

## DEMONSTRATION OF A HIGHLY ORDERED PATTERN UPON A MITOCHONDRIAL SURFACE

DANIEL C. PEASE. From the Department of Anatomy, School of Medicine, University of California at Los Angeles

Synaptic bodies (spherules) of the rod cells of the cat retina commonly contain several conspicuous mitochondria. These have been observed frequently to aggregate in an unusually intimate way, in pairs, or in small clusters of as many as seven (Fig. 2). An exceptional feature of this association is that the apposing surface membranes of adjacent mitochondria come to lie closely together with a strictly uniform spacing of approximately 125 Angstrom units between them. In such areas of contact the outer apposed osmiophilic membranes assume a markedly beaded appearance having great regularity (Figs. 1 and 3). The periodicity illustrated by these structured membranes is 160 Angstrom units. This latter measurement has been made with considerable accuracy, for it has been possible to count over 100 consecutive periods, and to compare their average length with a replica of a diffraction grating micrographed under conditions as nearly identical as possible. Poorly defined, osmiophilic cross-linkages are also sometimes visible from one membrane to the other across the uniform gap (Fig. 3). Thus, the two apposing membranes demonstrate a repeating lattice structure of constant dimensions.

Where adjacent membranes diverge from one another there is an abrupt change to smoothly contoured surface lines without evidence of periodic structure (Fig. 1). Thus the free surfaces of these mitochondria are in no way marked by unusual features. Also, the inner osmiophilic lines of the mitochondrial envelopes, as well as their reflections over all the surfaces of the cristae, are without a visible organizational pattern. The gap between inner and outer lines is not of particularly regular width, although of course distortions may have occurred during preparative techniques. Clearly, this space lacks the uniformly structured order of the gap between adjacent mitochondria. Thus, these spaces are fundamentally dissimilar in physical character.

Much of our present detailed knowledge of cytomembranes derives from our current understanding of the structure of nerve myelin and its relationship to the Schwann cell surface. Thus,

Robertson (5) has introduced the concept of the "unit membrane" and has studied in detail how their surfaces as found in plasma membranes can associate with neighboring membranes, at times to form truly fused or combined structures which he terms "compound membranes." The association between adjacent mitochondrial membranes described here, however, is not so intimate as in an "internal compound membrane" for the gap between the osmiophilic lines can be seen to be a direct extension of the general cytoplasm (Fig. 1).

It is only very rarely that a visibly ordered cross-linkage between adjacent plasma membranes has been described. But, in desmosome-like structures, particularly in invertebrates, patterns somewhat comparable to that described here occasionally have been noted. The most elaborate of these has been found by Wood in *Hydra* (9) where a repeating linear component was demonstrated. In the present study sufficiently oblique sections were obtained through the apposed membranes of adjacent mitochondria to reveal linear patterns had they been present. None was found, however, so it is concluded that here we are dealing with an arrangement different from that in the *Hydra* desmosome. It is perhaps more comparable with the periodic pattern observed in desmosome-like structures found in the axon sheaths of earthworms by Hama (2). Whether the latter is truly homologous with the presently described periodic structure is a moot question. It is evident that it has only been possible to visualize a marked periodicity of cytomembranes under very exceptional circumstances.

Synaptic bodies of mammalian retinal receptor cells have been examined in detail in three previous studies. Sjöstrand (7) has made a three dimensional reconstruction of the guinea pig synapse, based upon an exhaustive study of serial sections. The published illustrations indicate the presence of only a few small mitochondria in this area. Ladman (3) studied the synaptic bulbs of rats, and commented that there is regularly but one large mitochondrion in each spherule of this

species. In rabbits, De Robertis and Franchi (1) have emphasized specifically that mitochondria are completely absent in the spherules. Thus it is evident that there is considerable species variation, and it is likely that the cat is unusually well endowed with mitochondria in this locus.

It is worth comment that one finds unusually crowded mitochondria in the inner rod segment portions of these same receptor cells of the cat retina without being able to observe the intimate structured associations found in the spherules.

It is possible, but unlikely, that the preparative techniques employed influenced the results. The retinal material was collected from a cat, the head of which had been perfused *via* the aortic arch with buffered 10 per cent formalin, and was only later treated with buffered osmium tetroxide in a manner previously described by Pease (4). The perfusion was successful in blanching the eyes. The isolated retina reached the osmium fixative about 15 minutes after the start of the procedure. Embedding was in Araldite, followed by PbOH staining in the manner of Watson (8). Subsequent electron microscopic examination of all layers of the retina and choroid, as well as various brain areas, indicated that the tissue had in fact been well preserved by conventional standards.

Although the ordered structure of the mitochondrial membrane that has been described here seems like an almost unique observation, it does seem reasonable to generalize the conclusions. Thus it appears that it requires special cross-linking circumstances to reveal what must be a fundamental aspect of the mitochondrial envelope. The 160 Angstrom unit surface mosaic is unmasked only when there is interaction between adjacent membranes. Just as soon as the paired relationship is lost, as the membranes diverge away from one another, so is lost the visual evidence of pattern and order. In so far as intracellular membrane surfaces can be thought of as being covered with osmiophilic protein, it is tempting to suggest that the observed "beading" reflects the superficial distribution of macromolecular complexes, basi-

cally proteinaceous in character. The outer osmiophilic line of the mitochondrial envelope currently is regarded as a unit membrane by Robertson (6). Present observations are compatible with the thought that the periodic alterations as actually seen involve the entire molecular structure of such a membrane, including the lipid layers. Thus the dense spots may represent regions where there was less than the usual structural collapse upon fixation, in turn dependent upon the added support realized from the cross-linkages between adjacent membranes.

#### *Note Added in Proof*

After this article had been accepted for publication, this author learned of a remarkable visualization of an ordered mosaic seen in saponin-extracted plasma membranes by a negative staining technique (Dourmashkin, R. R., Dougherty, R. M., and Harris, R. J. C., *Nature*, 1962, 194, 1116). An hexagonal array of "pits" with center-to-center spacing of 140 to 160 Angstrom units was demonstrated on membranes of chicken liver and Rous sarcoma cells, as well as in erythrocytic ghosts. Since the spacing and regularity of this pattern seem similar to those reported here, it seems possible, and even likely, that a common interpretation eventually may be found. Although Dourmashkin *et al.* discuss their findings in relation to "mosaic" models of plasma membranes, such concepts are hard indeed to reconcile with conventional electron microscopic images of "unit membranes." However, F. S. Sjöstrand has some evidence for the compartmentalization of unit membranes in mitochondrial cristae after conventional preservation (presented at the V International Congress for Electron Microscopy, Philadelphia, 1962, and elsewhere, manuscript in preparation). So ultimately it may prove possible to construct a reasonably precise model of cytomembranes that will include an ordered mosaic of components.

Supported in part by a grant of the United States Public Health Service.

*Received for publication, June 18, 1962.*

References on page 388.

---

#### FIGURE 1

Part of a cluster of associated mitochondria from a spherule of a rod cell of the cat retina. The margin of the cluster is to the left. Apposed surface membranes of adjacent mitochondria traverse the figure. These exhibit a high degree of order. But where the outer mitochondrial membranes diverge from one another, the ordered structure ceases to be visible. Mitochondrial cristae and synaptic vesicles are evident.  $\times 98,000$ .



#### REFERENCES

1. DE ROBERTIS, E., and FRANCHI, C. M., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 307.
2. HAMA, K., *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 61.
3. LADMAN, A. J., *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 459.
4. PEASE, D. C., *Anat. Rec.*, 1962, **142**, 342.
5. ROBERTSON, J. D., *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 349.
6. ROBERTSON, J. D., Cell membranes and the origin of mitochondria, in *Regional Neurochemistry, Proceedings of the 4th Neurochemistry Symposium* (S. S. Kety and J. Elkes, editors), Oxford, Pergamon Press, 1961, 497.
7. SJÖSTRAND, F., *J. Ultrastruct. Research*, 1958, **2**, 122.
8. WATSON, M. L., *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 727.
9. WOOD, R. L., *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 343.

---

#### FIGURE 2

A cluster of six closely associated mitochondria from a spherule of the cat retina.  $\times 24,000$ .

#### FIGURE 3

Apposed surface membranes of associated mitochondria showing a 160 Angstrom unit repeating structure in the outer osmiophilic lines. Adjacent lines are sometimes in register with each other, and the gap between them is of constant width, the center-to-center spacing between them being approximately 125 Angstrom units. No such regularity is to be found in the gap width between inner and outer osmiophilic lines, or in the width of the cristae. Ill-defined osmiophilic cross-linkages between apposed membranes sometimes are visible, particularly so in the paired membranes in the lower right corner of this figure.  $\times 162,000$ .

