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# The unit membrane, the endoplasmic reticulum, and the nuclear pores are artefacts

Harold Hillman, Peter Sartory
Unity Laboratory, Department of Human Biology and Health, University of Surrey, Guildford, Surrey GU2 5XH, England
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**Abstract.** It is shown on the basis of solid geometry that the trilaminar appearance of membranes described by Robertson must be an artefact, although the membranes themselves are not. However, considerations of solid geometry as well as observations on living cells indicate that the endoplasmic reticulum and nuclear pores are artefacts resulting from preparation for electron microscopy. Suggestions for their genesis are proposed.

## 1 Introduction

Until the middle of the 1940s a cell was considered to consist of a membrane, cytoplasm, mitochondria, a nucleus, a nucleolus, and often a centriole and/or a Golgi apparatus. At that time, the electron microscope, which permitted magnifications two to three orders greater than was possible with the light microscope, was applied to cytology. A number of new features were seen, and they were summarised in the 'generalised cell'[7, 24, 29, 44, 50, 53]. Experiments relating the fine structure to the function of the living cell have been a major preoccupation of cytologists, electron microscopists, biophysicists, biochemists, and physiologists, and findings derived from them have been accumulated in the basic data bank from which experimental cellular pathologists—especially oncologists and geneticists—draw their currency.

The new features seen were:

(i) the single membrane of light microscopy was observed as being trilaminar—the 'unit' membrane[43];

(ii) an extensive endoplasmic reticulum[40] consisting either of a fine network[4, 37] or of flattened sacs[47, 48] was seen permeating throughout the cytoplasm, not necessarily uniformly; it was attached to the cell membrane and the nuclear membrane;

(iii) pores were described in the nuclear membrane[8];

(iv) the internal structure of the mitochondria was seen as shelf-like 'cristae'[46].

## 2 The unit membrane

Micrographs in modern standard textbooks[5, 6, 11, 13, 18, 19, 24, 27, 29, 33, 36, 42, 44, 49-51, 53, 56], or in any issue of such journals as the *Journal of Ultrastructure Research*, the *Journal of Microscopy*, the *Journal of Cell Biology*, all show the 'unit' membrane as two lines approximately the same distance apart throughout the tissue, and this appearance is shared by the nuclear membrane, the mitochondrial membrane, the cristae of the mitochondria, and the endoplasmic reticulum (please see below). We can find no publication in which the reality in the living cell of the 'unit' membrane has been doubted, although we have often been told that it is no longer believed. It should be emphasised that—except in the cases of the endoplasmic reticulum and the cristae—we are not denying the *existence* of the membrane. We are demonstrating here that the two-line, or 'trilaminar', appearance is an artefact.

If one were to cut sections of equally spaced concentric spheres through their common centre, each section would appear to be of uniform and equal distance apart. On the other hand, if the sections were cut away from the centre, the spacing

of the shells would appear to increase towards the periphery (figure 1). This simple consideration of solid geometry would not permit any layer to appear of constant thickness, irrespective of the orientation in which it had been cut. While the microtomist might deliberately cut a section precisely through the centre of a particular cell's membrane, it is quite inconceivable that all the cells in a section, as well as all their membranes, their endoplasmic reticula, their mitochondria and their cristae would be so arranged at all times that all their geometrical centres coincided.

The following explanation may be offered for the uniformity of the 'unit' membrane. A line is a geometrical abstraction; it has position but no thickness. A real layer, like a membrane, has two surfaces, so that if one looks at salts deposited on its surface, it will always appear as two lines if viewed with high enough magnification. However, the fact that the two lines always appear equally spaced throughout the tissue indicates that this appearance must have resulted from preparation of the tissue *after* the section had been cut. It represents a true two-dimensional picture.

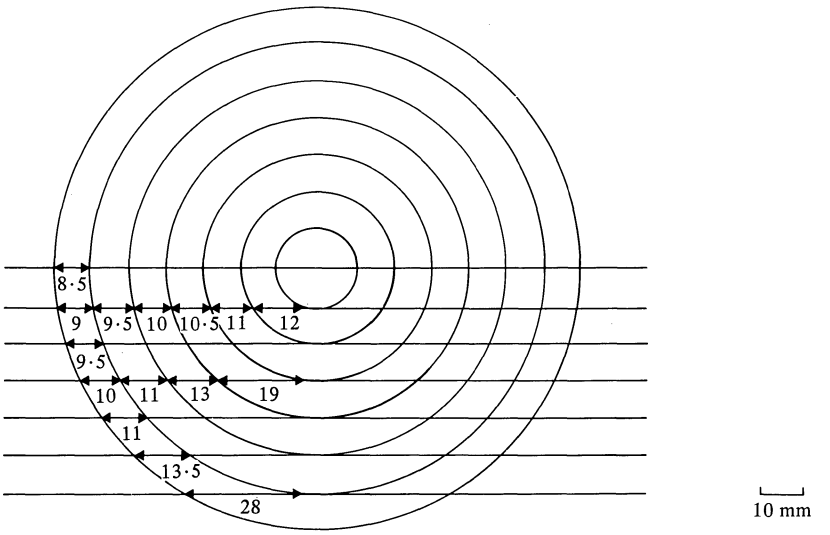



**Figure 1.** In a section made through equally spaced concentric spheres, the spheres will appear to be farther apart as the section is made farther from the geometrical centre.

### 3 The endoplasmic reticulum

The endoplasmic reticulum suffers from the geometrical disability discussed above, but there is another related geometrical reason for doubting its existence in the living cell. Each 'strand' of reticulum, as well as the two lines of which each strand appears to be composed, are in all cases seen in micrographs in perfect longitudinal section[4-7, 11, 13, 18, 19, 24, 27, 29, 36, 37, 40, 42-44, 46, 49-51, 53, 56]. Whether the reticulum is a real net[4, 37] or flattened vesicles[47, 48], it should sometimes appear in oblique or transverse section if it is randomly orientated throughout the cytoplasm. Other orientations in which one might expect to see it are shown (figure 2) but it is extremely rare to see views other than the well-known equally spaced parallel lines.

In response to this point, it has been said that electron microscopists only chose for publications those micrographs which show the reticulum in one orientation; this explanation would be acceptable if one were looking at a small part of the cell, but not if one views the *whole* cell[6, 13]; in such a picture one should see a complete random selection of sections of a three-dimensional structure. It is also sometimes

suggested that one only sees membranes on transverse section, because there is too little heavy metal in the pathway of the electron beam if the tissue is orientated longitudinally; if this were the case, one should not see longitudinal sections of muscle filaments[25] or of microtubules, which are of the same proportions[3, 12].

A third reason which throws doubt on the existence of the net concerns its attachment to the cell membrane. If the cell membrane appears as two lines, then the reticulum should always appear as four lines or 'cisternae' should be present; the reticulum usually appears as two lines. Alternatively, the reticulum could 'puncture' the cell membrane; this is not normally seen. The third possibility is that the reticulum is closed by the outer line of the cell membrane (figure 3). The latter is sometimes seen in electron micrographs, but does not correspond to the diagrams of the generalised cell, and would mean that the extracellular space was not in contact with the channel in the reticulum.

If one wishes to see living cells, one has to examine plant cells, protozoa, sperms, eggs, or tissue cultures. In all of these one sees intracellular movements, which are the main criteria by which one decides if the cells are alive or not; sometimes they must be observed by time-lapse photography. Most of these movements were

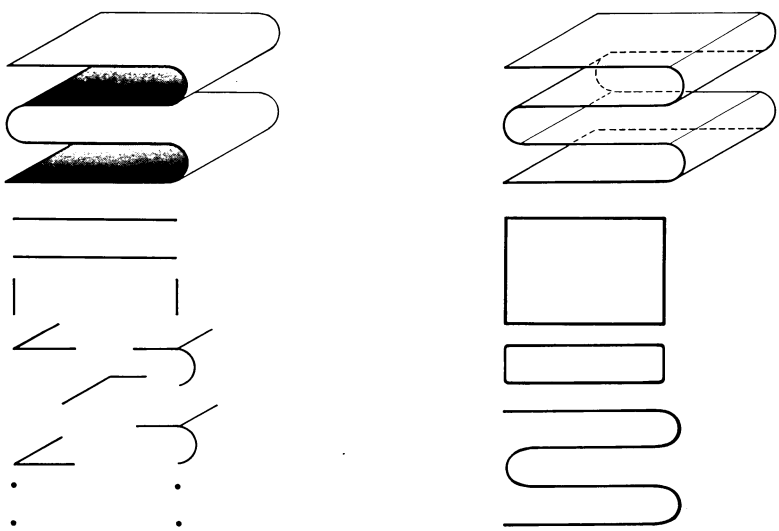



**Figure 2.** Appearances which would be expected if one cut sections of an endoplasmic reticulum randomly orientated within the cytoplasm, if the reticulum were composed of flattened vesicles or a real net.

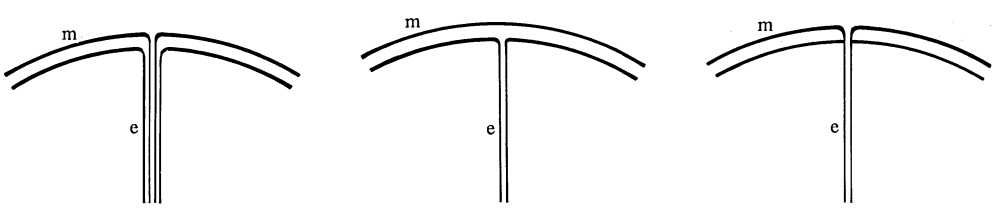



**Figure 3.** The three possible ways in which the two lines of the 'unit' membrane (m) could be attached to the endoplasmic reticulum (e). In the left-hand diagram, the reticulum would have to appear as four lines *or* cisternae would have to be present; in the middle diagram, the extracellular space would not be continuous with the channel in the reticulum; the right-hand diagram shows an appearance which is not seen in the literature.

observed during the 19th century with microscopes having magnifications of only 100 to 200 times. For example, streaming in plant cells[26], which are also supposed to have an endoplasmic reticulum[34], was first observed by Corti in 1774[9]. Brownian movement of small particles or bacteria was shown by Robert Brown to Darwin before he went on the voyage of the Beagle[10]. Diffusion of injected carbon particles and iron filings[23], nuclear rotation[39], pinocytosis[28], and phagocytosis[20], are all seen. These would not be possible if there were a three-dimensional net or system of flattened sacs which were attached to the cell membrane, and which required 100 to 1000 times the magnification to see. The electron microscopists say that one must have a 'dynamic' view of the reticulum[2,45] or, more specifically, that it is liquid; thus large particles could float through it. However, one liquid (the reticulum) suspended in another (the cytoplasm) can either float on it, sink in it, or be dispersed as a suspension. It *cannot* form shapes (i.e. a net) within it.

The endoplasmic reticulum probably consists of precipitate of the cytoplasm, which is an aqueous suspension in the living cell. During preparation for electron microscopy, whether classical transmission techniques[55] or freezing techniques[35] are used, dehydration always occurs[30]. If freezing is rapid, the ice may not be evaporated, but it *will* separate from the other constituents of the cytoplasm. Solutions only of potassium chloride, amino acids, and other organic materials, have been studied extensively by cryobiologists using both light and electron microscopy[32,41]. They show characteristic crystalline structures, often with repeating patterns, but no one would suggest that there is a fine network in a free solution of potassium chloride or alanine.

## 4 Nuclear pores

The nuclear pores always appear on transverse sections of the nuclear membrane as slits, or on plan view as circles; they are remarkably uniform in diameter and shape, even on tangential sections[8,16,17]. Why does one never see them in intermediate orientations, for example, as ovals (figure 4)? Furthermore their apparent size on tangential section of the nuclear membrane should depend upon how deep they are in the section (figure 5); their diameters should vary between zero and maximum.

If the nuclear membranes are punctured by pores which are 3%–32% of their total areas[15], the small ions could not be kept separate between the nucleus and the cytoplasm. Molecules with weights less than 10000 are less than 4 nm in diameter, and most inorganic ions are less than 0·9 nm in diameter[21], yet the pores in cells of different tissues are supposed to be 20–120 nm wide[1,8,14,15,22,38,52,54]. They are also supposed not to 'leak', but in most cells potential differences have been found across the nuclear membranes[31]; these potential differences would be short-circuited by pores.

The simplest explanation for pores is that the appearance on plan view is due to bubbles, which can often be seen in cytoplasm on inspection of micrographs.

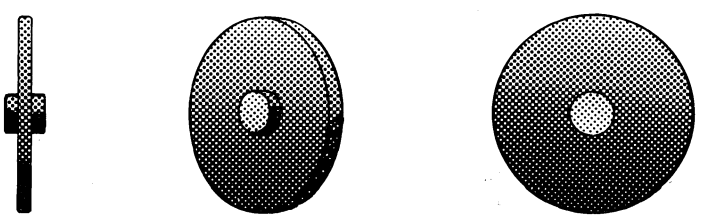
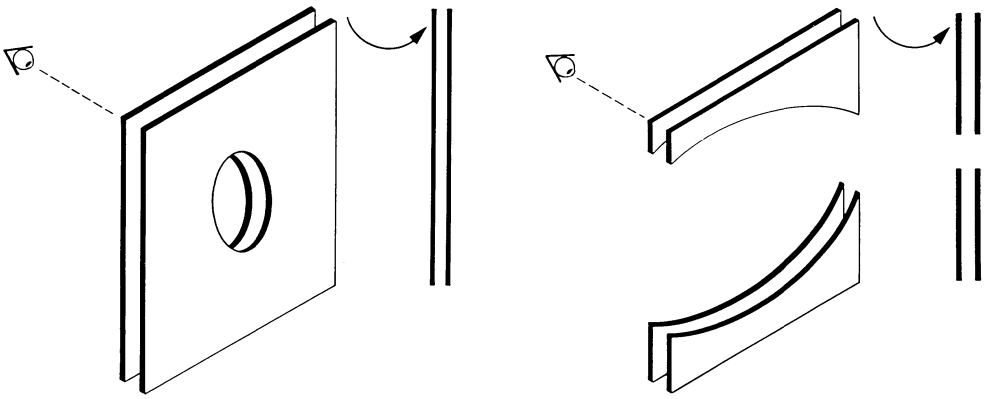



**Figure 4.** Nuclear pores as slits (a) or circles (c). Intermediate views (b) are not seen.

On transverse section, they could be cracks in the nuclear membrane that are due to shearing between the nucleus and cytoplasm; these two compartments have different concentrations of water and chemical constitutions, so they are likely to have different temperature coefficients of cooling.



(a) (b)

**Figure 5.** This diagram shows that nuclear pores will not be seen on transverse section of the nuclear membrane unless, firstly, their diameter exceeds the thickness of the section; secondly, the section cuts *both* sides of the pore. It also shows that on transverse section, the pore will appear to have the diameter of the smaller arc which is cut by the fact of the section, and thus one would not expect pores to appear to have uniform diameters.

## 5 Mitochondria

Mitochondrial cristae also appear the same width in whatever orientation they are cut[5, 6, 11, 13, 18, 19, 24, 27, 29, 33, 36, 42, 46-51, 53, 56], and so one would again offer the explanation that they are two-dimensional precipitates. Therefore, by analogy with the cytoplasm, it is likely that living mitochondria are membranous tubular structures containing a liquid 'mitochondrioplasm', which precipitates during dehydration for electron microscopy.

We would like to stress the following two points. Firstly, whereas the 'unit' membranes, the nuclear membranes, the mitochondrial membranes, the cristae, and the endoplasmic reticulum always appear two-dimensional, the whole mitochondria[42], and also muscle filaments can be seen on electron microscopy in various orientations in a single section[25]; thus the two-dimensional appearance is *not* a necessary artefact of electron microscopy as a technique. Secondly, our explanations for the appearance of structures which we regard as artefactual are put forward as simple hypotheses. Even if they are wrong, this would not in any way invalidate the evidence on geometrical and biological grounds that the structures could not exist in living cells.

We conclude that the *two-line appearance* of the cell membrane, the nuclear membrane, and the mitochondrial membranes—as opposed to the membranes themselves—are artefacts of preparation for electron microscopy, as are the endoplasmic reticulum and nuclear pores. They are not compatible with the universally accepted properties of living cells, or with solid geometry. It would be impossible to construct a three-dimensional model of the living cell based either on any published electron micrograph of a whole cell *or* on any diagram showing the above structures, if the model also permitted intracellular movements.

The centrioles, the Golgi apparatus, and the lysosomes have not been considered in this paper.

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