

## THE EFFECTS OF VINBLASTINE ON ISOLATED *XENOPUS* OOCYTES

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### INTRODUCTION

A recent survey of the effects of various physical and chemical parameters on yolk protein incorporation by isolated *Xenopus* oocytes indicates that the *Vinca* alkaloid vinblastine sulfate (VLB) is among the more effective inhibitors of this process (27). The *Vinca* alkaloids have received considerable attention because of their ability to disrupt the integrity of microtubules (1, 2, 21); therefore, they appear to be useful tools for examining the functions of microtubules in a variety of cells. Furthermore, they facilitate the isolation and purification of microtubular proteins for biochemical studies (4, 8, 17, 18). These properties of VLB, combined with its marked depressant effect on protein uptake by isolated oocytes, led us to examine VLB-treated oocytes for altered cytological characteristics. This communication describes alterations induced by VLB and discusses their possible significance in terms of the relationship of microtubules to the micropinocytotic uptake of protein.

### METHODS

*Xenopus laevis* were maintained in the laboratory under regulated conditions of temperature, light, and food supply (26). Females used as oocyte donors were injected with 1000 units of Human Chorionic Gonadotropin (HCG) at least twice within 2 wk before use. Oocytes (0.8–1.1 mm) were isolated under standard conditions in solution "O" and subsequently incubated for 17 hr in protein-containing medium with or without  $10^{-4}$  M VLB ("VELBAN", Eli Lilly & Co., Indianapolis, Ind.), as described previously (27).

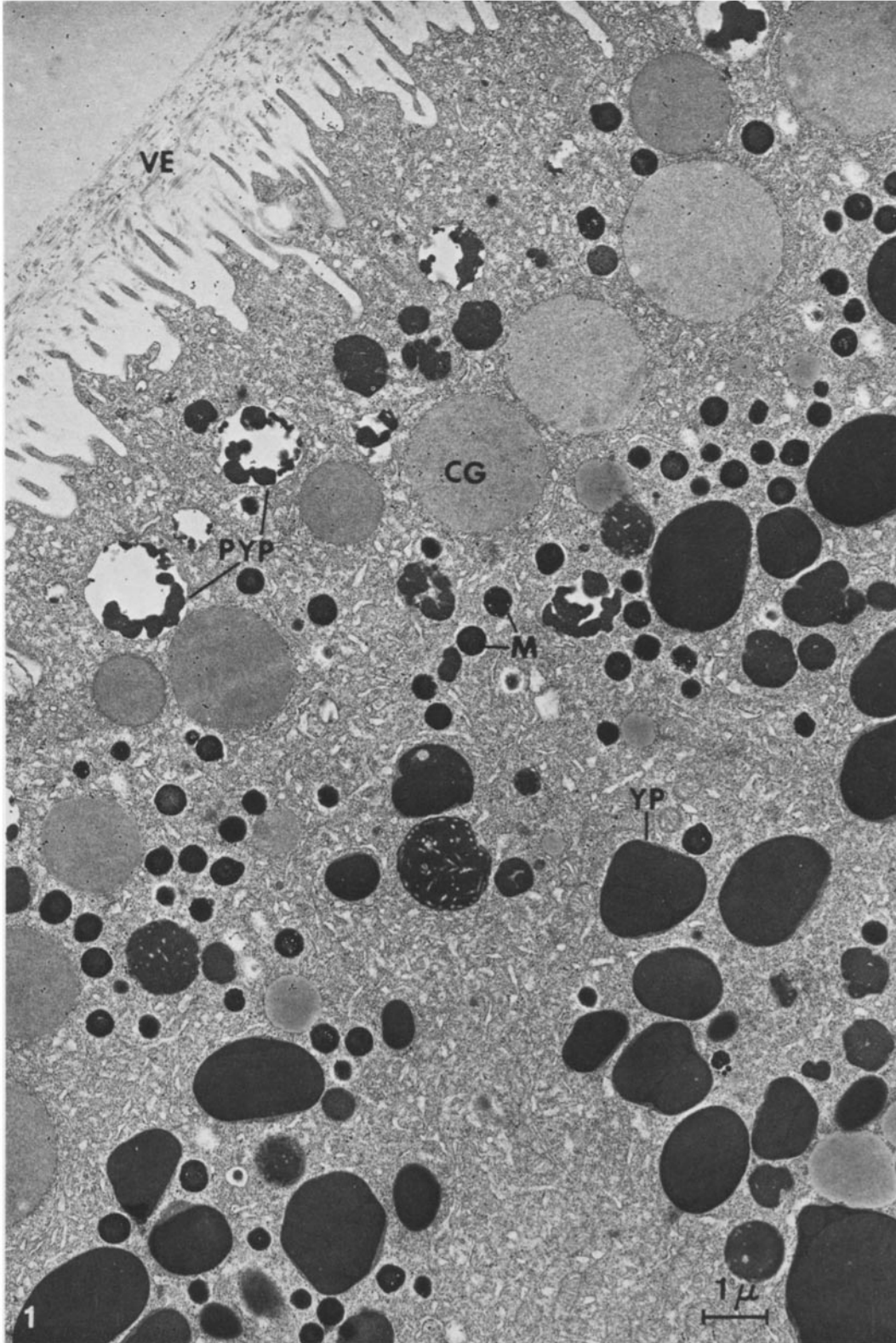
After incubation, oocytes were fixed in 4% glutaraldehyde in 0.05 M phosphate buffer (pH 7.3), washed overnight in the same buffer, and postfixed in 1% osmium tetroxide in phosphate buffer. The fixation procedures were carried out at 20°C. The fixed oocytes were rapidly dehydrated in a graded series of alcohols to propylene oxide, infiltrated, and embedded in Epon. Thin sections were made with a Porter-Blum MT-2 Ultramicrotome equipped with a diamond knife, placed on Formvar-coated, carbon-stabilized grids, stained with uranyl acetate and lead citrate, and examined with an Hitachi 11-E electron microscope operated at 75 kv.

### RESULTS

#### *Control Oocytes*

Oocytes dissected from their follicles and cultured in medium without VLB (Fig. 1) appear similar to those in vivo (compare with Fig. 1, ref. 25). Follicle cells, which cover the freshly isolated oocytes, are frequently absent after the 17-hr incubation period; the vitelline envelope, however, remains intact. The oocyte surface (oolemma) is highly folded and possesses deep crypts and many microvilli.

A typical feature of oocytes actively incorporating protein, whether under in vitro or in vivo conditions, is the presence of many micropinocytotic pits and vesicles (pinosomes). Pinosomes move centripetally, fuse with each other, and form primordial yolk platelets. The primordial yolk platelets further enlarge by fusion with others or with more pinosomes and eventually transform into yolk platelets which possess crystalline



**FIGURE 1** Control oocyte. Note the elaborate contours of the surface, micropinocytotic pits on the cell surface and vesicles (pinosomes) in the peripheral ooplasm are abundant. Primordial yolk platelets (*PYP*) appear swollen. Yolk platelets (*YP*) appear confined to discrete areas of the ooplasm, and melanosomes (*M*) lie in a band beneath the cortical granules (*CG*). Vitelline envelope (*VE*).  $\times 9100$ .

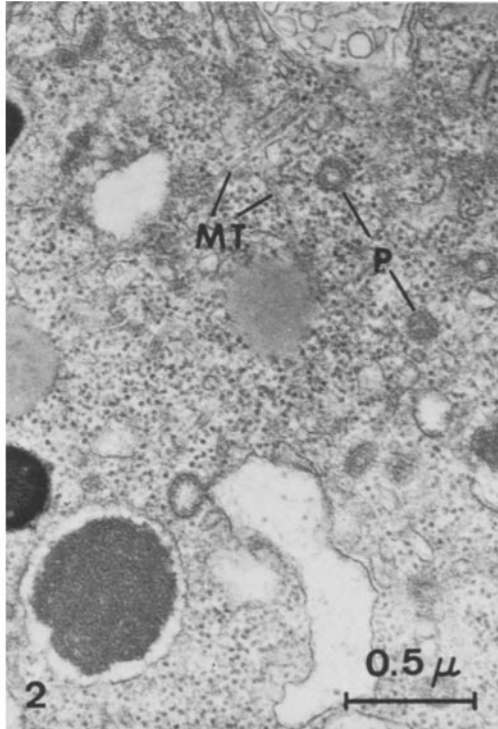


FIGURE 2 An electron micrograph taken near the surface of a control oocyte. Note microtubules (*MT*) and pinosomes (*P*).  $\times 33,600$ .

main bodies (10, 11). The primordial yolk platelets formed in all isolated oocytes (including those incubated in the presence of VLB) appear to be somewhat swollen. They contain dense aggregates of incorporated material, surrounded by optically empty spaces. Some microtubules are present and are generally located near the periphery of the oocyte (Fig. 2). Extensive linear arrays of microtubules, however, are not present.

#### *VLB-Treated Oocytes*

The most striking alteration of the oocyte after VLB treatment is the change in its topography (Fig. 3). The deep crypts disappear, and the oolemma becomes smooth between projecting microvilli. Further, the number of micropinocytotic pits and pinosomes is dramatically reduced. There are more primordial yolk platelets in VLB-treated oocytes than in controls. Many of them possess spherical or slitlike "holes" (Fig. 4) and are randomly distributed throughout the ooplasm. Only rarely in control oocytes are similar yolk platelet alterations observed. Many

of the smaller yolk platelets are also malformed and possess holes and are often very irregular in outline. Some contain uncrystallized condensations, presumably derived from fusion with primordial yolk platelets (Fig. 5).

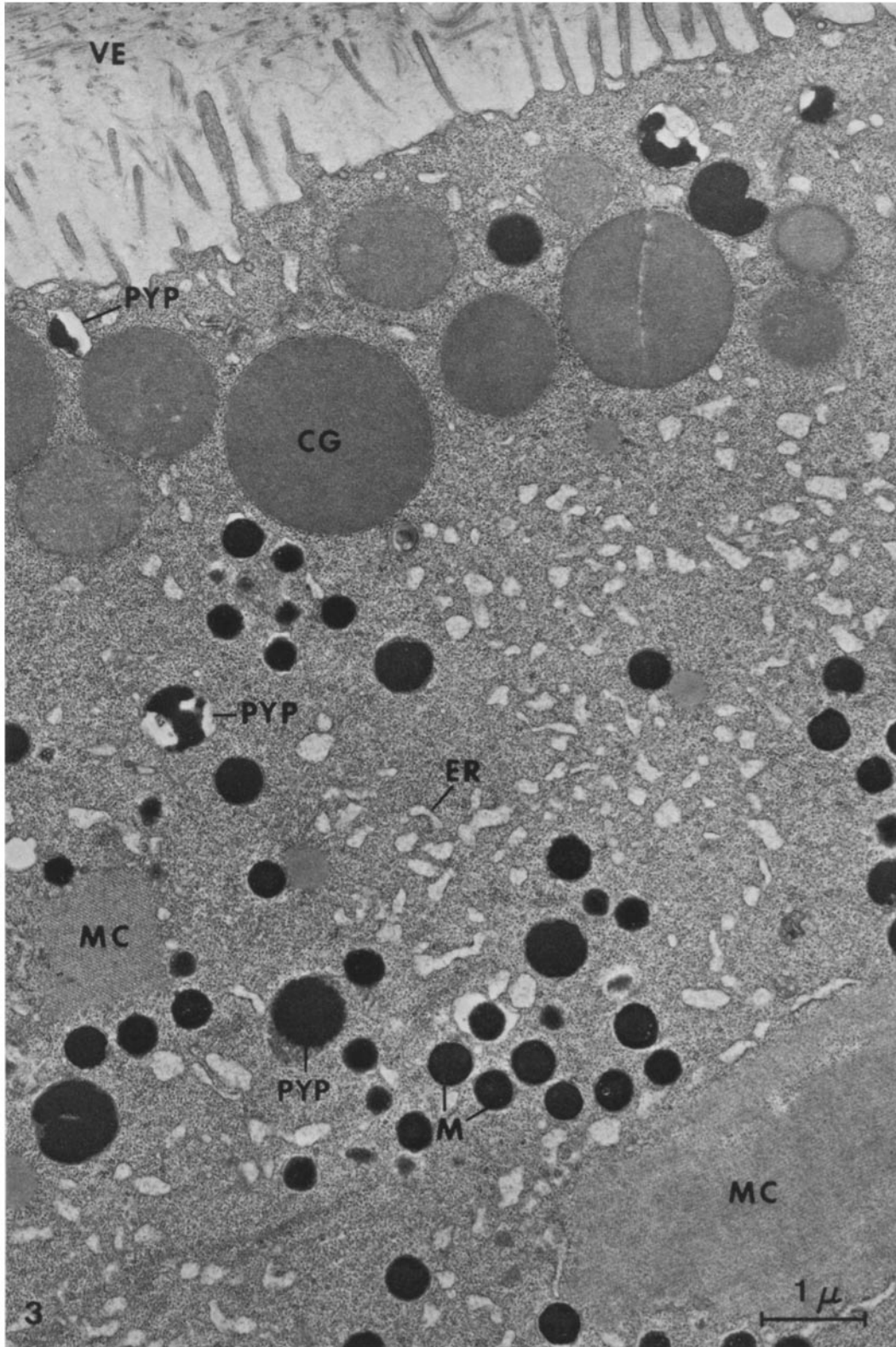
With the exception of the cortical granules, which maintain their position in the periphery of the oocyte, there is a general disorganization of the ooplasm. That is, mitochondria, small yolk platelets, and melanosomes which are normally segregated from each other become intermingled.

Large crystals presumably composed of microtubular proteins are present in VLB-treated oocytes. The structure of these crystals is similar to that described by others for microtubular protein crystals induced by VLB in other cells (3). Often, melanosomes and cisternae of the endoplasmic reticulum are closely apposed to the circumference of the crystals, an observation which reflects the general disorganization of the ooplasm.

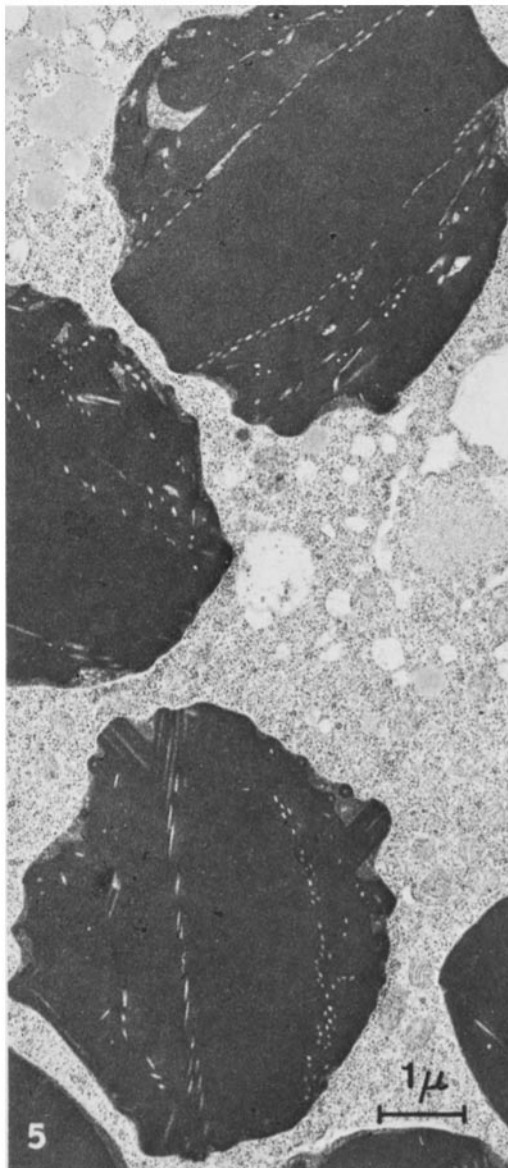
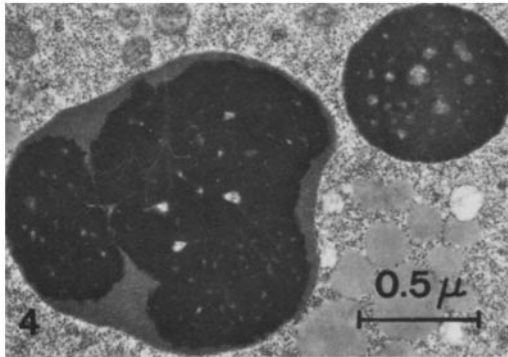
#### DISCUSSION

Microtubules participate in many cellular processes, ranging from development and maintenance of cell shape (23, 24) and regulation of cell movements and cytoplasmic particles (5, 6, 7, 12) to involvement in secretory processes (14, 15, 20) and mitosis (9, 13, 16, 19). Vinblastine, along with other mitosis-arresting drugs such as vincristine, colchicine, and colcemid, has the specific effect of destroying or depolymerizing microtubules and, as a result, affecting all of the above-mentioned cellular processes. From the cytological observations reported here, and in view of the depression of protein uptake by oocytes in the presence of VLB, it seems likely that microtubules play an important role in the normal processes of yolk acquisition and sequestration. Because of their location and orientation near the periphery of the oocyte, two possible roles are hypothesized: (a) a structural role in maintaining the elaborately contoured and micropinocytically active cell surface and (b) a role in directing the centripetal movement and subsequent fusion of pinosomes and primordial yolk platelets.

The observation that the number of micropinocytotic pits and pinosomes is reduced in VLB-treated oocytes undoubtedly accounts for the lack of protein uptake. Of greater interest, however, is the relationship of this process to the known effects of VLB in destroying microtubule integrity. Since the oocyte surface is altered by



**FIGURE 3** The elaborate contours of the oocyte surface are lost from VLB-treated oocyte. Although the cortical granules (CG) remain aligned beneath the oolemma, the primordial yolk platelets (PYP) and melanosomes (M) now appear randomly distributed. Microtubule-protein crystals (MC) are present and the endoplasmic reticulum (ER) appears swollen. Vitelline envelope (VE).  $\times 15,600$ .



VLB, one possible interpretation is that microtubules are necessary for maintaining the elaborate surface in a configuration which promotes micropinocytosis. Similar alterations of the cell surface, accompanied by a reduction of micropinocytotic activity caused by VLB and colchicine, have been reported by others, with the suggestion that microtubules acting as a cytoskeleton oppose the deformation of the cell surface (5, 6).

The second role hypothesized above for microtubules in *Xenopus* oocytes is that they establish boundaries within the ooplasm and aid in directing centripetal translocations of pinosomes and primordial yolk platelets, thus facilitating the more rapid fusion of these components by keeping them in proximity to each other. The observation of a general disorganization of the ooplasm and an abundance of primordial yolk platelets in VLB-treated oocytes supports this view. In other words, in the absence of microtubules, pinosomes and primordial yolk platelets are randomly distributed by cytoplasmic flow and as a consequence are not spatially confined. Thus, although micropinocytosis and the distribution of cytoplasmic components *per se* may not depend entirely on the presence of microtubules, both phenomena may be enhanced or promoted when microtubules are present.

Although large numbers of microtubules are not found in the normal oocyte, it would appear, simply from the size and number of microtubular crystals induced by VLB, that there is more microtubular protein present than can be accounted for by the number of microtubules observed. It may be that relatively large quantities of these proteins are synthesized by the oocyte and simply stored in a depolymerized state until needed for the construction of microtubules during meiosis, cleavage, and subsequent embryogenesis. It has been demonstrated that large amounts of colchicine-binding protein are present in the mature oocytes of *Rana pipiens* (22).

The relationship of the malformation of primor-

FIGURE 4 Primordial yolk platelet from a VLB-treated oocyte. Note the large "holes" which are characteristic after VLB treatment.  $\times 29,700$ .

FIGURE 5 Yolk platelets from a VLB-treated oocyte. Much of the crystalline pattern has been destroyed, their outlines are irregular, and they are now characterized by slitlike holes which appear to outline the main bodies.  $\times 11,400$ .

dial yolk platelets and some smaller yolk platelets to microtubules and/or the effects of VLB is unclear. It has been suggested that VLB, by virtue of its high charge, alters the molecular arrangement of microtubule proteins. It is possible that it has similar effects on the integrity of yolk crystals, and one may speculate that this highly charged molecule acts either by interfering with the normal crystallization of the primordial yolk platelets or by "melting" yolk crystals that have already formed.

Despite the interpretations offered in this report, the possibility that VLB acts (a) directly on the oolemma to alter its ability to incorporate by micropinocytosis, (b) by binding with yolk proteins to alter their incorporation and sequestration by the oocyte, or (c) by precipitating other structural proteins (28) must be considered. However, in light of the demonstrated effects of VLB on microtubules and its apparent ineffectiveness in changing other normal metabolic processes, such as protein synthesis, the interpretations enumerated above seem most reasonable at this time. In order to examine these alternatives, further studies of the effects of other microtubule-altering agents, such as colchicine, D<sub>2</sub>O, and reduced temperature, on the uptake of protein and on the structure of the oocyte are in progress.

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