms of the ER (see Fig. 3 and inset). In the freeze-etched preparation, the depressions strongly resemble nuclear pores (Fig. 6) or capillary fenestrae (9, 10). This resemblance is also apparent in well oriented thin sections (Figs. 7-9).

As described above, freeze-etched preparations of pancreatic exocrine cells offer clear evidence of the presence of depressions in the membrane faces of the ER. When one compares the images obtained with the freeze etching and sectioning techniques, it is reasonable to conclude that the depressions are indeed the fenestrae described by Palade (2).

The only intracellular structures resembling ER in disposition and having definite pores are the annulate lamellae (3). It is believed that the membrane faces described in this report are not annulate lamellae, for the following reasons: (a) the disposition of the sheets of exposed membranes in freeze-etched preparations strongly resembles the arrays of rough ER cisternae seen in conventional sections of exocrine cells; (b) annulate lamellae have not been described in normal pancreatic exocrine cells.

The circular depressions which have been presented as fenestrae should be clearly distinguished from the large holes in the ER sheets formed when the ER assumes the configuration of a branching tubular network. As expected, these holes are large and irregular and are permeated by the cytoplasmic matrix. Furthermore, these depressions should not be considered the result of fracturing of intermediate vesicles budding off from the ER. Seen on the B face of the membrane, the necks of fractured vesicles would appear as raised craters, each limited by a thin ridge. Moreover, entire vesicular profiles attached to the membrane face would also be revealed by the fracture.

In the tissues examined, the number of fenestrae is rather high but extremely variable from one cistern to the next. It is not known whether these

highly fenestrated profiles of ER are characteristic of the pancreatic exocrine cells and/or of the type of animal studied. Also to be determined is whether the fenestrae are permanent, stable structures in the ER membranes or are labile and appear and disappear at any point of the ER sheets. Although the functional significance of fenestrae in the rough ER (RER) is unknown, two hypotheses come to mind. Fenestrae might: (a) represent communication channels from one intercisternal space to the other; (b) compartmentalize the intracisternal space of the ER. Such compartmentalization could play a role in directing the flow of intracisternal secretory product.

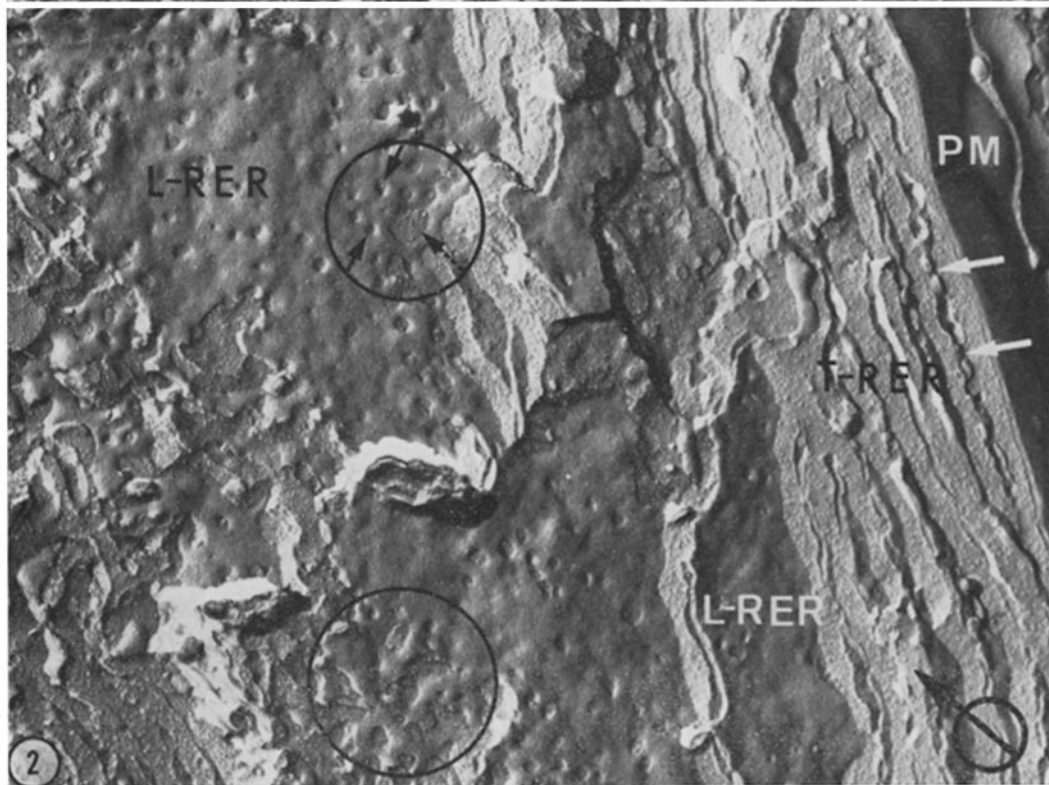
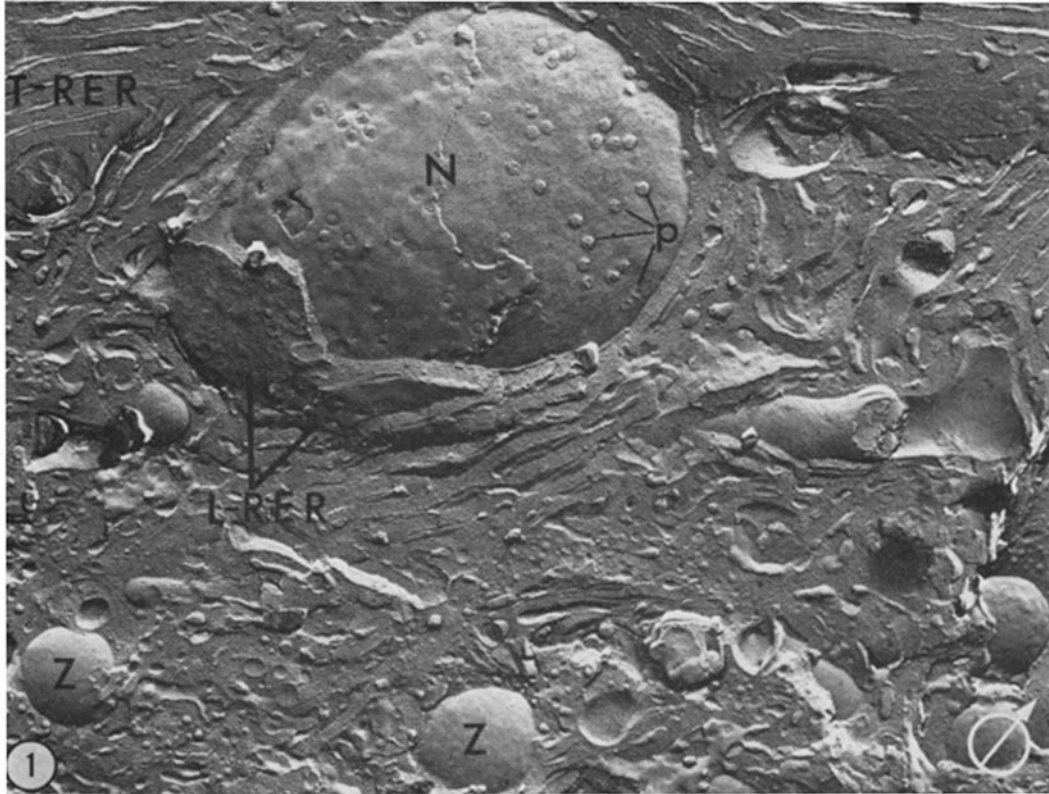
In summary, the above observations provide three-dimensional evidence of depressions occurring in the RER cisternae of the exocrine pancreatic cell. These depressions, distinct from the large openings of the branched ER network, most probably represent the fenestrae of the ER.

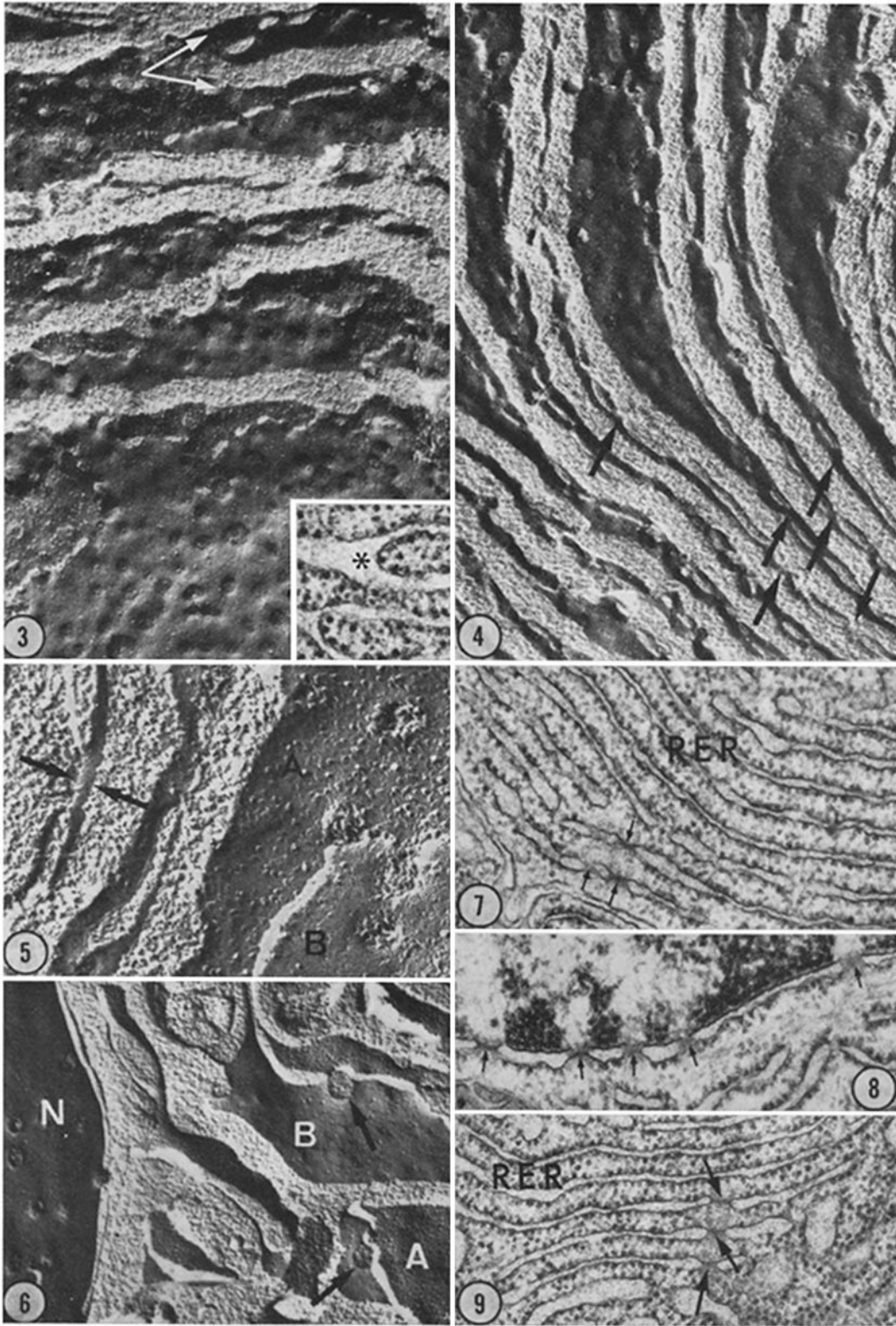
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FIGURE 1 Part of an exocrine pancreatic cell showing the main constituents of the cell cytoplasm after freeze etching. In the middle of the picture, the nucleus (N) can be seen with its characteristic pores (p). The smaller convex and smooth circular profiles represent zymogen granules (Z). The appearance of the RER depends upon the orientation of the plane of fracture: close to the nucleus, the membrane face containing small circular depressions is interpreted as a longitudinally split RER (L-RER), while the many ridgelike profiles interspersed within the cytoplasm are considered to be transversally fractured RER cisternae (T-RER). The numerous small circular profiles revealed by this technique probably represent the microvesicular component of the exocrine cell. The encircled arrow at the bottom of the picture indicates the direction of the platinum shadowing. All pictures are "positives," i.e., with the shadow appearing white. *Acomys* pancreas. $\times 18,000$.

FIGURE 2 Portion of an exocrine cell cytoplasm showing the RER in transverse and in longitudinal view. In longitudinal view, the split ER appears as large sheets of membrane faces. The plane of fracture reveals mostly the smooth leaflet of the ER membrane (B face, associated with the lumen of the cistern); in some areas, however, the particle-covered leaflet of the ER membrane is exposed (A face, associated with the cytoplasmic matrix). The longitudinally split ER sheets (L-RER) show the numerous circular depressions (arrows) believed to be ER fenestrae. Some regions of the ER do not have depressions but appear as shallow networks (encircled areas). These regions probably correspond to the branching ER (see Fig. 3 and inset). Note the difference between the holes of the branching ER permeated by the cytoplasmic matrix (dotted arrow) and the small depressions representing ER fenestrae (solid arrows). Near the plasma membrane of the adjacent exocrine cell (PM), which is uniformly smooth and without depressions, several profiles of RER have been fractured transversely (T-RER). They appear as parallel ridges of membranes showing several constrictions (white arrows). *Acomys* pancreas. $\times 25,000$.





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FIGURE 3 Longitudinally split ER. This replica shows successive sheets of ER pitted with small circular depressions (fenestrae). The uppermost sheet is not only fenestrated but appears also to be branched (double arrow). Inset: thin section of an exocrine cell showing the branching of the RER (asterisk). *Acomys* pancreas. $\times 42,000$; inset, $\times 55,000$.

FIGURE 4 Predominantly transverse fracture of the ER: with this orientation of the fracture, ER profiles appear as parallel ridges of membranes which are constricted at irregular intervals (arrows). The constricted regions probably represent the pores of the ER. In longitudinal fracture (upper part of the picture), the depressions are again revealed. *Acomys* pancreas. $\times 33,000$.

FIGURE 5 High magnification of ER profiles fractured transversely (left side of the picture) or longitudinally (right side of the picture). On the left side, the parallel ridges of ER membranes are interrupted at the presumed pore region by a band of finely textured material (opposite arrow). On the right side of the picture, ER sheets have been exposed longitudinally: fenestrae appear as depressions (B face) or as nipples (A face) on the exposed ER membranes. Dog pancreas. $\times 107,000$.

FIGURE 6 Replica from the perinuclear region of an exocrine cell. This fracture shows both the nuclear envelope (N) and sheets of RER. Arrows indicate fenestrae. ER sheets were split so as to reveal the A face (A) or the B face (B) of the membranes. Human pancreas. $\times 32,000$.

FIGURES 7 and 8 Thin sections of exocrine cells of *Acomys* pancreas. In Fig. 7, fenestrated RER profiles are apparent as well as bandlike structures bridging each of the fenestrae (arrows). Fig. 8 shows a nuclear envelope with characteristic pores (arrows). These strongly resemble the ER fenestrae described in Fig. 7. Fig. 7, $\times 36,000$; Fig. 8, $\times 46,000$.

FIGURE 9 Thin section of an exocrine cell of dog pancreas. This section clearly shows fenestrae in the RER profiles, as well as the band structures bridging the fenestrae (arrows). $\times 36,000$.