Keith R. Porter and the first electron micrograph of a cell

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Keith R. Porter died on 2 May 1997. Although he was especially renowned for the work on cell structure recounted here, his impact on cell biology was not confined to the early electron-microscopic studies of ultrastructure. To many, he was the father of cell biology, who helped establish many of the enduring institutions and ideas in the field. He had great biological intuition and feeling for a wide range of organisms and was greatly concerned with problems of cell shape and movement. He used ultrastructure and simple physiological or biochemical experiments to infer functional activities for cell organelles, including not only the endoplasmic reticulum, which he named, but the sarcoplasmic reticulum and T-tubules of muscle cells, microtubules, cilia, coated vesicles and more. He also pioneered cell studies with the high-voltage electron microscope, which led him to the idea of structural integration in the cell cytoplasm, an idea that is only now being pursued with success.

The transmission electron microscope was invented in the 1930s. It was not immediately obvious how this new instrument could be useful in the study of cell structure, despite the increase in resolution that the microscope afforded, which was, in practice, about two orders of magnitude more than the resolution of the light microscope. Use of the electron microscope in biology was also delayed by the Second World War. In 1945, the understanding of cell structure, more specifically structure of the cytoplasm, relied on a long series of observations and experiments undertaken earlier with the light microscope, summarized quite impressively by E.B. Wilson in his book *The Cell in Development and Heredity* [1].

There were many unsolved questions because of the inability of the light microscope to resolve structure at molecular dimensions. One interesting example was the controversy over whether yeast was a cell with standard organelles. No doubt bits and pieces of cells could be examined by electron microscopy, but it was important from a biological and biomedical perspective to build a bridge from the light-microscopic images of whole cells and tissues studied histologically or cytologically to the new images that were seen with the electron microscope.

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A major step was made by Porter *et al.* [2] in the images reproduced in Figure 1. The remarkable image on the left is the first published electron micrograph of a cell; the image on the right is a photomicrograph of a similar cell at not too different a magnification. The viewer is struck by the similarity of overall appearance and preservation of structure in the two images, but the increase in resolution, translated into sharpness of structural detail, in the electron micrograph, is also apparent. The images are of fibroblast-like cells, cultured from chick embryo tissues on slides coated with formvar film, fixed with osmium tetroxide vapour, air-dried and stained with Giemsa, for light microscopy, or directly transferred to a screen (grid) for electron microscopy. Porter later commented that, when he first put this specimen into the electron microscope, 'not much of worth was expected', but that to his delight the image contained 'more structural information than had been expected or could be interpreted' [3].

Osmium vapour fixation is the key to preservation of structure. When other standard fixatives for light microscopy were employed, fine structure was blown apart or otherwise destroyed [4]. The legend of the electron micrograph (Fig. 1a) points out a number of structural details, among which are filamentous mitochondria, osmiophilic structures 'especially abundant around the nucleus and presumably representing Golgi bodies' and a delicate lacework extending through the cytoplasm [2]. This continuous lacework, which did not penetrate into the cortical region of these cells, was later named the endoplasmic reticulum. A corresponding structure had been described by light microscopy in some secretory cells and in striated muscle, but it is quite difficult to see this in the corresponding photomicrograph (Fig. 1b).

For a decade or so after the article of Porter *et al.* had appeared, a number of laboratories laboured to translate these images into the standard electron-microscopic appearances of cell organelles that we know today. The laboratory of Porter and Palade at the Rockefeller Institute for Medical Research (now the Rockefeller University, NY, USA) was a leader in this endeavour, but technical advances from a variety of sources were utilized [3,5]. One necessary development was plastic embedding (methacrylate) [6], which, unlike paraffin embedding, permitted preservation of fine structure after hardening. A second development was the ultramicrotome. The mechanical-advance ultramicrotome designed by Porter and Blum [7] was a successful model that permitted methacrylate blocks to be cut into ribbons of thin sections. A third complementary development was the glass knife [8], which cut the sections over a boat filled with water on which the sections floated [9].

The reader is invited to compare an enlarged image of a region of whole-mounted cell (Fig. 2a), prepared in a similar fashion to the chick fibroblast of Fig. 1, with the result of a successful fixation, embedding and thin sectioning of a chick monocyte (Fig. 2b), taken from Porter's Harvey Lecture [10] on 'The submicroscope morphology of protoplasm' given in 1956. In the whole mount, the bacillus-like mitochondria can clearly be seen lying against the strings of bladders or vesicles that make up the endoplasmic reticulum. What the corresponding structures are in the thin section is not evident. Patient reconstruction and some good biological intuition were

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Figure 1 Plate 10 of Porter, Claude and Fullam (Ref. 2). (a) The first electron micrograph of a cell. The preparation shows a 'fibroblast-like cell and nerve fibers cultured from chick embryo tissue', studied with an RCA EM-B microscope. The nucleus is too thick for penetration by low-energy electrons, but the cytoplasm at the margins of the cell is thin enough to reveal organellar disposition. Besides preserving substructure, osmium fixation also helps to provide contrast in the specimen. Bar, 7.7 mm. (b) A corresponding cell prepared for light microscopy, fixed with 2% osmium tetroxide vapour but stained with Giemsa for contrast. Bar, 9.8 mm. (Reproduced, with permission, from [2].)

necessary to make the correlation between the images; familiarity with histological and anatomical profiles of sectioned structures also helped. As well as the cell nucleus (N) and sections of mitochondria (m), whose internal structure does not reproduce well here (but which is clear in the original), as Porter says, the figure 'shows circular or oblong profiles which represent sections' through the vesicular members of the endoplasmic reticulum (er). With such reconstructions, we moved from the first electron-microscopic image of a cell in 1945 to the standard thin-section images that we know today.

In addition to the novel efforts that the images here represent, which brought cell-structure studies from the limitations of the light microscope into the molecular era, Keith Porter was responsible for some of the instrumentation and much of the institutionalization of cell biology. His requirement for cell cultures for use in electron microscopy led to the development of the roller flask and inspired the commercial production of tissue-culture media [11]. His desire for an enlarged forum in which students of cell fine structure could present their work led to the foundation of the American Society for Cell Biology.

The path was not always easy. After many a lecture, Porter was asked why he thought that he was not dealing just with fixation artifacts. He usually answered that the electron-microscopic images of cells and cell constituents were consistent and reproducible from preparation to preparation and from cell type to cell type, and that, although one could not really distinguish a consistent artifact from reality, even a consistent artifact must have some meaning in terms of molecular arrangements and overall function.

The take-home lesson from the early electron-microscopic studies of cells is that the basic construction of organelles – such as mitochondria or endoplasmic reticulum – is the same in all plant and animal cells. Elsewhere, I have called this, now transparent, conclusion 'the organelle doctrine'. In molecular terms, the organelle doctrine says that the macromolecules that assemble in specific ways to form what we recognize as the substructure of an organelle are the same or very similar in all eukaryotic cells. This substructure is a feature of common descent and evolution, which makes possible the related biochemistry and physiology of an organelle and the construction of superfamilies of protein molecules. The structural correspondence is the reason why we recognize one set of molecules as endoplasmic reticulum and another as mitochondria in cells as different as yeast, *Tetrahymena* and human liver. The first electron micrograph

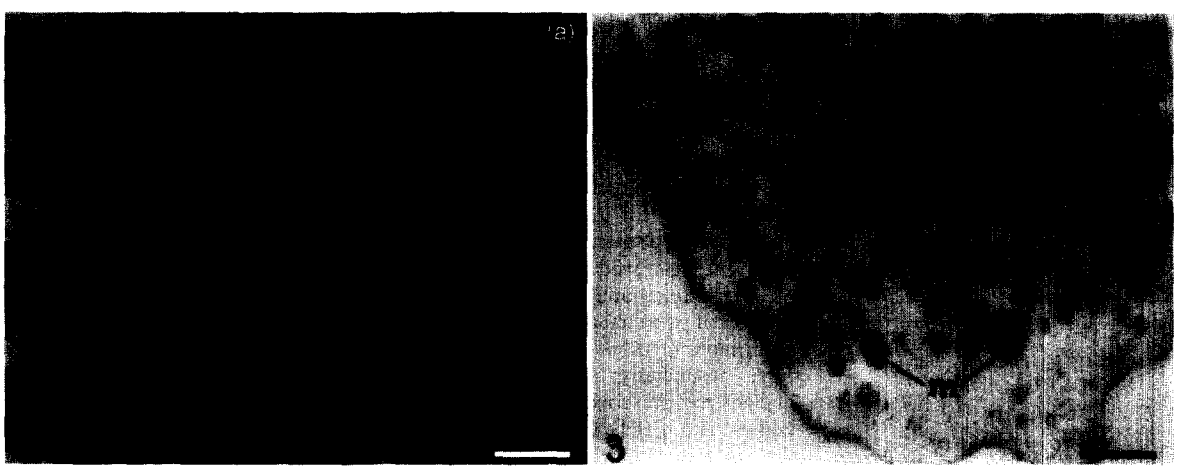

Figure 2 (a) Fig. 2 of Porter's Harvey Lecture[10]. An enlarged area of the margin of a whole-mounted chick macrophage, showing details of cytoplasmic structure, essentially as in the electron micrograph of Fig. 1a. The reticular nature of the endoplasmic reticulum (er) is particularly clear. Bar, 0.67 μm. (b) Fig. 3 of Porter's Harvey Lecture[10]. A corresponding thin-section micrograph of a chick monocyte. This preparation was fixed in buffered osmium tetroxide, dehydrated, embedded in n-butylmethacrylate and sectioned. The continuity of the endoplasmic reticulum is lost in the section. In his article[10], Porter demonstrated that the overall appearances of the various organelles in thin section in this preparation, in liver and in onion root-tip cells are the same. Bar, 0.56 μm. (Reproduced, with permission, from [10].)

of a cell was a landmark, which eventually led to a molecular understanding of cell evolution and organelle function.

As indicated here, Porter's work formed the basis for cell-biological electron microscopy. Much of our modern structural and functional understanding of smooth and rough endoplasmic reticulum, cilia and centrioles, endocytosis, lipid adsorption in the intestine, stimulus–contraction coupling in muscle, and microtubules, among others, comes from his laboratory. The organelle doctrine, the single encompassing idea of fine structural study, follows directly from his extensive comparative studies of so many types of cells. Despite all this, and the special distinction represented by his achievement in taking the first electron micrograph of a recognizable cell, the work was overlooked by the Nobel Committee honouring the pioneers of cell biology. It seems probable that history, like many cell biologists from those early years, will judge this to be an unfortunate oversight.

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