Intercellular junctions: sites of permeability barriers and cellular communication

Following the classical study by Farquhar and Palade, in which the tripartite junctional complex that joins many kinds of epithelial cells was described, numerous investigations of the structure and function of these and other kinds of intercellular junctions have provided greater understanding of the permeability properties of cellular and vascular layers. Two electron microscopic techniques have become particularly useful for visualizing various types of intercellular junctions. These are: (1) the electron-dense tracer methods used to probe the extracellular space, and (2) the technique of freeze-fracture of cell membranes, which permits en face visualization of junctional elements. The application of these techniques to ocular tissue is resulting in some interesting new concepts of functional dependency and cooperation among cells in the eye.

The permeability properties of vascular beds and epithelial or endothelial layers are revealed when solutions of electron-dense tracer molecules of selected diameters, such as ferritin (110 Å), myoglobin (33 Å), microperoxidase (25 Å), or lanthanum nitrate (~ 3 Å) are introduced either into the blood or into cavities such as the vitreous or anterior chamber. Fixation of specimens at various time intervals thereafter permits visualization of the sequential progress of the selected molecules through the vessel walls into the tissue or, conversely, from the cavity into neighboring structures. When careful experimental technique is observed, the results of tracer experiments can yield valuable information regarding routes for the passage of large and small molecules into and out of various ocular compartments.

The freeze-fracture technique takes advantage of the fact that frozen biological material, when cleaved with a knife, tends to fracture along a plane between the two hydrophobic phospholipid leaflets of cell membranes. In this way the intramembranous structure is exposed, and if some of the frozen water is sublimed off, these structures stand out in relief. By making a shadowed metal replica of this freeze-etched surface, the intramembranous structures can be studied in the electron microscope. These freeze-fractured or freeze-etched preparations provide a view of a large area of cell surface, and when cell junctions are included in the specimen, an en face image of intercellular particles and strands is seen.

**Tight junctions.** The tripartite junctional complex is an elaborate apicolateral intercellular junction, the terminal bar of tight microscopy. Its most apicolateral intercellular junction, the terminal bar of light microscopy. Its most apicolateral intercellular junction,
the zonula (belt) occludens or tight junction, characterized in sectioned material by fusion of the outer leaflets of the apposing plasma membranes, and in freeze-fractured preparations by a network of anastomosing strands which lie in the plane of the fused membranes. The intermediate portion of the junction is a strongly adherent band, the zonula adherens, characterized by a curiously wider (250 Å) intercellular space than occurs in nonadherent regions between the cells. The third and most basal element of the junction is another adherent spot, the macula adherens or desmosome, characterized by an electron-dense plaque in each cell subjacent to the inner leaflet of the plasma membrane with tonofilaments radiating from the plaques. The mechanical strength of the two adherens-type junctions has been demonstrated in osmotic experiments. The desmosome provides the principal structural resistance to lateral shearing forces.

The permeability of the zonula occludens varies with its structural complexity. Cells joined by wide occluding bands that have a complex network of intramembranous strands permit minimal paracellular flux of water and ions. These epithelial sheets (for example, stomach and bladder epithelia and the pigment epithelium of the retina) have a high transepithelial electrical resistance. Narrow occluding zonules, or those with mere focal fusions of apposed membranes, have simple, sometimes discontinuous, intramembranous strands. These are leaky epithelial sheets and have low transepithelial electrical resistance (for example, the epithelium of the proximal convoluted tubule of the kidney). Thus the morphology of the intercellular junction can now be used to predict the physiologic properties of a cellular sheet. For example, the endothelial lining of Schlemm’s canal with its focal occluding junction would be leaky and provide little transendothelial electrical resistance.

Capillary permeability. Capillary networks vary greatly in permeability, owing to regional structural differences. Typically visceral capillaries (e.g., those of the choriocapillaris and at the base of the pigment epithelium in the ciliary processes) have a discontinuous endothelium provided with apertured fenestrae 300 to 800 Å wide. These capillaries are freely permeable to macromolecules. The function of the aperture diaphragm bridging the fenestrae is unknown. Somatic capillaries occur in skeletal muscle and in the central nervous system and are relatively impermeable. In the eye they are found in the ciliary muscle, iris stroma, limbal arcade, and retina. They have a continuous nonfenestrated endothelium and neighboring cells are joined by zonulae occludentes, thus the passage of large and small molecules is greatly restricted. Large molecules together with a small volume of solute are ferried across the endothelial cell in pinocytic vesicles.

In the eye the fenestrated capillaries allow large molecules to leak into the interstitium at the base of the pigment epithelial layer of the retina and ciliary body. The intercellular junctions between the epithelial cells restrict the further passage of these molecules into the intracellular cavitites. Several studies have shown that within minutes peroxidase injected into the blood flows out of the choriocapillaris, across Bruch’s membrane, and through the intercellular spaces of the pigment epithelial cells, but is stopped at the zonula occludens of the tripartite junctional complex at the apicolateral border of the pigment epithelial cells. Peroxidase injected into the vitreous cavity, within minutes, traverses the intercellular spaces of the entire neural retina (including the zonulae adherentes of the outer limiting “membrane”) and is stopped by the encircling zonulae occludentes of the pigment epithelial cells. Thus the principal diffusional barrier of the retina is the series of occluding junctions of the pigment epithelium (Verhoeff’s membrane). Disruption of this retinal barrier by photocoagulation is followed by a healing process that does not re-establish the zonulae occludentes. Peroxidase studies carried out after the
The retina has healed show unrestricted passage of the tracer from choroid to vitreous and vice versa. The disappearance of suband intraretinal fluids after photocoagulation is due, therefore, to creation of a drainage route rather than to formation of a tighter seal in the scar.1

In the ciliary body, the occluding junction that restricts the passage of peroxidase is at the apicolateral borders of the nonpigmented epithelial cells, i.e., near pigment epithelial cell surface rather than the posterior chamber surface. Peroxidase freely penetrates the intercellular spaces of the pigment epithelial cells, moves across their apical surfaces, in and around the gap junctions that bridge the two apposed apical surfaces of the pigmented and nonpigmented cells, and is stopped at the apicolateral border of the nonpigmented epithelial cells. Conversely, peroxidase introduced into the anterior chamber flows freely across the internal limiting membrane of the ciliary epithelium and down the intercellular spaces to the apicolateral tight junctions. Previous reports that paracentesis opens up these junctions and allows a protein-rich fluid to accumulate in the anterior chamber as secondary aqueous have been contradicted recently by a carefully designed tracer study in monkeys. This study shows that these junctions and those in iris capillaries remain entirely impermeable to peroxidase during paracentesis. The source of the plasma proteins of secondary aqueous is the episcleral venous blood via retrograde flow from Schlemm's canal.5

In the pars plana there is overlap of the two patterns of tight junctions. Tight junctions between pigment epithelial cells, i.e., the characteristic retinal pattern, continues forward about 100 μ into the ciliary body. The nonpigmented epithelial cells overlying these pigment epithelial cells are joined by tight junctions, the characteristic ciliary body pattern. Across the apical surfaces of these two layers, therefore, there is a passageway unsealed by tight junctions, which may permit some leakage between the intercellular spaces of the ciliary body pigment epithelial cells and the retinal interphotoreceptor space. Accumulation of fluid in the peripheral retina could lead to cystoid degeneration or retinoschisis.6

Gap junctions. Another type of intercellular junction that is of great interest to biologists is the gap junction or nexus. This junction, which unfortunately has a misleading name, provides an intercellular channel for the passage of ions and molecules. Wherever gap junctions are demonstrated, lateral electrical, metabolic, and informational communication among cells of the epithelial sheet can be anticipated. The gap junction is not so easily recognized in routine electron microscopic thin sections (500 A thick) since its distinguishing feature is a 20 A intercellular space in a junction that otherwise resembles an occluding junction. But unlike the occluding zonule, periodic gaps that are permeable to the tracer, lanthanum, are present, and from this property it got its name. The gap junction is readily recognized in freeze-fracture preparations. It is characterized by a small (0.1 to 5 μ) cluster of hexagonally arrayed intramembranous particles. In glandular epithelia and in the pigment epithelium of the retina, gap junctions are included in the zonula occludens of the tripartite junctional complex, thereby complicating the semantics of the classification of junctional structures. In other kinds of cells gap junctions assume a variety of locations with respect to the polarity of the cell, for example, they couple the apical poles of the nonpigmented and the pigmented epithelium of the ciliary body. Gap junctions also occur between cells that have no tripartite junctional complexes, for example, smooth muscle cells of the iris and ciliary body, and between photoreceptors.9

The hexagonally arrayed intramembranous particles of the gap junction are visualized as hollow tubes that constitute the intercellular passageways.7 The intercellular spread of electrical current in cells coupled by gap junctions has been demonstrated with the use of microelectrodes in

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the pigment epithelium of the retina and other well-coupled cells. Gap junctions between photoreceptors in the outer plexiform layer may account for some of the short-range transfer of electrical events from cones to neighboring rods and cones, and from numerous rods onto a central cone observed in a number of electrophysiologic studies.

Fluorescein injected into one cell can be observed by light microscopy to pass through gap junctions into neighboring cells. Substances having molecular weights up to 100,000 traverse the gap junction. Informational molecules such as nucleotides may pass these junctions, and they could influence the growth, embryologic development, or performance of the neighboring coupled cells. Some solid epithelial tumors have been shown to consist of electrically uncoupled cells which means that their gap junctions are missing or faulty. Moreover, in tissue culture these tumor cells were unable to establish intercellular junctions as could their normal counterparts. These data suggest that in some cancerous growths the loss of control of proliferative mechanisms may be due to defects (genetically determined?) in the maintenance of normal intercellular junctions.

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Automated refraction

Sophisticated instruments for automated clinical refraction have recently appeared on the market, and still others will appear within the next year. The field of refraction is ready for change; there have been few major advances in half a century. But what are these instruments? How do they work? Why do they cost five to ten times as much as conventional refracting instruments? Do they measure up to the glowing specifications printed handsomely on the manufacturers' brochures? Most importantly, will they improve the quality of visual care that we can provide? Some of

REFERENCES