A DOUBLE LAYER OF PROTEIN IN MITOCHONDRIAL CRISTAE

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When mitochondrial cristae membranes are treated with detergents they are split into two membranous fractions each of which is thinner than the original membrane. The thicker membrane is reformed when the two fractions are recombined to restore NADH oxidase activity. We suggest that the cristae membrane is made up of two lipoprotein layers of different composition.

Disruption of mitochondrial cristae by a variety of solvents and detergents gives rise to a similar pattern of fragmentation in all cases. This pattern can best be described as a red-green split. Separation of the red cytochromes, b and c_1, from the green cytochromes, a and a_3, by amyl alcohol (Green et al 1955) and deoxycholate treatment (Crane and Glenn 1957) has been described. A procedure has recently been developed by Jacobs et al 1966a which achieves the same separation by use of the non-ionic detergent Triton X-114.

Thin sections of the membranes in the red and green fractions show unit membrane structure with thinner cross sections than the original cristae membrane. The cristae membranes show an average overall cross section of 80 Å (figure 1). The cytochrome oxidase ($a + a_3$) membrane ranges from 40 Å to 60 Å cross section with an average of 55 Å (figure 2). The red fraction membrane ($b + c_1$) show cross section from 35 Å to 55 Å with an average of 45 Å (figure 3).

Fig. 1. Section of beef heart mitochondria fixed for 2 hours in osmium tetroxide and post stained with $KMnO_4$. Marker 200 Å. Note the dark line in the center of the membrane at the arrow. Insert mitochondria cristae section. Marker 100 Å.

The cross sections of the membranes are consistent with the globular structure seen in each membrane by negative staining. Cristae membranes contain 90 Å basepiece units (Green and Perdue 1966). Cytochrome oxidase membranes contain 50 Å globular units which are consistent with the 70,000 molecular weight of cytochrome oxidase (McConnell et al 1966). Membranes which contain cytochromes b and c_1 show 35-40 Å globules which are consistent with molecular weights of 30,000 (Bomstein et al 1961) and 40,000 (Goldberger et al 1961) proposed for cytochromes b and c_1 respectively. Thus the thickness of the membranous fragments is consistent with the size of their

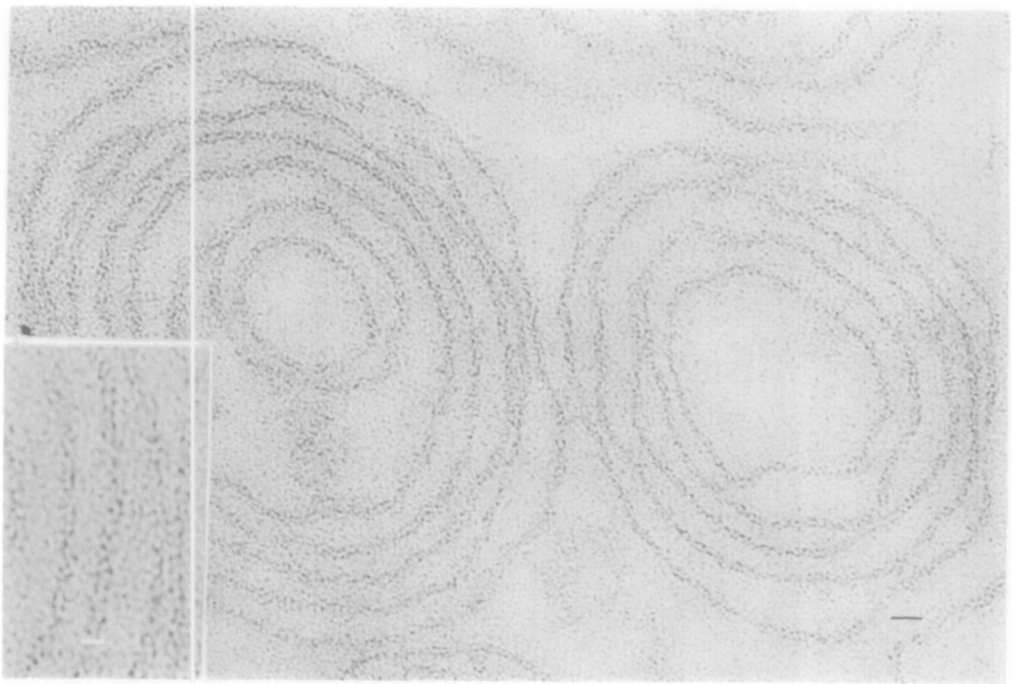

Fig. 2. Cytochrome oxidase section prepared as for figure 1. Marker 200 Å. Insert enlarged. Marker 100 Å.

component globules which in turn are of a size appropriate to their molecular weight. The red-green split can be explained on the basis of separating the cristae membrane longitudinally into two thinner membranes. In the original membrane there could be interdigitation between the globules of each membrane layer to the extent of 20 Å to account for the fact that the cristae membrane appears thinner than the sum of the two parts.

When the red fraction and green fraction are recombined according to the method of Hatefi et al 1962 to restore NADH oxidase activity much of the membrane is again 75 Å thick in sections so that it corresponds to the original cristae. Some areas still show the thinner membrane elements which indicates that the reconstitution is not complete.

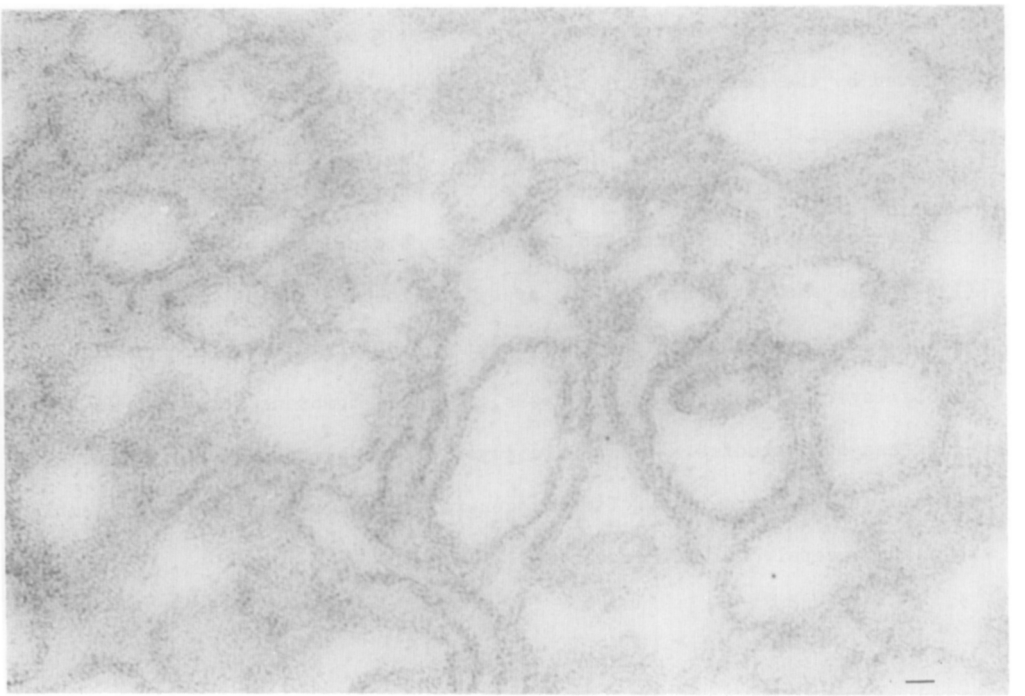

Fig. 3. Section of red fraction prepared by deoxycholate treatment of mitochondria. Prepared as in figure 1. Marker 200 Ä.

Lipid free cytochrome oxidase does not form membranes (Jacobs et al 1966b) and shows dispersed 20 Å strings by negative staining. As lipid is added the strings cluster into 50 Å globules in small groups. When the lipid content reaches 8% one observes membranes made up of closely packed globules. As the lipid content is increased to 50% the globules are more widely dispersed over a smooth sheet. From this we infer that the globules are lipoprotein elements of cytochrome oxidase which can cluster together to form a membranous sheet. Additional lipid can then be inserted between the globules.

Evidence for globular structure in mitochondrial cristae has also been presented by Sjostrand (1963). In sections he shows 40 Å globules in a single row along the membrane. In the figures which he presents one can also

observe sections of membrane with double rows of 40 Å globules. We interpret this to mean that the fixation procedure can lead to a separation of the double layer membrane into single layers equivalent to the separation achieved by the red-green split.

Fragmentation of chloroplasts with digitonin (Henninger et al 1967) and Triton (Vernon et al 1966) also shows separation of a thick membrane into thinner fragments. Freeze-etch studies of chloroplasts by Branton and Park (1967) also show evidence for two types of globules inside the membrane structure. Frey-Wyssling and Muhelethaler (1965) have also proposed a double layer of globules as the basis of plasma membrane structure as a result of freeze-etch studies. The double layer of lipoprotein globules found in mitochondrial cristae may reflect a general structure for the thick membranes found in several cellular organelles.

Acknowledgements

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