

## FUSION OF UNIT MEMBRANES OF THE ENDOPLASMIC RETICULUM IN THE GILL EPITHELIAL CELL OF SHRIMP, *CARIDINA*

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### INTRODUCTION

During the course of an investigation of the gill of the shrimp, *Caridina*, a specific kind of differentiation of the membranes of the endoplasmic reticulum was observed. It is represented by two apposed, fused cisternae which have bifacial, granular, and agranular, profiles. With progress

in electron microscopy a number of important observations have been made on the differentiation of the biological membrane, one of which is the close contact or fusion of unit membranes, frequently observed in various vertebrate tissues (1-7). However, it seems noteworthy to indicate

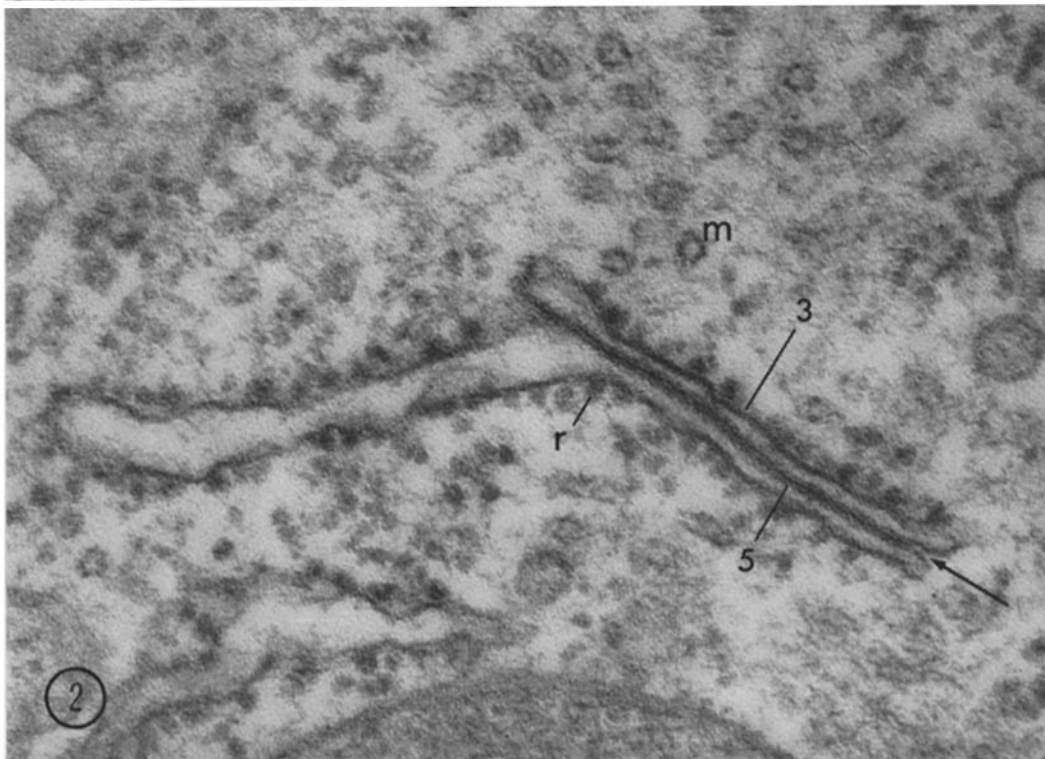


FIGURE 1 Basal portions of gill epithelial cells of shrimp. Note the closely apposed endoplasmic cisternae (arrow) among ordinary organelles such as mitochondria, granular endoplasmic reticulum, free ribosomes (*r*), vesicles, lysosomal bodies, and microtubules (*m*).  $\times 34,000$ .

FIGURE 2 High magnification of the closely apposed cisternae shown in Fig. 1. Note that localized areas of the confronting cisternal membranes are free of ribosomes and fuse to form a pentapartite-layered structure (indicated by 5), and that the width of the two apposed membranes is consistently twice (or slightly less) that of a single, separated cisternal membrane which shows a trilaminar structure in thin section (indicated by 3). *m*, microtubules; *r*, ribosomes.  $\times 112,000$

that membrane fusion has been reported to occur between adjacent plasma membranes or their derivatives, but that it appears not to have been previously observed to occur between intracytoplasmic membranes. Since the situation appears to be the same with the invertebrate tissues, it was felt that a report of this observation would be of interest.

#### MATERIALS AND METHODS

Gills were obtained from the shrimp, *Caridina*, without anesthesia, and were fixed for 2 hr at 4°C in a 2% glutaraldehyde solution buffered to a final pH of 7.4 with *s* collidine buffer. The tissue was then rinsed in the same buffer and subsequently postfixed at 4°C in 1% osmium tetroxide pH 7.4 (*s* collidine buffer). Dehydration was carried out in a graded series of ethanol at 4°C, followed by embedding in Epon. Blocks were sectioned on a Porter-Blum MT-1 ultramicrotome and stained with both uranyl acetate and lead citrate at room temperature, and examined in a Hitachi HL-12 electron microscope at 75 kv.

#### RESULTS AND DISCUSSION

In the micrograph presented in Fig. 1 are shown the basal portions of gill epithelial cells of the shrimp. Among the various ordinary organelles such as mitochondria, vesicles, microtubules, and lysosomal granules is observed a considerable amount of granular endoplasmic reticulum and free ribosomes. As indicated by an arrow, there are two, closely apposed cisternae of the granular endoplasmic reticulum. It is to be noted that the localized area of contact between the membranes of the two cisternae is free of ribosomes.

As shown at high magnification (Fig. 2), the closely applied membranes apparently make direct contact without any space left between them, for the width of the two apposed membranes (approximately 145 Å thick) appears to be consistently twice (or slightly less) that of a single, separated unit membrane. A pentapartite-layered structure is clearly observed in the area of close contact, and its profile is approximately 500 m $\mu$  in length in sectioned material and reveals an intermediate dense line approximately 40 Å in thickness, which is interpreted to be the result of fusion of the cytoplasmic leaflets of the apposing unit membranes.

The electron-transparent and electron-opaque layers on both sides of the intermediate dense line are approximately 30 Å and 25 Å thick, respectively. The frequency of the appearance of two fused, bifacial cisternae is interpreted to be very low, because the structure shown in this report is the only instance observed in a number of sections made from 228 embedded specimens obtained from 93 shrimps.

Recently, an interesting paper was published on the fusion of the limiting membranes of adjoining mucigen granules (3). In that report, it appears that fusion of two unit membranes results in the formation of a triple-layered structure instead of a pentapartite-layered one. Since the details concerning the exact method of membrane fusion are not clearly demonstrated in the micrograph presented, it is conceivably possible that only one of the two limiting membranes is shown, and that the other one is completely broken away at the area of contact between the two adjoining mucigen granules.

Although the functional significance of the bifacial, fused cisternae reported in the present study remains quite obscure, it might be suggested that the structure represents a differentiation toward formation of granular endoplasmic reticulum by splitting.

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