

ENDOTHELIAL CELL JUNCTIONS

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In the course of a freeze-cleave study on intercellular junctions in the regenerating rat liver, we observed an unusual array of intramembranous particles located in regions of contact between endothelial cells lining the hepatic sinusoids. These arrays were characterized by an accumulation of particles which resembled a zonula occludens in their linear deployment but differed in that the contact regions were composed of individual particles which remained separated from each other by regular particle-free intervals.

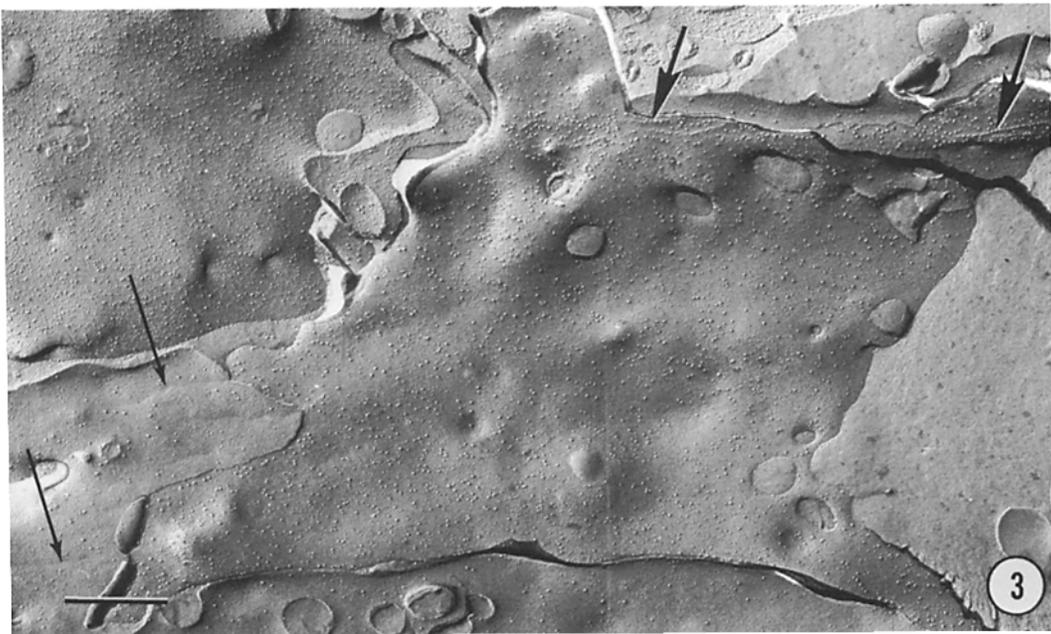
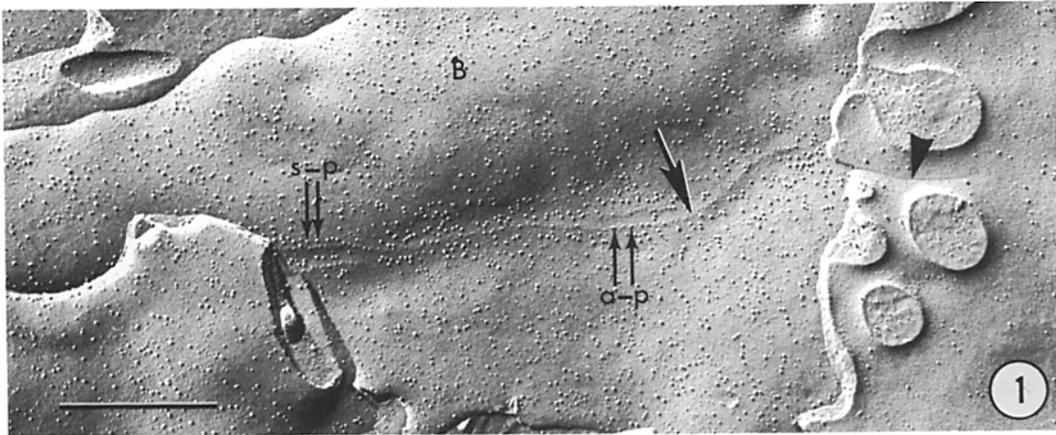
MATERIALS AND METHODS

3 to 4-week old rats (Charles River Breeding Laboratories, Wilmington, Mass.) were partially hepatectomized according to the method first described by Higgins and Anderson (3). Animals were killed at 2-h intervals, from 28 to 40 h after hepatectomy. Small wedges of right lateral lobes of the livers were fixed in Karnovsky's formaldehyde-glutaraldehyde fixative (4) for 1-2 h. The tissues were then equilibrated in 25% glycerol for 2 h before freezing in Freon 22. Tissues were fractured and shadowed with platinum in a standard Balzers' freeze-etch machine (Balzers High

FIGURE 1 A freeze-cleave replica of endothelial cell junctions in the regenerating rat liver. In the region of a cell bulge (*B*), there is a slight elevation of the membrane to form a linear crest (large arrow). On the apex of the crest, single intramembranous particles in linear array can be seen (*a-p*). In addition, linear particle aggregates can be seen on either side of the crest, upon its slopes (*s-p*). At the arrowhead, the **B** fracture face of the endothelial junction is represented as a thin groove in register with the linear crest of the **A** fracture face. Calibration = 0.5 μm ; \times 43,000.

FIGURE 2 A freeze-cleave replica of endothelial cell junctions in the regenerating rat liver. In this micrograph, a distinct membrane crest is absent, but particle aggregates forming linear arrays are clearly visible (arrow). On the right, at the arrowhead, short linear arrays alternating with randomly dispersed particles form distinctive patterns. Calibration = 0.5 μm ; \times 42,000

FIGURE 3 Both **A** and **B** fracture faces are present in this micrograph of the endothelial cell junctions in the regenerating rat liver. Linear particle aggregates characterize the **A** fracture face (large arrows) and thin tenuous particle-free grooves are present on the corresponding **B** fracture face (small arrows). Calibration = 0.5 μm ; \times 27,000.



Vacuum Corp., Santa Ana, Calif.). Carbon was evaporated onto the shadowed surface to provide support. Before digestion in Clorox, the samples were routinely defatted in dimethylformamide overnight (8). The replicas were examined in a Siemens IA electron microscope, operated at 80 kV.

RESULTS

In regions of contact between endothelial cells lining the hepatic sinusoids in the regenerating rat liver, the surface of one cell often protruded slightly to form a bulge which extended towards the neighboring cell. On the A fracture face, the bulge often had slight elevations of its membrane which formed linear crests (Fig. 1). On the apex of the crest, we observed an aggregation of particles some 8-9 nm in diameter which formed distinct linear arrays. The particles were often separated from each other by regular spaces about the width of one particle. Sometimes, particle rows were seen on either or both sides of the crest, upon its slopes (Fig. 1); at other times, the crest was absent, but the linear particle aggregates could still be seen (Figs. 2 and 3). In addition, short arrays formed linear patterns on flattened membrane surfaces with single particles randomly distributed in between the arrays (Fig. 2).

On the B fracture face, the junctional region was characterized by a narrow continuous groove which was in linear register with the crest on the A fracture face of the apposing cell (Fig. 1). The grooves were particularly noteworthy for their tenuous appearance and for their lack of particulate material (Fig. 3). Although these junctions could be quite extensive and could run for long distances on the endothelial cell membrane, they did not form zonulae or continuous belts and therefore must be considered primarily focal in nature.

In control unoperated animals, it was much more difficult to observe these junctions between endothelial cells. The few that were observed seemed to differ from those in operated animals in that the particle arrays were less distinctive and seemed, at times, to be only suggestive of a linear deployment (Figs. 4 and 5).

DISCUSSION

We have observed an unusual arrangement of junctional constituents between endothelial cells of the regenerating rat liver. The linear arrangement of single membrane particles suggests that the

regions of specialized intercellular contact between endothelial cells are discontinuous and punctate in nature. The close association of particles to form rectangular or linear ropes or ridges similar to those found on A fracture face of a zonula occludens (2, 5) were rarely observed; when they were noted, however, they were extremely short, consisting of only two or three particles. That these particles are indeed individual and not merely the result of irregular cleavage of ridge material leaving particles adhering to the complementary fracture face is supported by the complete lack of particulate material within the thin grooves. True zonulae occludentes were not observed between endothelial cells.

Many of the junctions observed were focal contacts, limited to regions of bulges between apposing cells. It is, therefore, unlikely that these junctions play a role in preventing the passage of material between endothelial cells which would be the case for true occluding junctions. In addition, since liver endothelial cells are fenestrated, exhibiting large gaps up to 1 μm in diameter (6), the necessity for an occluding seal is doubtful. More likely, the endothelial cell junctions described here, which are divisible into two components, linear membrane crests and aggregates of intramembranous particles, may represent small areas of intercellular adherence or of simple contact. Both the crests and the particle aggregates may function in concert as regions of cell recognition and as anchors for holding the cells together in a loose endothelial sheet. In this context, it is worth noting that these junctions are more prominent in the regenerating liver than in the unoperated animals, perhaps because in the regenerating liver the endothelial cells are under greatly increased mechanical stress brought about by the rapidly dividing liver cells (1).

Intercellular contacts resembling the junctions described here have also been found in young chick embryos.¹ Mugnaini and Schnapp (7) have reported a zonula occludens in nerve myelin but their illustrations seem to resemble our endothelial type of junction more than a real zonula occludens because it consists of a linear array of single particles. The possibility exists, therefore, that the junctions we describe are a new type of intercellular contact. The ease with which one can find such junctions in the regenerating liver and in young

¹ Revel, J. P., and S. Brown. Manuscript in preparation.

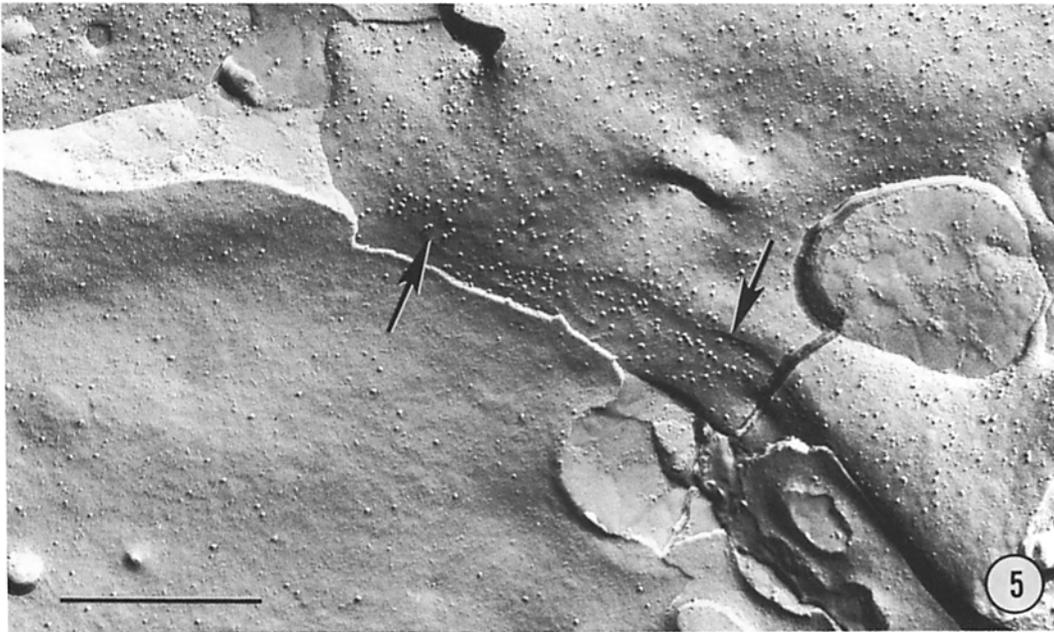
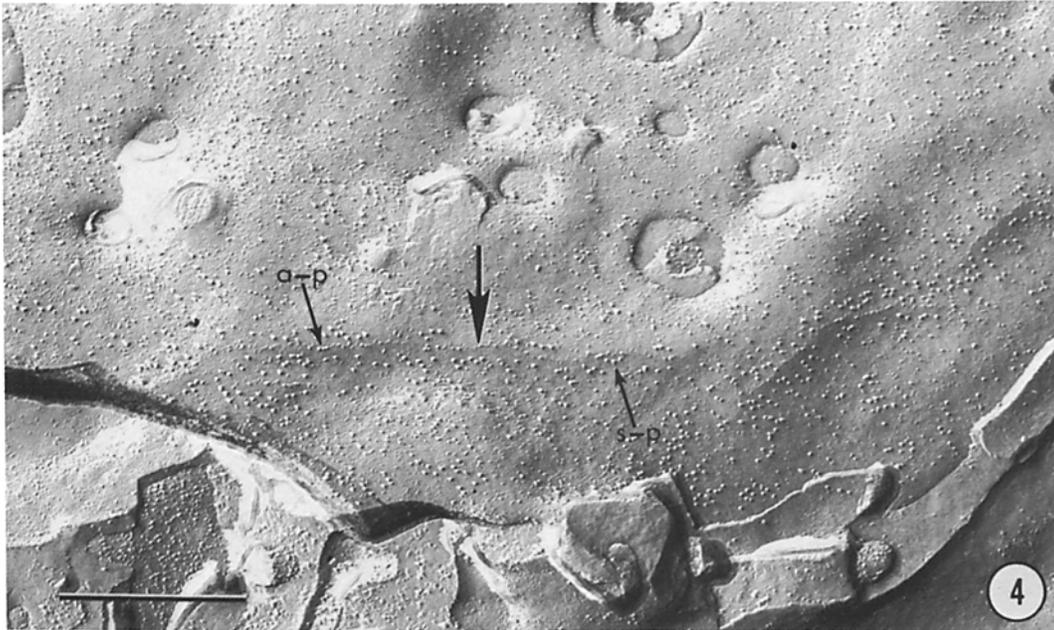


FIGURE 4 Freeze-cleave replica of endothelial cell junctions from normal unoperated rat. The linear membrane crest (large arrow) with particle aggregates on the apex (*a-p*) and upon its slopes (*s-p*) is present, although the linear arrangement of the particles is not quite so distinctive as that seen between sinusoidal cells in the regenerating rat liver. Calibration = $0.5\ \mu\text{m}$; $\times 49,000$.

FIGURE 5 Endothelial cell junctions in the normal unoperated rat similar to Fig. 4. Several membrane crests (large arrows) with randomly distributed intramembranous junctional particles are evident. Again, their linear deployment is not so distinctive as in the regenerating rat liver. Calibration = $0.5\ \mu\text{m}$; $\times 53,000$.

embryos suggests that this junction could also be a step in the formation of a zonula occludens. No evidence can be found for this idea, however, and it is perhaps better at the present time to consider this as a separate junctional type.

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