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Freeze-Fracture Replica Study of Fenestrated Capillaries in the Parathyroid Gland

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The fenestrated capillaries of the rat parathyroid gland were studied using the freeze-fracture replica method. The population density of the fenestrae on the capillary wall was 11 to 42 per μm^2 and its average value was 28 per μm^2. The average diameter of the fenestrae was 65 nm. Comparing these results with the previous data of other organs, the capillary fenestrae of the rat parathyroid gland were ranked, in respect of the population density, above those of the exocrine pancreas and below those of the kidney.

INTRODUCTION

The freeze-fracture replica studies on the parathyroid gland have been performed in humans[13], rats[7] and hens[11]. However, these descriptions are limited to either the parenchymal cells or[13] their intercellular junctions[7][11], and the blood capillaries in the interstitial connective tissue are as yet disregarded.

Freeze-fracture replicas are the most suitable method for the observation of the capillaries, because they allow us the surface view of the latter.

The present study should provide several data for the morphology of the blood capillary of the rat parathyroid gland, using the freeze-fracture technique.

MATERIALS and METHODS

Healthy, adult male Wistar-strain albino rats, three to five months old, were used for the present study. They were anesthetized with Nembutal and perfused through the left ventricle with Karnovsky's solution containing 2.5% glutaraldehyde and 2% paraformaldehyde buffered with sodium cacodylate (0.1 M). After 10 minutes of perfusion, the parathyroid glands were removed with part of the thyroid tissue and

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placed in the same fixative for 3 hours. The tissues were washed overnight in the same buffer containing 7% surcrose, and kept at 4°C in the refrigerator.

For freeze-fracture replicas, each tissue was trimmed into a cylindrical shape containing an entire gland supported by the thyroid tissue and then transferred to a 30% cacodylate-buffered glycerol solution for 3 hours. The glycerinated tissues were frozen in Freon 22 cooled by liquid nitrogen and fractured by a EE-FED-B freeze-fracture apparatus (JEOL, Japan), at $-130\sim-140$°C under a pressure of 2×10^{-6} Torr. The fractured surfaces were replicated with platinum-carbon, and the replicas were cleaned with graded concentrations of sodium hypochlorite for one hour. After washing in distilled water, the replicas were mounted on copper grids.

For thin section preparations used for comparative observations, some of the glands fixed in Karnovsky's solution were post-fixed in 1% osmium tetroxide in cacodylate buffer for one hour. These were followed by the process for making conventional thin sections. Replicas and thin sections were examined in a JEOL-100B or JEM-T8 electron microscope.

For the measurment of diameter of capillary fenestrae, the long and short diameters of a number of fenestrae, which are selected rondomly from their groups, were measured on the printed photographs at a final magnification of 50,000, and the half value of the sum of these two diameters was recorded as the diameter of each fenestra.

RESULTS

The rat parathyroid gland consists of cords of parenchymal cells, which are compactly arranged, interspersed with a rich network of blood capillaries. Therefore, in the freeze-fracture replica preparations of this gland, either cross fractures or en face fractures of the capillary wall are frequently encountered. In cross fractures through the capillary wall, fenestrations and caveolae are easily distinguished by the following features: the cytoplasm containing numerous fenestrae is extremely attenuated and seems to be lacking at the sites of fenestrations (Fig. 1 inset), whereas the caveolae mainly occur in the thick cytoplasmic area and appear as small vesicles continuous with the plasma membrane (Fig. 2).

On the other hand, the en face fractures of the capillary wall exhibit numerous crater-like, low elevations of about 50 to 75 nm in diameter (Figs. 4, 5) and circumvallate papilla-like, shallow depressions of about 52 to 77 nm in diameter (Figs. 3, 5) on the E fracture face and on the P fracture face of the endothelial cell plasma membrane, respectively. The former elevations are frequently mingled with domes of about 48 (46-61) nm in diameter (Fig. 5); the latter depressions are lower than the general level of the surrounding area and their bases likely correspond to a diaphragm of fenestrae or caveolae. Also, elevations on the E fracture face and the depressions on the P fracture face are considered to be in a complementary relation with each other. The domes on the E fracture face can be identified as caveolae (Fig. 5); the circum-

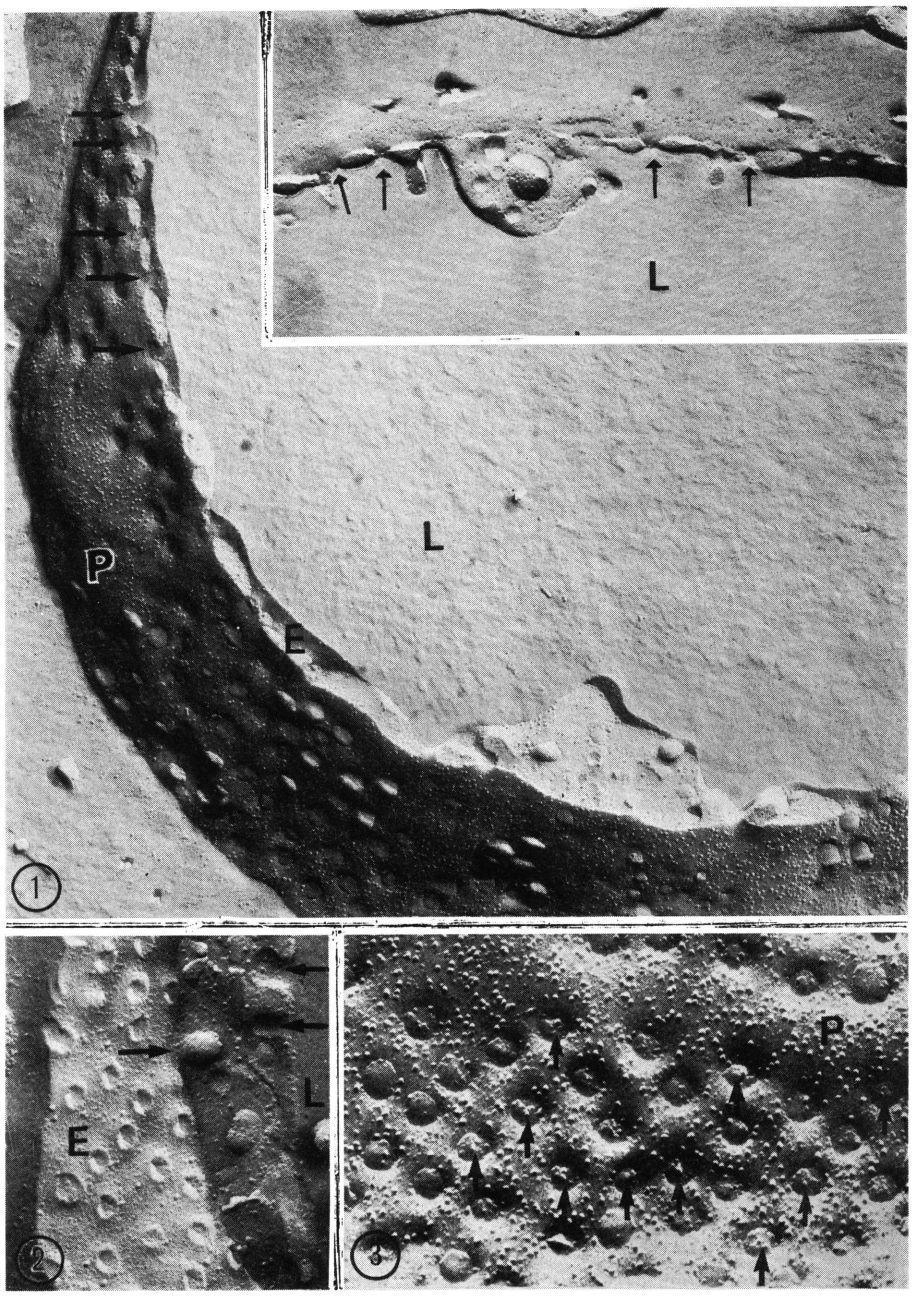
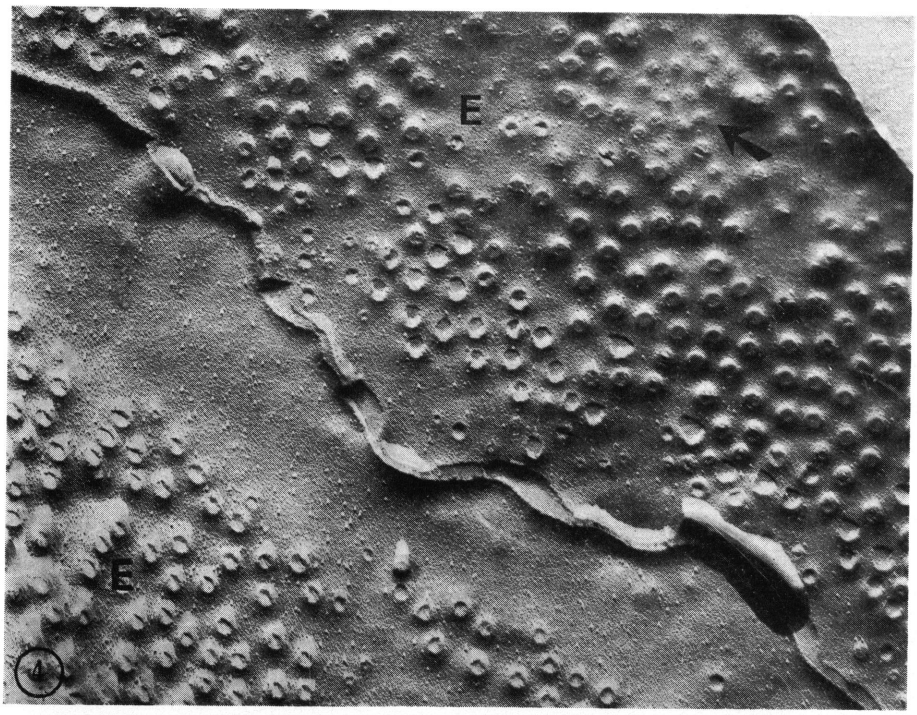


Fig. 1. Nearly cross fracture through a blood capillary.
The fracture plane passes along the P fracture face of the basal plasma membrane (P), through the cytoplasm and along the E fracture face of the luminal plasma membrane (E) of the endothelial cell. Arrows indicate fenestrae which are opened toward the E face of the luminal plasma membrane. X 46,000.
The Inset shows many fenestrae (arrows) at the thinnest portions of the cytoplasm of the endothelium. X 23,000. L:capillary lumen.

Fig. 2. Nearly transverse fracture through a part of the capillary endothelium.
The fracture path moves along the E face of the basal plasma membrane (E) and through the cytoplasm, showing caveolae (arrows) continuous with the luminal or basal plasma membrane as well as caveolae on the E face. X 61,000.

Fig. 3. P fracture face of the capillary endothelial pasma membrane showing a large number of fenestrae which appear as circumvallate papilla-like structures with a central knob (arrows). X 60,000.



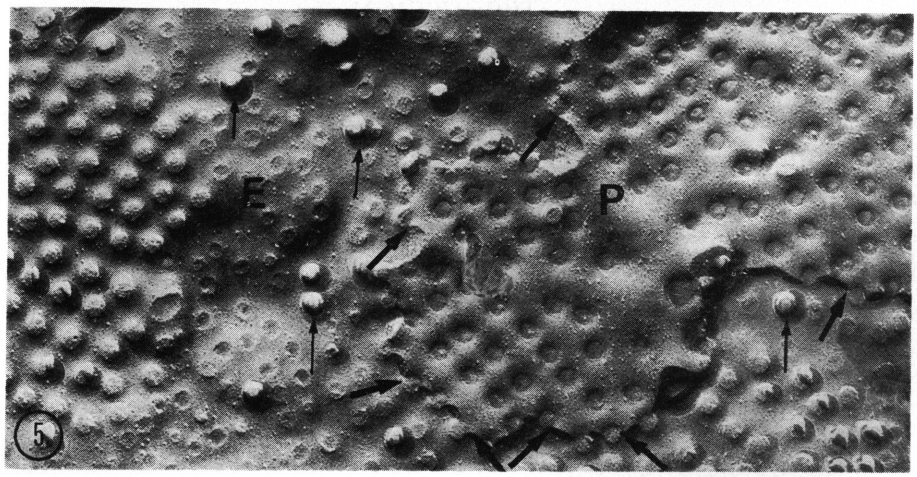


Fig. 4. E fracture face of the capillary endothelial plasma membrane (E) showing a tendency to grouping of fenestrae. A group of smaller low elevatons on the top right(arrow) may be caveolae. Note the presence of a fenestra-free, belt-like zone along the cell boundary, but in some places, such a zone is partially occupied by groups of fenestrae. Slight variations in the depth of the craters are observed due to the level of the fracture plane. X 36,000.

Fig. 5. E and P fracture faces of the capillary endothelial pasma membrane (E, P). Note that the E and P faces are fused with each other at the margins of papillae or craters (large arrows). This is morphological evidence that these structures may correspond to fenestrae. Dome-like elevations indicate caveolae (small arrows).

vallate papilla-like structures on the P fracture face, when many of them have a central knob[5] on the diaphragm, may be fenestrae (Fig. 3). However, it is difficult to decide with certainly whether on the E fracture face such elevations represent caveolae or fenestrae, except either when they appear associated with the P fracture face in the same cell (Fig. 5), or when the fracture plane passes through both the plasma membrane and the cytoplasm in the same cell (Figs. 1, 2).

Generally, in the capillary endothelium of the rat parathyroid, the fenestrae (50-77nm) are somewhat larger in diameter than the caveolae (46-61nm) and more regularly distributed. In the extensive area occupied by numerous fenestrae, the fenestrae indicate a tendency to be distributed in groups, and each group seems to be separated by fenestra-free narrow area (Fig. 4). The population densities of fenestrae calculated in each group were 11 to 42 per μm^2 and their average value was 28 per μm^2.

On the other hand, the caveolae are rather randomly dispersed and variable in number from one area to the other on the same endothelial surface, showing no constant distribution pattern. The boundaries of the endothelial cell surface are surrounded by a fenestra or caveola-free, belt-like zone to 1 μ in width, but in some places they are occupied by the groups of these structures (Fig. 4).

DISCUSSION

It is well known that endocrine glands possess fenestrated capillaries. In the rat parathyroid gland also, fenestrations have been observed in thin section preparations[9]. In the freeze-fracture replicas, however, the location, size and distribution pattern of fenestrations are clearly demonstrated on the endothelial cell surface: the fenestrae are mainly distributed in the peripheral, thin cytoplasmic areas away from each side of the nuclear area; they indicated a tendency to grouping; in each group their population densities are 11 to 42 per μm^2 (average 28); the average diameter of the fenestrae in each group measures 50 to 77 nm.

In the hamster adrenal gland, the capillary fenestrae are reported to occur in endothelial tracts separated by ribs devoid of fenestrae[10]. Additionally, in the proximal tubules of the rabbit kidney the fenestrae are described never to exist near the region of the endothelial cell border[14]. In the present study, however, they are slightly grouped and sometimes present also in the endothelial cell border regions, exhibiting no such prominent ribs. It is unclear whether such differences between distribution patterns of fenestrae are related to permeability.

Comparing the results calculated in the present study with previous data reported in other organs[1)~6)8)10)12)], the fenestrae in the rat parathyroid capillaries are similar in diameter to those of all other organs previously described except for the adrenal cortex and small intestin, but their population density is higher than that of the thyroid gland and somewhat lower than that of the adrenal gland (Table 1).

Recent experimental study on the permeability of the intestinal capillary wall

Table 1 Size and Frequency of Fenestrae in Capillary Endothelium

Organ	Animal	Diameter(nm)	Frequency/um²	S/F*	Authors
Kidney	mouse	70	30	S	Rhodin (1962)
Kidney	mouse	65	60	F	Friederici (1969)
Kidney	rat	69		F	Moul (1571)
Kidney	rat	65		F	Kühn et al. (1975)
Pancreas	rat	66	15(2−31)	F	Simionescu et al. (1974)
Small intestin	mouse	35−45		S	Clementi & Palade (1969)
Jejunal mucosa	rat	67	26(6−56)	F	Simionescu et al. (1974)
Thyroid	rat		20	F	Fujita et al. (1975)
Adrenal medulla	rat	50		S	Elfvin (1965)
Adrenal cortex	hamster	57−166	35	F	Ryan et al. (1975)
Adrenal medulla	hamster	52,5−78		F	Ryan et al. (1975)
Parathyroid	rat	65(60−77)	28(11−42)	F	Setoguti et al. (1982)

* S, sectioned specimens; F, freeze fractured specimens.

using peroxidase and ferritin has suggested that the fenestrae are the most effective portions in the endothelial cell surface for capillary permeability[1]. Therefore, the number of fenestrae per μm^2, in addition to the diameter of the fenestrae, may reflect the intensity of the permeability of capillaries in the given organs. In this connection, it seems resonable to suggest that the average population density of fenestrae in the parathyroid gland may be ranked above that of the exocrine pancreas[12] and below that of the kidney[3].

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