

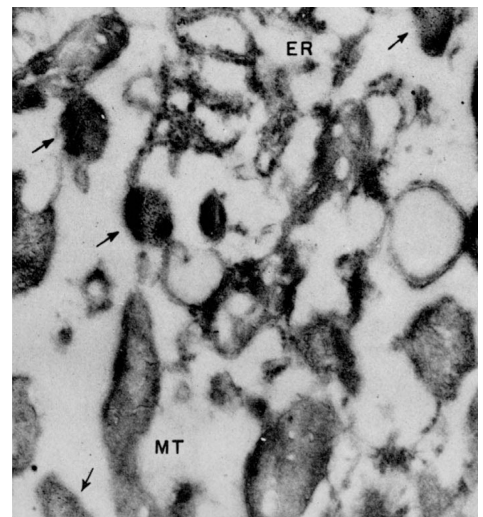
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## Catching sight of lysosomes

“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



A fraction rich in lysosomes (arrows) plus the odd mitochondria (MT).

DE DUVE

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## The invention of freeze fracture EM and

Russell Steere believed that different was good. His new method of freeze fracture electron microscopy (EM) might not be perfect, but “if artifacts”—the bane of all EM work—“are produced by this type of fixation they should at least differ from those resulting from chemical fixation.”

With this modest claim, Steere et al. (1957) introduced a method that has played “an absolutely critical role in the elucidation of membrane structure,” according to L. Andrew Staehelin (University of Colorado, Boulder, CO). Steere started with frozen samples and then combined a series of known methods—using an ultramicrotome for cutting, freeze drying to etch and expose surface features, and finally creating a replica with heavy metals. After Steere’s initial demonstration of the method on virus particles, a group in Zurich converted Steere’s rather home-made contraption into a more robust instrument (Moor et al., 1961; Moor and Mühlethaler, 1963).

The Zurich group was most interested in nuclear and chromosomal structure—the area, says Staehelin, “where freeze fracture has been least successful.” But Daniel Branton seized on it for investigating membrane structure. His study materials were onion roots and yeast (the latter isolated “by centrifugation from the starch used as a binder” in Fleischmann’s yeast cakes).

From this material, he concluded that membranes were bilayers, because his freeze fracture EM split membranes in half, thus revealing either of two internal membrane faces (Branton, 1966). He based this conclusion on three pieces of evidence. First, tangential fractures of variable width showed small portions that were clearly neither outer nor inner membrane surfaces, but something in between. More extensive faces of this kind, whether convex or concave, could not be etched, indicating that both faces were membranous. And neither face showed features characteristic of true

membrane surfaces, such as bound ribosomes.

Branton’s conclusion met with some resistance, which reflected the confused nature of the literature of the time. Stoekenius and Engelman (1969) castigated the field for its profusion of models that “seem to be taken too literally” and “are seldom critically and impartially enough presented. Few authors seem to be aware of all of them.” They concluded that “none of the many experimental results and arguments advanced in support of a given structure is compelling.”

Membrane structure studies had gotten off to a roaring start with the findings of Gorter and Grendel (1925). As they stated in the first sentence of their crisp, five-page paper, “we propose to demonstrate in this paper that the chromocytes of different animals are covered by a layer of lipoids just two molecules thick.” After measuring lipid quantities, cell numbers, and cell surface areas, they did just that: the result was a near-perfect 2:1 correlation.

Ten years later, Danielli extended this model to include protein layers on either side of the lipid bilayer, with the protein layers producing the observed low interfacial tension. Later studies showed that phospholipid head groups alone could produce the same effect. As Stoekenius and Engelman (1969) stated: “Despite the tenuous evidence upon which the model was originally proposed, it was widely accepted.”

The earliest electron micrographs of membranes from Robertson (1957), who saw a pair of dense lines, could also be seen as support for the Danielli model, although the laws governing heavy metal deposition were recognized as being obscure.

Meanwhile, other “subunit” theories had arisen, based on the concept that membranes could be built from lipoprotein subunits just as virus structures were built from protein sub-

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 correlate 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**

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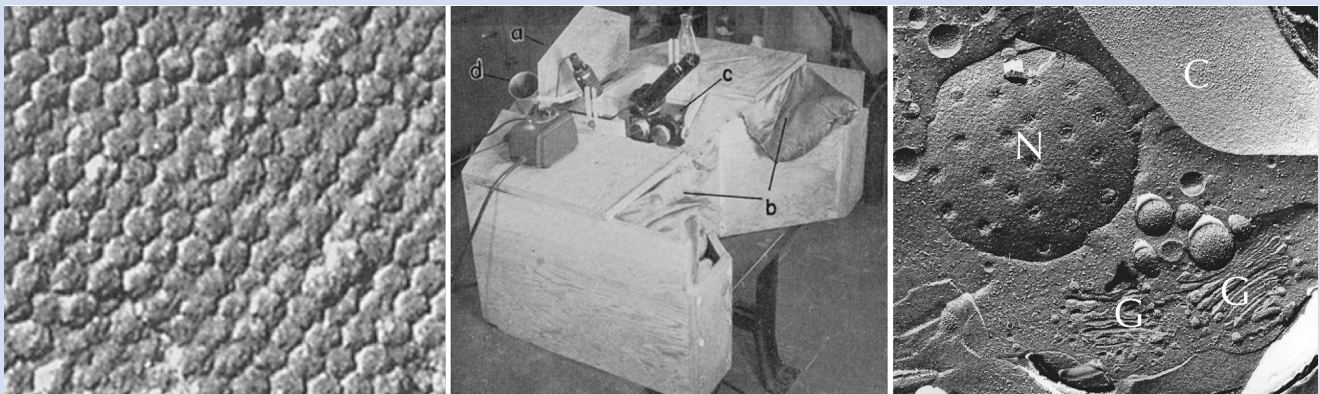
## the determination of membrane structure

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**

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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.