Endothelial tight junctions form the blood-brain barrier

The concept of a barrier between blood vessels and the brain and spinal cord had existed since the late 1800s, when immunologist Paul Ehrlich found that intravenously administered dyes failed to stain certain regions of the brain, whereas other body tissues were stained. Ehrlich thought the dyes did not have a staining affinity for the brain, but his student, Edwin Goldman, showed that the dyes could stain brain tissues but could not cross a barrier into the brain.

But until 1967, it was not clear whether the structural basis for the barrier was at the level of the endothelium, the astrocytes or glial cells in the brain, or the basement membrane. It took the high resolution of electron microscopy, the development of sensitive tracer methods, and a fortuitous lunch date between Thomas Reese and Morris Karnovsky to show that the endothelial cells in the brain vasculature, and more precisely the junctions between these cells, form the cellular correlate for the so-called blood–brain barrier (Reese and Karnovsky, 1967).

"The study was a result of having a place where faculty could get together and talk," says Karnovsky. "I met Reese by chance at the faculty dining room at Harvard. He was working on the anatomy of the brain and I was developing better tracers. As we were walking back from our lunch, we decided that the problem that needed to be tackled next was the blood–brain barrier."

Karnovsky had already extended the horseradish peroxidase (HRP) tracer method of Werner Straus (1958) to the light and electron microscope. HRP was relatively small, and it could be detected by allowing the enzyme to act on a suitable substrate to yield an electron-opaque reaction product. The product could then be localized by microscopy of tissues fixed at various times after HRP injection (Graham and Karnovsky, 1966).

The first experiments were disillusioning: after intravenous injection of mice with HRP and fixing by perfusion, the authors saw nothing. They came close to giving up, even considering that Ehrlich may have been right that some dyes, or in this case HRP, didn’t work in the brain because of a lack of affinity for the tissue. But then Reese found a vesicle in the endothelium with HRP reaction product in it. That led them to do a slice immersion experiment where they cut out a chunk of tissue and dropped it in fixative. With this experiment they could, finally, see the HRP in the lumen of the vessels. This convinced them that the experiment was working but HRP could not cross the endothelium, and, incidentally, was being washed out of the lumen by the perfusion fixation.

They could see that the reaction product was blocked by tight junctions (Reese and Karnovsky, 1967) and concluded that the blood–brain barrier existed at the level of the vascular endothelium. In a paper Karnovsky published the same year in the JCB, he determined what made the brain special. Peroxidase was seen passing through the vascular endothelium of heart and skeletal muscle, apparently through or around the looser cell junctions in these tissues (Karnovsky, 1967), with a possible contribution from vesicles (Palade, 1953).

"Since passage of peroxidase through the vascular endothelium could occur in other tissues, we proposed that the blood–brain barrier was due to the fact that the cell junctions in the vascular endothelium in the brain were tight," says Karnovsky. Indeed, unlike cell junctions found in other endothelia, the cell junctions of endothelial cells in the brain appeared to be extensive and were surmised to form an unbroken belt between cells.

A second characteristic feature of the endothelium of cerebral vessels observed by Reese and Karnovsky was the low frequency of vesicles associated with the transport of materials across endothelia, but the authors did not think this played a major role in the blood–brain barrier. "Even those vesicles that were there rarely seemed to fill with peroxidase, and no peroxidase seemed to penetrate beyond the luminal surface of the endothelium, so our feeling was that the junctions were the main barrier," explains Karnovsky.

Graham, R.C., and M.J. Karnovsky. 1966. J. Histochem. Cytochem. 14:291–302.
Karnovsky, M.J. 1967. J. Cell Biol. 35:213–236.
Palade, G.E. 1953. J. Appl. Phys. 24:1424.
Reese, T.S., and M.J. Karnovsky. 1967. J. Cell Biol. 34:207–217.
Straus, W. 1958. J. Biophys. Biochem. Cytol. 4:341–550.
Defining gap junctions

In the 1960s there was adhesion and there was direct current transfer—a strange neuronal phenomenon whose mechanism was unknown. The two fields only gradually drifted together, but with the report by Revel and Karnovsky (1967) they were united around a distinct, structural correlate soon to be named the gap junction.

Eight years before, Fursphand and Potter (1959) had reported that subthreshold electrical stimulation (insufficient to elicit an action potential) still gave current transfer between some nerve cells. This apparently passive flow of current was seen in crayfish giant synapses and later in other cells. Robertson (1961) thought this phenomenon might be mediated by the membrane adhesions that he saw. In his words, “the elimination of the gap between the paired axon membranes…may conceivably be sufficient of itself to account for the apparently pure electrical transmission properties of this synapse.” Two years later he found a repeating structure, a hexagonal array in frontal view, that seemed to be in the right place to do the job (Robertson, 1963).

A contact zone called the “nexus” seemed to function in the same way between smooth muscle cells (Dewey and Barr, 1962) and many other excitable cells (Dewey and Barr, 1964), but there were no structural details. The union of adhesion and ion permeability in one structure was also emphasized by Loewenstein and Kanno (1964).

Hexagonal arrays were spotted by a second group, but they mistook them as either possible micellar rearrangements of the plasma membrane (Benedetti and Emmelot, 1965) or components of tight junctions (Benedetti and Emmelot, 1968).

Karnovsky’s interest, meanwhile, was not in current transfer but in the permeability of different types of cell–cell junctions (see “Endothelial tight junctions form the blood–brain barrier” JCB. 169:378). He came up with a new tracer—a polymer of oxidized lanthanum salts—based on some chemistry he remembered from his undergraduate days in South Africa. The new tracer was smaller than the bulky HRP, but electron opaque and large enough to stay fixed in one place.

It was after 2:00 a.m. when Karnovsky got his first tangential sections showing the hexagonal packing of gap junctions in cardiomyocytes. “I didn’t realize at the time what this could indicate, because I hadn’t read the literature,” says Karnovsky. “I took the wet plates…and showed [Jean-Paul] Revel in the neighboring lab.” Revel was so excited that with the liver samples he says he “cut some sections and, lo and behold, there they were, and then they were gone as the hurriedly prepared samples broke in the beam.” According to Karnovsky, Revel said the images “resembled the Benedetti structures.”

Benedetti and Emmelot (1965) had proposed that their structure might represent a “micellar arrangement” of lipids—an alternative to the lipid bilayer structure of membranes. But Karnovsky and Revel did not see the hexagonal arrangement as a generalized structure covering large areas of unspecialized membrane. “We realized by looking that this was extremely localized,” says Karnovsky. It was localized to adhesions. With improvements in electron microscopy came the ability to differentiate between various types of adhesions (see “Defining junctional complexes” JCB. 168:989). Revel and Karnovsky (1967) were able to see that their objects of interest were “cell junctions in which there is a minute gap between the external leaflets.” Despite repeated use of the word “gap” in the paper, this was the closest they came to actually calling the structures “gap junctions.” “We never used this term in the original paper,” says Karnovsky, although they did introduce the term in an abstract published soon after (Revel et al., 1967). Revel calls it an “oxymoron,” but the attribution has stuck to this day.

What Karnovsky and Revel did show was that these junctions were in nonneuronal tissues such as liver and heart. And, unlike tight junctions, the junctions did not act as a barrier: they allowed diffusion of staining salts around them. The distinction between gap and tight junctions was emphasized in more detail by Goodenough and Revel (1970).

Karnovsky was too busy with other pursuits, both administrative and scientific, and did not study gap junctions further. Daniel Goodenough went on to purify and determine a preliminary structure of gap junctions (Makowski et al., 1977), with the final structure coming from Unger et al. (1999). Norton “Bernie” Gilula contributed to this latter structure paper and to many other detailed structural and chemical studies, including the classic demonstration of cell-to-cell communication of hormone responses via gap junctions (Lawrence et al., 1978). WW