Catching sight of lysosomes

All we wanted was to know something about the localization of glucose-6-phosphatase, which we thought might provide a possible clue to the mechanism of action, or lack of action, of insulin on the liver cell.” Thus begins Christian de Duve’s discovery of lysosomes, which he first visualized in a 1956 paper in this journal (Novikoff et al., 1956).

Glucose-6-phosphatase was soon left behind when irregularities showed up with a control enzyme, acid phosphatase. After a gentle cell fractionation procedure, this activity was present at only one tenth of the level expected based on more violent extraction procedures. The activity then reappeared if extracts were left for several days in the refrigerator. As de Duve wrote, “...we could have rested satisfied with this result, dismissing the first series of assays as being due to one of those troublesome gremlins that so often infest laboratories, especially late at night. This would have been a pity, since chance had just contrived our first meeting with the lysosome.”

de Duve concluded that the acid phosphatase activity was latent because of a membrane-like barrier—initially believed to be the mitochondrial membrane. But analyzing the distribution of a single enzyme over many fractions, not just the contents due to one of those troublesome gremlins that so often infest laboratories, especially late at night. This would have been a pity, since chance had just contrived our first meeting with the lysosome.”

de Duve concluded that the acid phosphatase activity was latent because of a membrane-like barrier—initially believed to be the mitochondrial membrane. But analyzing the distribution of a single enzyme over many fractions, not just the contents due to one of those troublesome gremlins that so often infest laboratories, especially late at night. This would have been a pity, since chance had just contrived our first meeting with the lysosome.”

de Duve concluded that the acid phosphatase activity was latent because of a membrane-like barrier—initially believed to be the mitochondrial membrane. But analyzing the distribution of a single enzyme over many fractions, not just the contents due to one of those troublesome gremlins that so often infest laboratories, especially late at night. This would have been a pity, since chance had just contrived our first meeting with the lysosome.”

de Duve concluded that the acid phosphatase activity was latent because of a membrane-like barrier—initially believed to be the mitochondrial membrane. But analyzing the distribution of a single enzyme over many fractions, not just the contents due to one of those troublesome gremlins that so often infest laboratories, especially late at night. This would have been a pity, since chance had just contrived our first meeting with the lysosome.”

The invention of freeze fracture EM and...
of a single fraction as did many investigators, he found subtle differences in the distribution of acid phosphatase and mitochondrial enzymes. The differences were clinched when a centrifuge component broke, resulting in the use of a weaker table-top centrifuge that sedimented mitochondria but not the lighter lysosomes.

By 1955, five enzymes related to degradation had been localized to this fifth fraction, which had been added to the Claude’s earlier quartet of nuclear, mitochondrial, microsomal, and supernatant fractions. The new entities were named lysosomes (de Duve et al., 1955). de Duve now had enough confidence in the biochemistry to enlist the EM expertise of Alex Novikoff, and together they tentatively identified a class of “dense bodies” as the probable structural correlates of biochemically defined lysosomes (Novikoff et al., 1956). Notwithstanding the presence of what Dorothy Bainton termed a few “excessively sad looking mitochondria” in these EM images (Bainton, 1981), the identification proved valid, and was supported by an independent study of “small droplets” by Straus (1956).

The de Duve approach was an excellent complement to that of George Palade, who started with EM pictures and then tried to ascribe functions to what he saw. de Duve, by contrast, started with the function (biochemistry) and studied it to prove the necessary existence of the structure—an approach that would also lead to the discovery of peroxisomes. JCB

Bainton, D.F. 1981. J. Cell Biol. 91:66s–76s.
de Duve, C., et al. 1955. Biochem. J. 60:604–617.
Novikoff, A.B., et al. 1956. J. Biophys. Biochem. Cytol. 2:179–184.
Straus, W. 1956. J. Biophys. Biochem. Cytol. 2:513–521.

the determination of membrane structure
units. Candidates for the subunits, other than a few proteins such as rhodopsin and mitochondrial ATPase, were hard to come by, and Branton’s fracture images helped to reaffirm the idea of a continuous bilayer. Multiple papers from George Palade had also reported that different lipids and enzymes were both made (Dallner et al., 1966) and destroyed (Omura et al., 1967) at different rates, thus puncturing the idea of a “standard membrane” unit that was synthesized in one unified action.

Branton answered critics of his earlier paper by labeling membrane surfaces with ferritin, and showing that ferritin was never observed on fracture faces (Pinto da Silva and Branton, 1970). But still there were those mystery bumps (though no holes) on the fracture faces. Branton himself was hesitant to claim that these might be proteins, and Stoeckenius and Engelman (1969) dismissed them as “possible artifacts.” These authors allowed that “at special sites the protein may reach deeply into the lipid phase or penetrate to the opposite surface,” but asserted that “protein is predominantly arranged on the surface of the lipid.” It was not until the masterful summary by Singer and Nicolson (1972) that Branton’s bumps were recognized as proteins swimming in the lipid bilayer of the now familiar fluid mosaic model. JCB

Branton, D. 1966. Proc. Natl. Acad. Sci. USA. 55:1048–1056.
Dallner, G., et al. 1966. J. Cell Biol. 30:97–117.
Gorter, E., and F. Grendel. 1925. J. Exp. Med. 41:439–443.
Moor, H., et al. 1961. J. Biophys. Biochem. Cytol. 10:1–13.
Moor, H., and K. Mühlethaler. 1963. J. Cell Biol. 17:609–628.
Omura, T., et al. 1967. Proc. Natl. Acad. Sci. USA. 242:2389–2396.
Pinto da Silva, P., and D. Branton. 1970. J. Cell Biol. 45:598–605.
Robertson, J.D. 1957. J. Biophys. Biochem. Cytol. 1:1043–1048.
Singer, S.J., and G.L. Nicolson. 1972. Science. 175:720–731.
Steere, R.L., et al. 1957. J. Biophys. Biochem. Cytol. 3:45–60.
Stoeckenius, W., and D.M. Engelman. 1969. J. Cell Biol. 42:613–646.

Plane through a crystal of tobacco ringspot virus (left) made using Steere’s do-it-yourself cold operating box (middle). A later freeze fracture image is shown at right.