HYPERTROPHY OF GRANULAR ENDOPLASMIC RETICULUM AND ANNULATE LAMELLAE IN EARLE'S L CELLS EXPOSED TO VINBLASTINE SULFATE

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INTRODUCTION

A large amount of granular endoplasmic reticulum, the striking characteristic of cells actively synthesizing protein (e.g. pancreatic exocrine cells, plasma cells, hepatocytes), is not a normal feature of Earle's L-929 mouse fibroblasts in monolayer cultures (19). Similarly, annulate lamellae, which often show a structural resemblance to

nuclear membrane with its pores and show a continuity with membranes of the granular endoplasmic reticulum, are characteristic of germ cells, particularly oocytes (1, 15, 24, 28), and of fetal cells (22, 26), virus-infected cells (17, 23), tumor cells (2, 3, 6, 9), and have been reported occasionally in cells of active secretory glands (8).

Induced hypertrophy of agranular endoplasmic reticulum in hepatocytes of animals injected with phenobarbital, ethanol, and some other drugs has been described recently (13, 25, 27). Similarly, a large amount of annulate lamellae has been described in hepatocytes after injection of the animal with β-3 furylalanine (10), in hyperplastic liver nodules of rats treated with carcinogens (20, 21), rarely in pancreatic acinar cells of animals treated with azaserine (11), and in myocardial cells of 18-day chick embryos incubated at 90°F (22).

An earlier time-lapse study (18) showed that a large number of L cells arrested in mitosis by vinblastine sulfate (Velban; Eli Lilly & Co., Indianapolis, Ind.) would give rise, on continued incubation, to multimicronucleated cells, some of which contain as many as 20 or more nuclei. These multimicronucleated cells are viable and, when washed and reincubated in fresh medium, they are capable of apparently normal cell division. The present brief report describes the hypertrophy of granular endoplasmic reticulum and the large number of annulate lamellae seen in cells from cultures treated with metaphase-arresting but nonlethal doses of Velban (0.01 μg/ml).

MATERIALS AND METHODS

Earle's L-929 fibroblasts were grown in glass prescription bottles and were fed with Eagle's minimum essential medium supplemented with 10% fetal calf serum and 100 units/ml of penicillin. Vinblastine sulfate (Velban) was added to monolayer cultures at a concentration of 0.01 μg/ml, and the cells were removed by gentle scraping after 6–44 hr of incubation. The medium was centrifuged, and the cell buttons were fixed in 2–5% cacodylate-buffered glutaraldehyde (pH 6.8–7) at room temperature (21°–23°C) for 30–60 min. The cell buttons were washed in buffered 6% sucrose solution, postfixed in 2% phosphate-buffered osmium tetroxide (pH 6.8–7) for 1–2 hr, and dehydrated in a graded acetone series. The material was embedded in an Epon-Araldite mixture. Thin sections stained with lead citrate were examined in a Philips 300 electron microscope.

OBSERVATIONS

During the first 16 hr of incubation in medium containing Velban, L cell cultures show progressive accumulation of cells arrested in mitosis (C mitosis). On continued incubation in the same medium these C-mitotic cells, which are recognizable as rounded cells with prominent chromosomes, gradually extend their pseudopods, spread on the glass surface, and resume their normal fibroblastic appearance. A large number of these postC-mitotic cells are multimicronucleated and may have as many as 2–20 or more micronuclei.

At the ultrastructural level cells treated with Velban (0.01 μg/ml) for 24–44 hr are large, often two to five times larger than a normal L cell, and show many lysosomes, many lipid-like storage granules, and a great amount of fibrous material in the cytoplasm. Except for their small size, the micronuclei in most of these cells are indistinguishable in their fine structure from the nuclei of control L cells. In Fig. 1, sections of one small and three large nuclei of a multimicronucleated cell are seen; one of the nuclei encloses a cytoplasmic invagination containing a fibrous, lamellar body. Many of the micronuclei in Velban-treated cells contain one or more nucleoli. However, it is in the cytoplasm of the Velban-treated cells that hypertrophied collections of cytomembranes, normally not seen in L cells from untreated cultures, are seen. Fig. 2 shows a number of concentric double lamellae of the granular endoplasmic reticulum (ER) in a cell exposed to Velban (0.01 μg/ml) for 44 hr. Many membrane-associated and free ribosomes can be recognized in this micrograph; the arrows point to short lengths of ER which are not arranged in concentric stacks and which contain electron-opaque material. The large concentric lamellae of granular ER are seen in approximately 30–50% of cell sections scanned. Some of these cells have so much granular ER that they take on a superficial resemblance to plasma cells or pancreatic exocrine cells. Besides having an abundance of ER, many of these cells contain a large number of annulate lamellae. In some of the thin sections scanned, practically every cell section had one or more stacks of these lamellar structures in the cytoplasm. These annulate lamellae, though of common occurrence in oocytes, are normally not seen in untreated L cells. Fig. 3 is a micrograph of a Velban-treated cell with large stacks of annulate lamellae in the cytoplasm. Fig. 4 shows a number of transversely cut lamellae at higher magnifica-

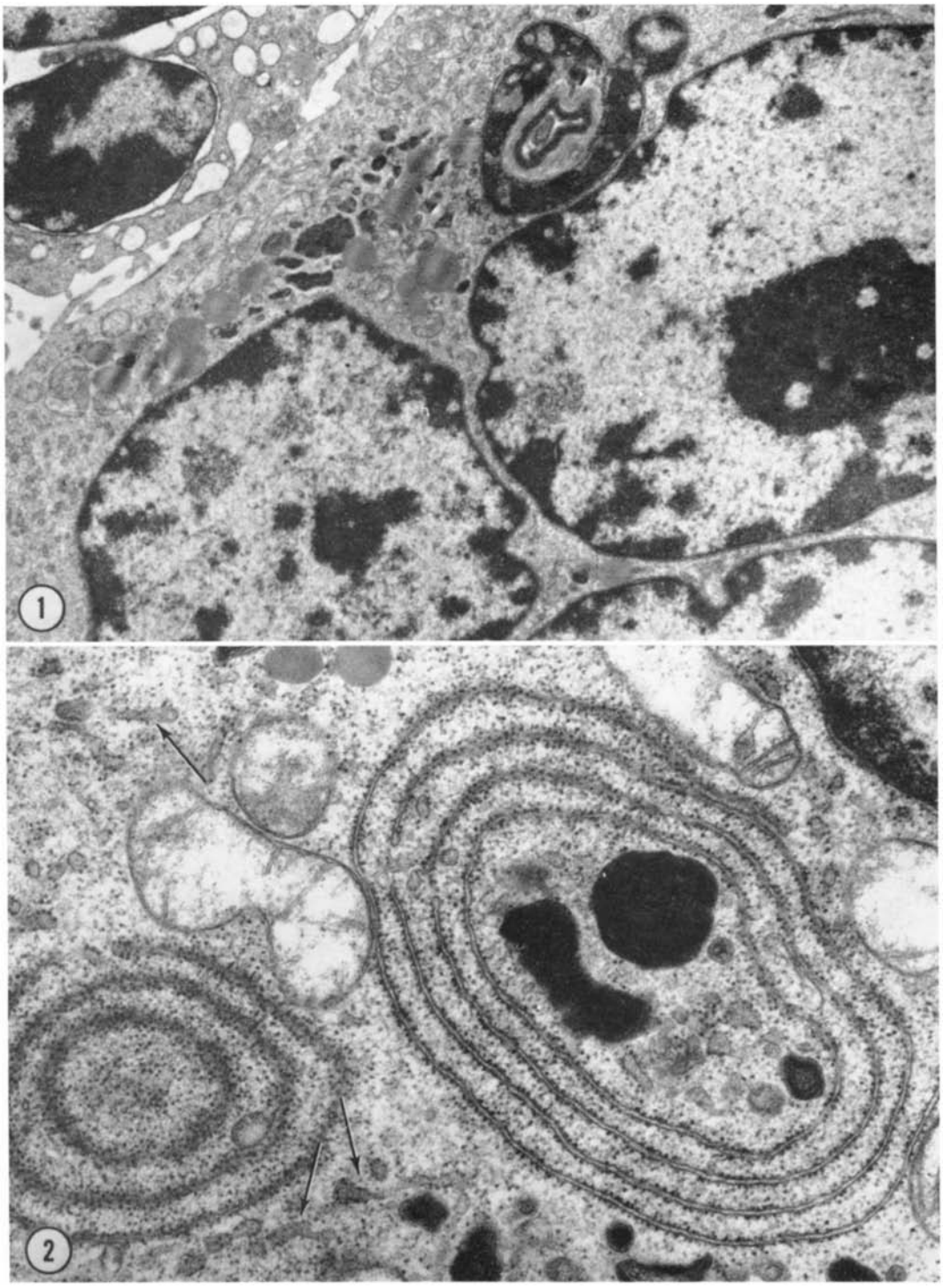

FIGURE 1 Portion of a multimicronucleated cell showing sections of four nuclei. The micronuclei as seen in this micrograph are indistinguishable in their fine structure from nuclei of normal cells. × 10,000.

FIGURE 2 Large stacks of granular ER with associated ribosomes are seen in this cell from a Velban-treated culture. Arrows point to lamellae of granular ER which are not arranged in concentric stacks and which contain electron-opaque material. × 57,600.

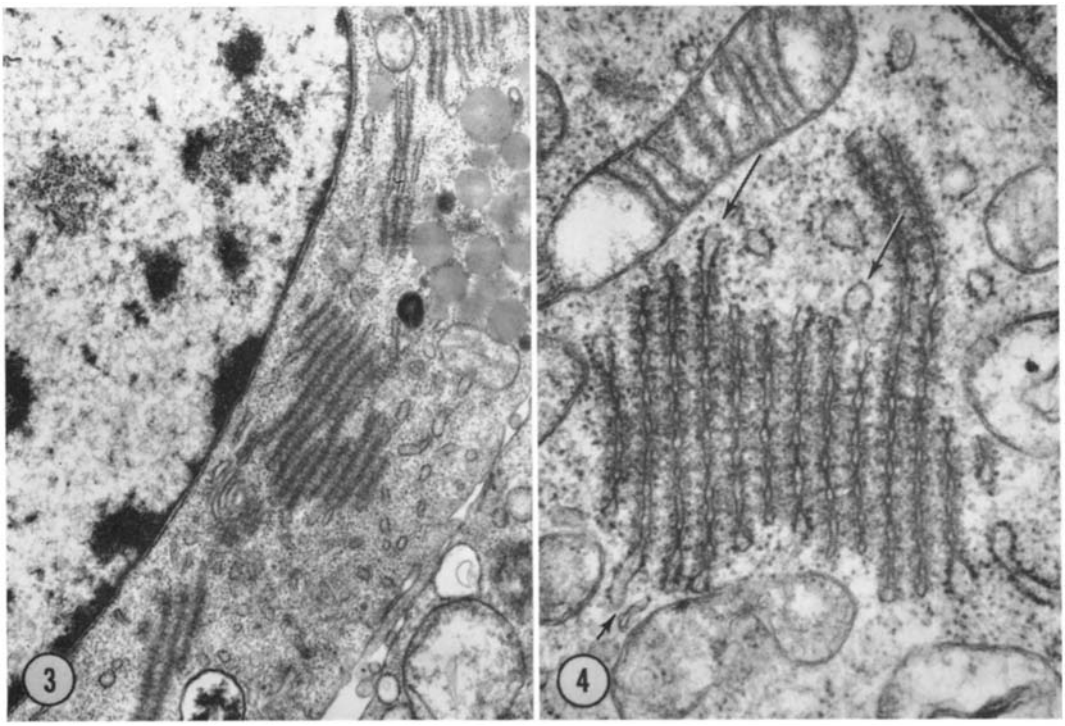

FIGURE 3 A large number of annulate lamellae can be seen in the cytoplasm of this Velban-treated cell. × 16,500.

FIGURE 4 A higher magnification of annulate lamellae. Electron-opaque material is seen between the lamellae. Arrows point to areas of continuity between the membranes of annulate lamellae and the membranes of granular ER. × 39,700.

tion. Electron-opaque material is seen between the lamellae, and at many places (arrows) a continuity between the membranes of the annulate lamellae and the membranes of the granular ER can be seen. Fig. 5 shows a section parallel to the surface of the annulate lamellae. A large number of pores, many of them showing a dense granule (100–125 A) in the center, are seen in this micrograph. These transversely cut pores are reminiscent of similar structures in nuclear membranes.

DISCUSSION

Vinblastine sulfate belongs to the group of *Vinca rosea* alkaloids which are known to have a mitosis-arresting effect on cells in vivo and in vitro. A number of studies have demonstrated the inhibitory effect of Velban on the incorporation of labeled precursors into RNA, DNA, and proteins of mammalian cells (4, 5, 14). Interference with metabolic pathways leading from glutamic acid to urea via ornithine, arginine, etc., and from glutamic acid to the citric acid cycle via α-ketoglutamic acid has been suggested as the possible mode of action of Velban on mammalian cells (12). The teratogenic effects of Velban on pregnant animals, often leading to congenital malformations in embryos, have been well documented (7). Cells arrested in mitosis by Velban show the absence of spindle microtubules in association with centrioles and centromeres, and many cells released from the Velban-induced mitotic block show abnormal cytokinesis and multipolar divisions (18). Some C-mitotic cells in cultures treated with mitosis-arresting but sublethal doses of Velban (0.01 μg/ml) re-form into large multimicronucleated cells. Time-lapse studies have shown that these cells are apparently viable and capable of entering and completing cell division after they have been washed and incubated in fresh medium (18). As seen in the present study, such cells contain unusu-

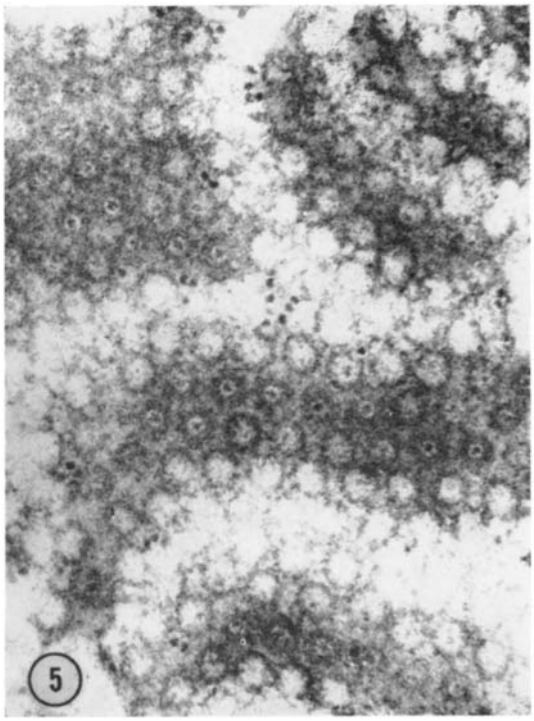

FIGURE 5 A section cut parallel to the membranes of the annulate lamellae. Regular pores, many of them with a 100–125 A central dense granule, are recognizable. × 95,600.

ally large amounts of hypertrophied granular ER and annulate lamellae, structures which are normally characteristic of actively synthesizing cells such as plasma cells and pancreatic acinar cells. A recent study has shown that there is a substantial increase in the incorporation of thymidine-^{3}H into the DNA of cells from the guts of pregnant mice injected with Velban, but that there is a decrease in the incorporation of thymidine-^{3}H into the DNA of cells from the whole embryo (29). It has been suggested that the different effects of Velban on DNA synthesis in adult and embryonic tissues may be responsible for the embryocidal action of Velban (29). Similarly, it is possible that mitosis-arresting but sublethal doses of Velban, as used in the present study, instead of having an inhibitory effect, might stimulate the synthetic activity of these cells as manifested by the large amounts of granular ER, associated ribosomes, and storage granules.

The other population of cytomembranes seen in these cells, namely the so-called annulate lamellae, are characteristic of actively growing cells or of cells from stimulated hyperplastic tissues. The extensive basophilia and proximity to the nuclear membrane of the annulate lamellae, as well as the presumed presence of particulate and nonparticulate RNA associated with these membranes, have led to the suggestion that annulate lamellae may be the means of mass transfer of nuclear material and information from the nucleus to the cytoplasm (see reference 16 for discussion). The presence of annulate lamellae extensively in oocytes and in actively growing tissues, including fetal cells, further lends weight to the theory that these structures are associated with an increased amount of nucleocytoplasmic exchange.

The evidence presented here suggests that L-cell cultures enriched in annulate lamellae by exposure to sublethal doses of vinblastine sulfate would be useful for studies on intermediary metabolism with labeled precursors and on the isolation and chemical characterization of these structures after cell fractionation.

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