FINE STRUCTURE OF NEGATIVELY STAINED PLANT MITOCHONDRIA

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Recently investigators have reported knob-like particles on mitochondrial membranes isolated from different organisms (1, 2, 4, 5). This report presents observations on the structure of plant mitochondria, negatively stained with phosphotungstic acid, which show knob-like projections on the membranes.

MATERIALS AND METHODS

Castor beans (*Ricinus communis*) were soaked for 24 hours in distilled water and sown in moist vermiculite. The seeds were allowed to germinate for 4½ days in the dark at 29°C. At the end of this period, mitochondria were isolated from endosperms according to Wiskich and Bonner (6), except that the final suspension was made in de-ionized water. Geronimo and Tanner (personal communication) of this department have obtained high enzymatic activity for endosperm mitochondria isolated from castor beans in the same manner.

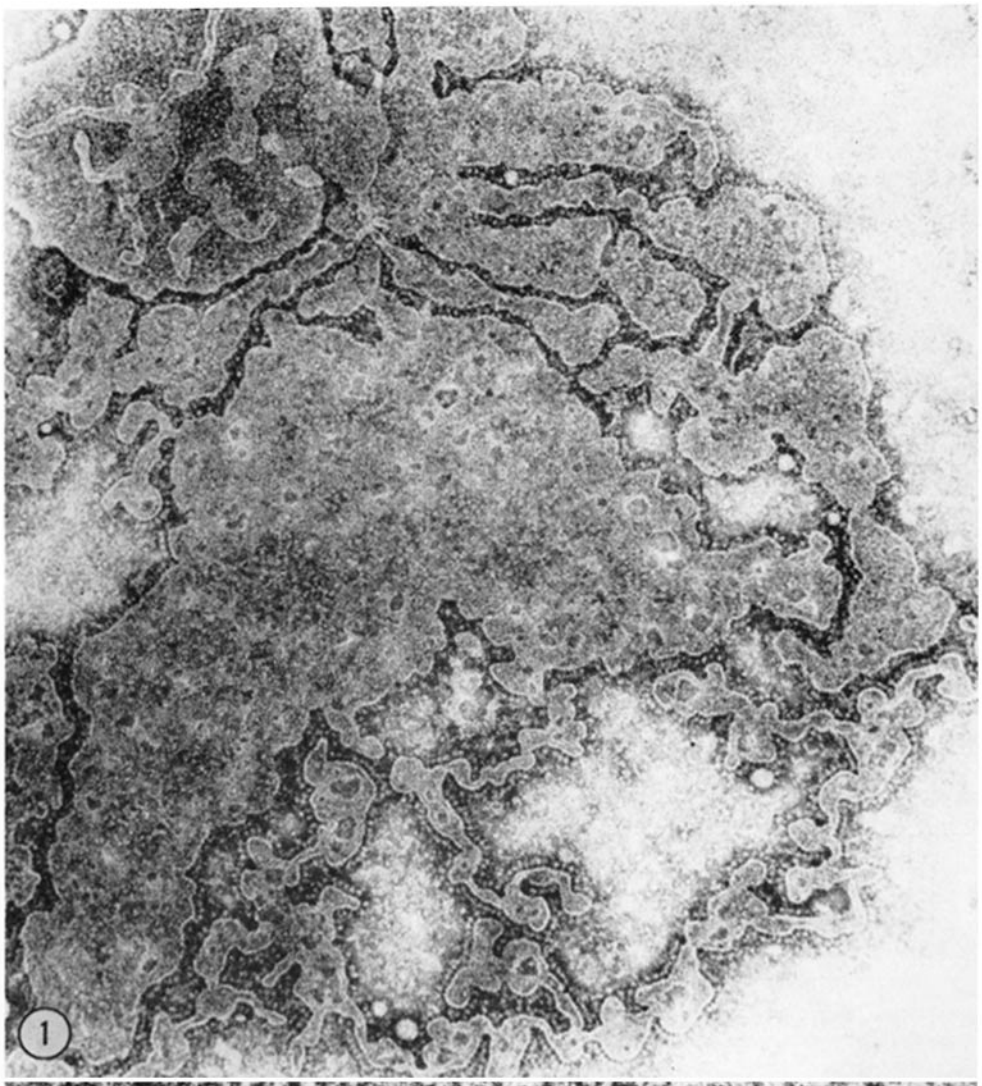
A small drop of the mitochondrial fraction in water was placed on an electron microscope grid with a supporting film of carbon. A filter paper was used to remove the excess water until only a thin film remained on the grid. A drop of 2 per cent phosphotungstic acid in water, adjusted to pH 6.5 with 10 per cent potassium hydroxide, was then added to this film and the excess removed as above. The grid was allowed to dry at room temperature.

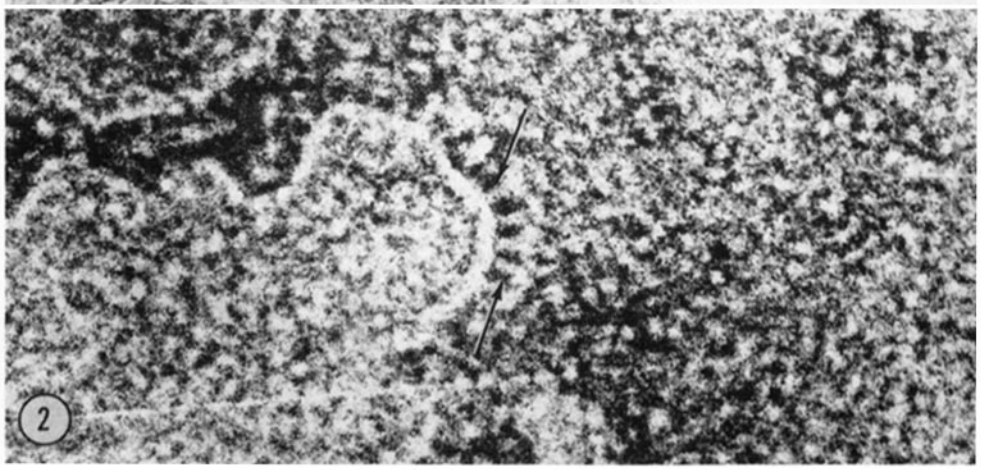
The electron micrographs were made in a Hitachi HU 11-A microscope using double condenser, and the photographs were taken at a screen magnification of 25,000. Structural details of mitochondrial membranes and cristae were distinguishable at an optical magnification of 140,000.

RESULTS

Examination of the negatively stained mitochondrial fraction in the electron microscope showed intact mitochondria as well as membrane fragments of varying size. Usually, the surfaces of membranes in intact and disrupted mitochondria showed lollipop-like particles (Fig. 1). These particles were more pronounced on the irregular ribbon-like structures in the disrupted mitochondria than in the intact ones. No particles were seen on the outer membrane of the mitochondria.

The particles seem to be composed of roughly spherical heads attached to the mitochondrial membrane by narrow stalks. The diameter of the heads falls within the 75–95 A range, and the length of the stalks, which are only occasionally clearly distinguishable (Fig. 2), within the 40–60 A range. The particles seem to be distributed at the rate of 100 per micron length of membrane surface. Occasionally, areas of the membrane appeared to be lacking the particles.





DISCUSSION

Mitochondria isolated from castor bean endosperm and negatively stained show structural details not observed in fixed and sectioned materials from both plant and animal tissues. The negative stain allows the visualization of spherical particles, with an average diameter of 85 A, attached to the mitochondrial membranes by means of stalks averaging 50 A in length. The knob-like particles were not seen on the outer membrane of the intact mitochondria as postulated by Green (3). The measurements of the size of these particles, especially of the stalks, should be considered as approximate values, since the particles may be oriented in varying angles with respect to the membranous base in the phosphotungstic acid. The frequency distribution of the particles per unit length of membrane surface corresponds closely to the findings of Chance and Parsons (1) from their study of mitochondria isolated from adult bees. The continuous nature of the variable ribbon-like structures indicates that they may be cristae mitochondriales expanded irregularly due to osmotic shock followed by disruption of the mitochondrion. These cristae were observed to be of more uniform nature in intact mitochondria than in disrupted mitochondria. The presence of spherical particles in the background, with dimensions roughly similar to those of the particles on the membrane, may explain the infrequent occurrence of particle-free membranes. The absence of knob-like particles in sectioned material has been suggested by Stoeckenius (5) to result from deleterious effects of fixatives on the particles.

Since the functional role of mitochondria is common to all organisms, it is significant that plant mitochondria show structural similarity to mitochondria from other organisms.

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