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Vinblastine-Induced Ultrastructural Changes in Perisinusoidal Cells of the Rat Liver

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Summary: Vinblastine, given intravenously to rats, leads to disappearance of microtubules, formation of paracrystalline inclusions and accumulation of fat droplets in the cytoplasm of the perisinusoidal cells of the liver suggesting that microtubules of the perisinusoidal cells play a role in lipid metabolism.

Key words: Vinblastine - Liver - Perisinusoidal cell - Microtubules- Electron microscopy

Perisinosoidal cells (Ito cells, fat storing cells), located in the space of Disse between the sinusoidal endothelium and hepatocytes, are regarded as representing a distinct cell type in the liver. Differing from endothelial and Kupffer cells, not only in location but also in morphology, they are assumed to play a role in synthesis and storage of various lipids including vitamin A (BRONFENMAJER *et al.* 1966; HRUBAN *et al.* 1974), in intralobular fibrillogenesis (McGEE and PATRICK, 1972), and in reinforcement of the endothelial lining of the sinusoids (ITO and SHIBASAKI, 1968).

While investigating the ultrastructure of liver of rats treated with vinblastine, an antimicrotubular agent, the perisinusoidal cells were found to exhibit conspicuous changes. The aim of the present paper is to describe these alterations and to call attention to the microtubular system which may affect the functional activity of perisinusoidal cells.

MATERIAL AND METHODS

Eight male Sprague-Dawley rats with a mean initial body weight of 450 g, were given 5 mg/100 g body weight of vinblastine (VELBAN, LILLY) dissolved in isotonic NaCl and benzylalcohol, through the jugular vein under light ether anesthesia. The animals were sacrificed 2, 4 and 8 hours later without anesthesia by dislocation of the cervical spine. Six untreated rats were used as controls. A small piece of liver was removed from the left lateral lobe, fixed in 2.5% glutaraldehyde and 1.5% formaldehyde, postfixed in 1% osmium tetroxide,

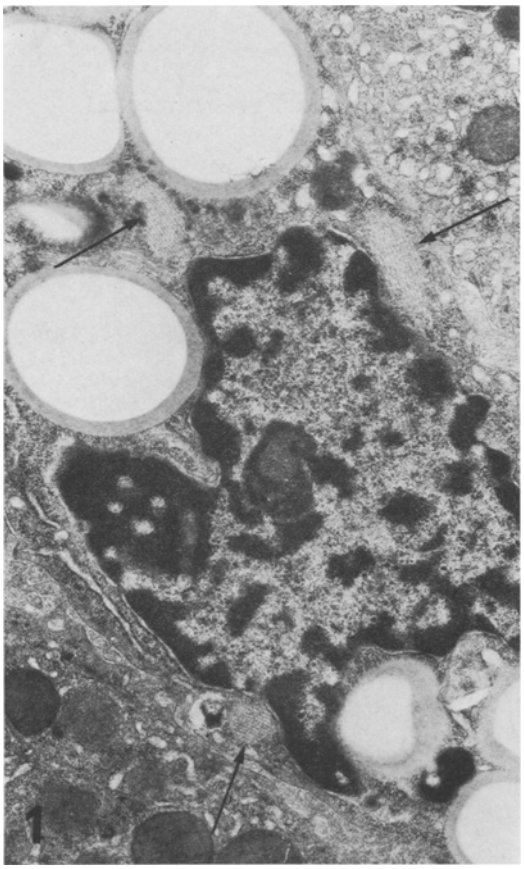

Fig. 1. Liver: Electron micrograph of a perisinusoidal cell showing paracrystalline inclusions (arrows) in the cytoplasm. Rat was given vinblastine 8 hours before sacrifice. x 13.000

dehydrated in graded ethanol and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and studied with a Philips 300 electron microscope.

RESULTS

In agreement with previous findings (ITO and SHIBASAKI, 1968; ITO, 1973), perisinusoidal cells were detected by electron microscopy in the control rat livers under the endothelium, in the space of Disse, between the sinusoidal walls and hepatocytes. Surrounded by a few reticulin fibers, they were elongate and possessed an oblong nucleus not uncommonly indented by lipid droplets. The cytoplasm contained a few rough surfaced endoplasmic reticulum membranes,

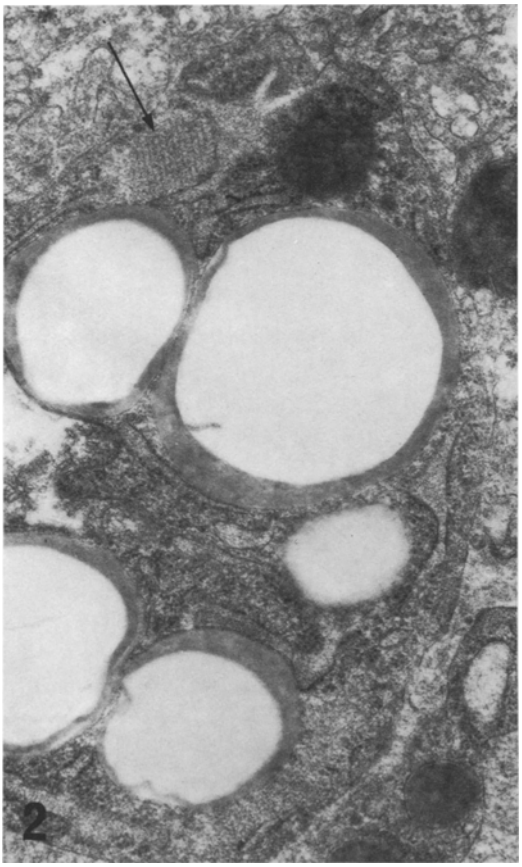

Fig. 2. Liver: A typical paracrystalline inclusion (arrow) is seen in the cytoplasm of a perisinusoidal cell. Rat was given vinblastine 8 hours before sacrifice. x 24.400

numerous free ribosomes, well developed Golgi apparatus and several medium sized lipid droplets occupying a considerable part of the cell. Mitochondria were scarce and were rod-shaped with lamellar cristae. Microtubules, with an average diameter of 250 Å, were inconspicuous in the dense cytoplasm.

At 2, 4 and 8 hours following vinblastine administration microtubules had completely disappeared from the cytoplasm. The most striking change was the appearance of cytoplasmic paracrystalline inclusions (Figs. 1-3). These non-membrane bound formations were elongate or irregular in shape, and were located adjacent to the nucleus close to the Golgi complex. They were composed of thin straight parallel strands spaced at an average distance of 360 Å on longitudinal sections and exhibited a honeycomb-like structure on cross sec-

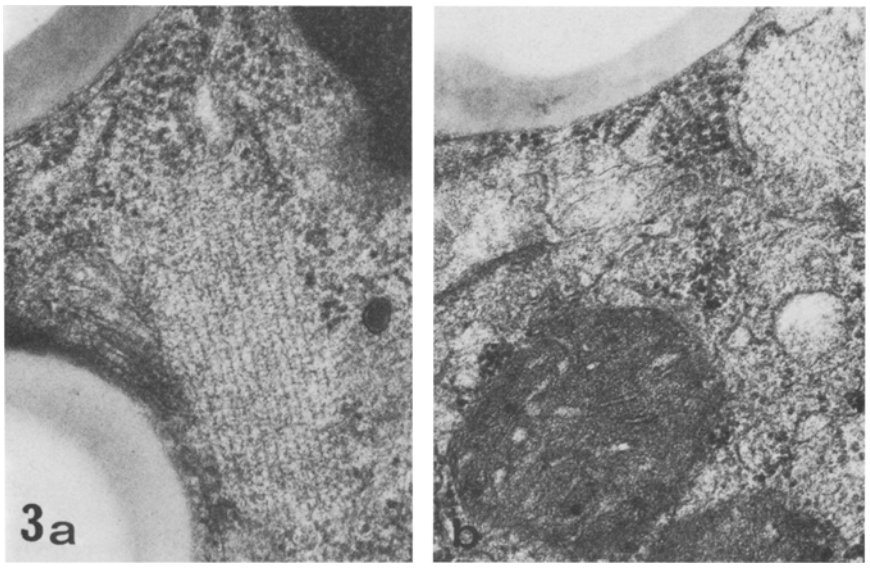

Fig. 3. a. and b. a) A high power view of paracrystalline inclusions on longitudinal section. x 49.700. b) A high power view of paracrystalline inclusions on cross section. x 49.700

tion. The fine structural features of the nucleus and cytoplasmic organelles did not differ from those of the controls. Cytoplasmic lipid droplets were, however, increased in size and number.

DISCUSSION

Vinca alkaloids, such as vinblastine and vincristine, depolymerize microtubular protein and disrupt microtubules resulting in the formation of paracrystalline inclusions which are detectable in several organs by electron microscopy (BENSCH and MALAWISTA, 1969; TYSON and BULGER, 1973). In the liver, disruption and disappearance of microtubules as well as the formation of paracrystalline inclusions have been reported in the cytoplasm of hepatocytes, sinusoidal endothelium and Kupffer cells (ORCI *et al.*, 1973), but to our knowledge the present work is the first in which this process has been revealed in the perisinusoidal cells.

The functional role of microtubules and the significance of the vinblastine-induced alterations in relation to perisinusoidal cell activity are not known. In other tissues microtubules are thought to interfere with intracellular migration of secretory granules (LACY *et al.*, 1968), with entry of cholesterol

into mitochondria (TEMPLE and WOLFF, 1973), and with the synthesis and release of some proteins and lipoproteins (ORCI *et al.*, 1973; LE MARCHAND *et al.*, 1974). The fact that in the perisinusoidal cells the vinblastine-induced alterations of microtubules are associated with lipid accumulation seems to indicate that in perisinusoidal cells microtubules are involved in lipid synthesis and/or transport.

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