THE DEVELOPMENT OF CYTOPLASMIC STRUCTURES
IN THE EMBRYO SAC OF LILIUM CANDIDUM,
AS OBSERVED WITH THE ELECTRON MICROSCOPE

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With 8 Figures in the Text

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Summary

Specific cytoplasmic bodies are formed out of the cores of the concentric membrane arrangements in the embryo sac of Lilium candidum. After vesiculation of the outer membranes in a concentric membrane system, a large number of cytoplasmic bodies and areas of vesicles are found in the postmeiotic embryo sac. In most of these bodies the contents gradually disappear, leaving optically empty spaces. At least part of the vesicular endoplasmic reticulum, present in the central zone of the maturing embryo sac, tends to fuse together forming a system of tubular ER.

The embryo sac of the lily develops in accordance with the Fritillaria type discovered by Bambacioni (1928). The object was cytologically studied with the light microscope by Mottier (1898), Bouin and Bouin (1898), Orman (1912), Flint and Johansen (1958), Eyme (1965) and others who observed karyological and cytoplasmic processes during megasporogenesis and gametophytogenesis. Abundant ergastoplasm appears in the cytoplasm of the megasporocyte, first in the form of parallel strands and then of coils. Such ergastoplasm appears also in gametophytogenesis of the Ranunculaceae plants (Eyme, 1961, 1962).

In the megasporocyte of the lily (Lilium candidum) the ergastoplasm observed in the light microscope corresponds to the membranes of the endoplasmic reticulum (Rodkiewicz and Mikulska, 1963). The position and character of the cytoplasmic membranes in the megasporocyte and in the embryo sac in its early development undergo considerable changes (Rodkiewicz and Mikulska, 1965, Mikulska and Rodkiewicz, 1964). On the whole, the changes correspond to those of the ergastoplasm observed in the light microscope.

Cisternae of ER, in young megasporocytes, form parallel arrays around the nucleus, and then the cistern arrangements begin to change their position forming in the cytoplasm raylike structures — tending away from the nucleus — or parallel structures leading in all directions. Subsequently, the parallel arrangements of cisternae yield to a coiling process upon which they appear as multilayered concentric bodies.
Material and Methods

Ovules of *Lilium candidum* were fixed with a 2 per cent OsO₄ solution in veronal buffer at pH 7.2 and transferred into a mixture of butyl and methyl metacrylates. The preparations for electron microscopy were stained with uranyl acetate and examined with a Tesla electron microscope.

Results

In the lily megasporocyte the membranes of the endoplasmic reticulum participate in the construction of the concentric bodies (Fig. 1). Concentric arrangements of the ER enclose a large portion of the cytoplasm which may optically present a somewhat denser structure than the ground cytoplasm. Towards the end of the first meiotic prophase the external membranes of the multilayered bodies become vesiculated (Fig. 2), and finally, of the multilayered body only the core, limited within a double membrane, is left. As a result of this process numerous dense cytoplasmic bodies (cores) and large areas of vesicles are formed in the cytoplasm of the postmeiotic cell (Fig. 3). With the further development of the embryo sac the contents of most of the cytoplasmic bodies undergo partial, or complete destruction. A three-dimensional reconstruction made after examining serial sections of the cytoplasmic bodies reveals their spherical structure (Fig. 4a, b).

It is very difficult, by observing preparations with the electron microscope, to determine beyond any doubt the actual stage of development of an embryo sac older than the megasporocyte. Nevertheless, the authors are inclined to believe that it was the embryo sac in its first and its second four-nucleate stage that was most frequently met with, while the intermediate stages would be rare as the four-nucleate stages are longerliving than intermediate ones. The later, cellular stage of embryo sac development could be easily determined with the electron microscope.

The cytoplasm of the embryo sac in its first four-nucleate stage contains numerous elongated, oval, and, less frequently, irregular bodies enclosed by one, two, or more concentric membranes (Fig. 3). Within the membranes thick granular cytoplasm can be seen which often contains mitochondria, osmophilic bodies and, sometimes the profiles of tiny vesicles. The vesicles may have originated by disintegration of inner membranes of the multilayered systems (Fig. 4a, b, bottom right). In a further stage a prevailing number of the cytoplasmic bodies is enclosed by a double membrane, and at this stage the bodies no longer contain

Fig. 1. Profiles of multilayered bodies in the lily megasporocyte by the end of first meiotic prophase ($\times$ 10,000)

Fig. 2. Multilayered body with vesiculating outer membrane ($\times$ 8,000)

Fig. 3. Cytoplasmic bodies and vesicular ER in the lily embryo sac in the first four-nucleate stage ($\times$ 10,000)

Abbreviations: c cytoplasmic body, ve vesicles, m mitochondrion, n nucleus, v vacuole