

thyroidectomized hypercalcemic rats⁶. It was suggested that the origin of the urinary hypocalcemic factor was thyroid or parathyroid or from both glands. The present study (experiment 1) revealed that the urine specimens of hypophysectomized hypercalcemic rats contains no hypocalcemic factor. The above experiments indicate that, after the removal of the pituitary gland, the functional capacity of the thyro-parathyroid glands to produce the hypocalcemic factor is lost. The results also indicate that hypercalcemia causes the release of a pituitary factor which stimulates a hypocalcemic factor from thyro-parathyroid. Furthermore, experiment 2 shows the presence of a calcium lowering substance in the pituitary gland. This substance is found to be effective only in the presence of thyro-parathyroid glands (experiment 3). The pituitary hypocalcemic factor is different from known pituitary hormones, as suggested by NATELSON et al.¹⁰ and as observed in experiment 4. The hypocalcemic effect of the guinea-pig pituitary extract, under the present experimental conditions, is much faster than that observed by NATELSON et al. This discrepancy may be due

to the species difference of animals used and/or to the mode of administration of pituitary extract. Loss of its activity by boiling or by tryptic digestion may suggest that this pituitary factor is a protein or a polypeptide.

Zusammenfassung. In der Hypophyse wurde ein Faktor festgestellt, welcher die Ausschüttung von Thyreocalcintonin fördert. Die Ausschüttung dieses Kalzium-senkenden Hormones scheint einer hypophysären Regulation unterstellt zu sein.

M. S. ZILELI, G. KANRA,
G. ÜRÜNAY, T. GÜNER
and S. CAGLAR

*Metabolic Division, Hacettepe Medical School,
Ankara (Turkey), 30 October 1967.*

¹⁰ S. NATELSON, J. B. PINCUS and G. RANNAZZISI, *Fedn Proc. Fedn Am. Soc. exp. Biol.* 22, 420 (1963).

Observations on the Ultrastructure of the Non-Articulated Laticifers of *Jatropha podagrica* (Euphorbiaceae)

One of the most enigmatic cell types in the Angiospermae is the non-articulated laticifer. Despite a wealth of light microscope information on the morphology of adult laticifers and considerable biochemical information on the synthesis of rubber, an understanding of the role of this system in the living plant is lacking. In addition, no description of the fine structure of non-articulated laticifers has been published other than MOOR's detailed analysis of the walls of laticifers of *Euphorbia splendens*¹. Information on the ultrastructure of the cytoplasm of laticifers might give some clue to their function. This communication contains some of the early findings of an electron microscope investigation of non-articulated laticifers.

Immature 2.0 mm embryos of *Jatropha podagrica* were dissected from seeds and fixed for 4 h in cold 6% glutaraldehyde in 0.06 M Sorensen's phosphate buffer at pH 6.9. After fixation they were washed with several changes of the same buffer. Embryos for light microscopy were dehydrated with a graded acetone series and embedded in epon. Embryos for electron microscopy were post-fixed for 30 min in unbuffered 2% OsO₄ and dehydrated with acetone. These embryos remained overnight in 70% acetone containing 1% uranyl nitrate before final dehydration and embedding. Thick 1½ µ sections for light microscopy and thin sections for electron microscopy were cut with Dupont diamond knives. The thin sections were stained on grids for 3½ min with REYNOLDS lead citrate² before being examined with a Zeiss EM 9a.

Laticifer distribution at this stage of embryogeny consists of a ring of elongate cells surrounding the provascular cylinder in the hypocotyl with extensive cortical ramification in the nodal region and a continuous cotyledonary system. In both the hypocotyl and cotyledons there is an intimate relationship between the developing vascular tissue and the major laticifer branches. The laticifers already have a thickened wall, a prominent central vacuole, and are multinucleate. Laticifer nuclei in this species assume the form of elongated spindles and are generally oriented with their longitudinal axes parallel to the longitudinal axis of the laticifer. Light microscopy has

shown that laticifer nuclei frequently occur in closely packed groups of 2 or more. Electron micrographs of such groups of nuclei demonstrate that the outer nuclear membranes of adjacent nuclei are connected by short segments of rough endoplasmic reticulum (Figure 1). Such interconnections would explain the linear arrays of nuclei

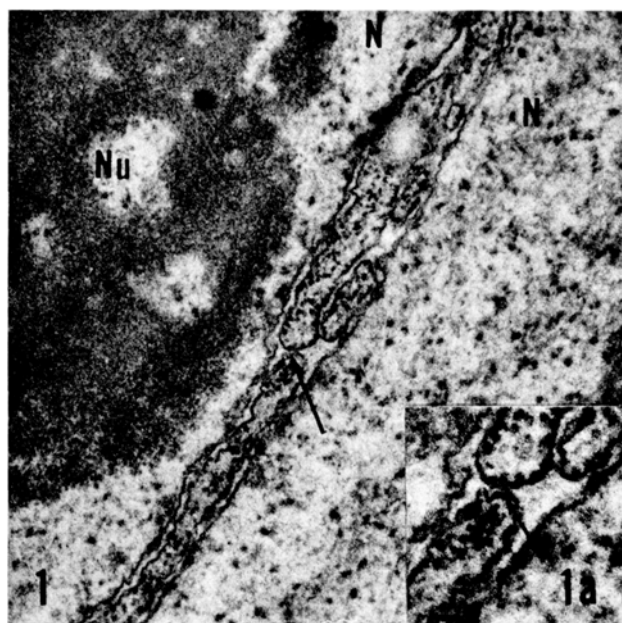


Fig. 1. Electron micrograph of 2 laticifer nuclei showing a bridge of rough endoplasmic reticulum (arrow) between the nuclei. N, nucleus; Nu, nucleolus. $\times 32,500$. 1a. Higher magnification of the same nuclear interconnection (arrow). $\times 61,750$.

¹ H. MOOR, *J. Ultrastruct. Res.* 2, 393 (1959).

² G. S. REYNOLDS, *J. Cell Biol.* 17, 208 (1963).

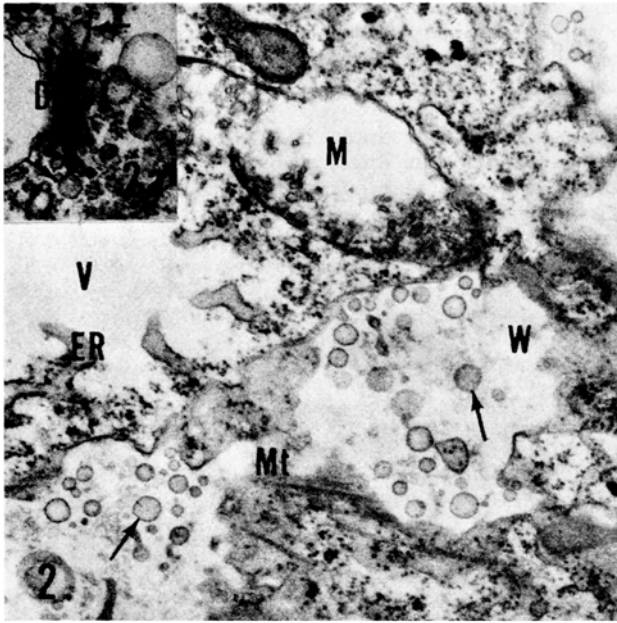


Fig. 2. Electron micrograph of a laticifer wall thickening. Note the wall inclusions (arrows) and microtubules (Mt). ER, endoplasmic reticulum; M, mitochondrion; V, vacuole; W, wall. $\times 22,750$. 2a. Dictyosome (D) photographed elsewhere in the laticifer. Compare the appearance of the dictyosome vesicles (arrows) with the spherical wall inclusions below. $\times 22,750$.

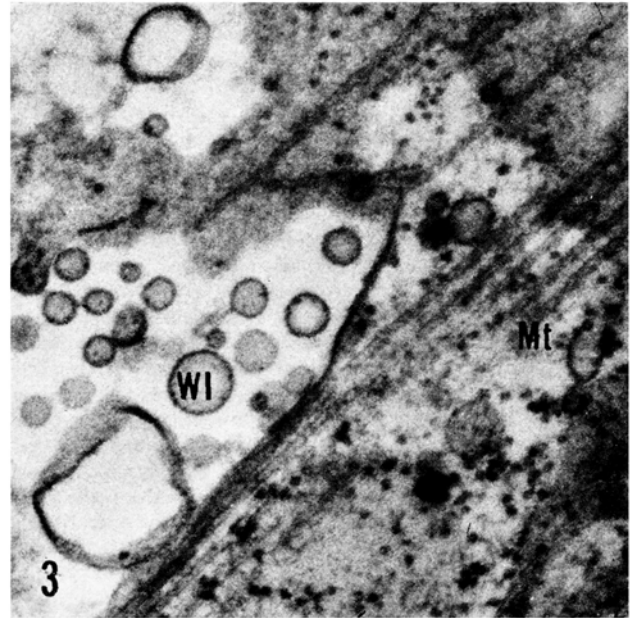


Fig. 3. High magnification electron micrograph of a thin section cut parallel to a thickened area in the laticifer wall, showing several microtubules (Mt). WI, wall inclusion. $\times 61,750$.

commonly observed in non-articulated laticifers. It is probable that these nuclear interconnections are ephemeral and that they are disrupted during subsequent laticifer elongation. There is no evidence that these nuclear interconnections represent early stages of fusion between nuclei.

Electron micrographs of thin sections parallel to localized thickenings in the walls of laticifers of this species show that these wall thickenings contain single-membrane-bound spheres which closely resemble dictyosome vesicles (Figures 2 and 3). Although it is impossible to establish a definite relationship between dictyosomes and these spheres at the present, it does appear that these wall inclusions are of cytoplasmic origin and that they may be involved in the deposition of new wall material. Microtubules (Figures 2 and 3) with a diameter of 240 \AA and lengths up to 7μ or more are particularly abundant in the cytoplasm adjacent to the wall thickenings, but may occur elsewhere in the peripheral cytoplasm. Frequently they are in parallel arrays, but occasionally one or more microtubules may be oriented at an angle to the others. According to the work of LEDBETTER and PORTER^{3,4} the disposition of microtubules near the cell periphery suggests that they may be involved in at least 2 processes. The highly-ordered parallel arrays closest to the cell surface are thought to regulate the orientation of the deposition of new wall material. The fine structure observations reported here on laticifer wall thickenings and associated microtubules appear to support that hypothesis. Randomly-oriented microtubules in the peripheral cytoplasm of cells not undergoing cytokinesis are considered by LEDBETTER and PORTER^{3,4} to be in a favorable position to control cytoplasmic streaming. The possibility of cytoplasmic streaming in non-articulated laticifers could have important implications in their function and should receive further attention.

Throughout the early phases of this investigation of non-articulated laticifers I have seen no indication of the incorporation of adjacent cells into laticifers. The lack of such cell fusion is evidence supporting the concept of non-articulated laticifers as coenocytes developing by apical intrusive growth. This concept has been challenged recently by MILANEZ⁵⁻⁷ who maintains that he has repeatedly observed cell fusions during the development of non-articulated laticifers of several species. Recent observations of MOOR¹, MAHLBERG⁸⁻¹¹, and those reported here have failed to confirm the occurrence of cell fusion as an aspect of the development of non-articulated laticifers¹².

Zusammenfassung. Einleitende elektronenmikroskopische Untersuchungen der ungliederten Milchröhren von *Jatropha-podagrica*-Embryonen haben das Vorkommen von Verbindungen der Zellkerne, augenfälligen Dictyosomen und reichliche Mengen von Mikrotubuli in diesen aussergewöhnlichen Zellen gezeigt. In den Milchröhren dieser Art wurde kein Vorkommen von Zellverschmelzung beobachtet.

D. D. CASS

Department of Botany, University of California, Berkeley (California 94720, USA), 8 April 1968.

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⁴ M. C. LEDBETTER and K. R. PORTER, *Science* 144, 872 (1964).

⁵ F. R. MILANEZ, *Rodriguesia* 15, 163 (1952).

⁶ F. R. MILANEZ, *Rodriguesia* 18-19, 351 (1956).

⁷ F. R. MILANEZ, *Rodriguesia* 21-22, 347 (1959).

⁸ P. G. MAHLBERG, *Phytomorphology* 9, 110 (1959).

⁹ P. G. MAHLBERG, *Am. J. Bot.* 48, 90 (1961).

¹⁰ P. G. MAHLBERG and P. S. SABHARWAL, *Am. J. Bot.* 54, 465 (1967).

¹¹ P. G. MAHLBERG and P. S. SABHARWAL, *Am. J. Bot.* 55, 375 (1968).

¹² This research was supported by U.S.P.H.S. Postdoctoral Fellowship No. 5-F2-GM-33, 474-02.