

STUDIES OF SPERMATOGENESIS IN THE HEPATICAE

III. Continuity between Plasma Membrane and Nuclear Envelope in Androgonial Cells of *Blasia*

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ABSTRACT

An ultrastructural study of late-stage androgonial cells of *Blasia pusilla*, a thallose liverwort, showed the nearly spherical nuclei often lying close or appressed to the cell walls. In some cells the two membranes comprising the nuclear envelope separated, the inner membrane continuing intact as a limiting boundary of the nucleus and the membrane on the outer, cytoplasmic side recurving away from the nucleus to continue without evident interruption around the periphery of the cell as the plasma membrane. It is believed that *Blasia* offers the first completely convincing demonstration of the heretofore problematic continuity of cytoplasmic membranes. A possible sequence of events leading to this unusual relationship between nucleus and cytoplasm is suggested. The sequence includes blebbing of the outer membrane of the nuclear envelope and subsequent membrane proliferation, apparent isolation of cytoplasmic ground substance, fusion of internal membrane with the ectoplast, and migration that finally brings the nucleus into flat contact with the wall. While this manifestation of membrane continuity may be anomalous, it is not presently considered the result of cell injury.

INTRODUCTION

More than a decade ago, Robertson (14) proposed a concept of general cellular organization that was characterized in large part by membrane continuity. The concept linked plasma membrane with nuclear envelope through the endoplasmic reticulum, and concomitantly postulated an open system of cytoplasmic membranes. Widely popularized by Brachet (4), Robertson (15), and others, the hypothesis was slow in finding unequivocal supporting evidence and was eventually modified by placing added emphasis on the putatively intermittent nature of membrane continuity (16). Continuity between nuclear envelope and endoplasmic reticulum has been long recognized (20) and frequently illustrated in a wide variety of plant and animal materials. But it has been far more

difficult to demonstrate continuity between the plasma membrane and the more internal membranes. Perhaps the best illustrations are found in certain secretory cells. For example, Sedar (18) has shown that the intracellular extensions of the plasma membrane in gastric oxyntic cells of bullfrog are continuous with a tubular system having apparently similar membrane structure. Somewhat comparable intracellular extensions of the plasma membranes of chloride cells and pseudobranch gland cells of a marine fish have been reported by Ritch and Philpott (13). However, this is not to say that the tubular system in these cases has been shown to be continuous with the endoplasmic reticulum. Claims of continuity between plasma membrane and nuclear envelope, as in the early

reports of McAlear and Edwards (11) and Epstein (7), have tended to be morphologically inadequate and too inferential to serve as proof.

The observations presented here were made during an investigation of male gamete development in liverworts, a group of primitive, haploid land plants, and provide an unambiguous, graphic demonstration of the heretofore problematic continuity of cytoplasmic membranes.

MATERIALS AND METHODS

Male specimens of the dioicous liverwort *Blasia pusilla* L. were collected in late June 1968 at Portland Arch, Fountain County, Ind. The plants bore ovoid antheridia averaging 0.3 mm in length and 0.2 mm in widest diameter. These were dissected from the gametophyte thallus and immediately transferred to 6% glutaraldehyde in 0.1 M Sørensen's phosphate buffer at pH 6.8, where they were kept for 2 hr at room temperature. After thorough washing in buffer solution, the material was postfixed in 2% aqueous osmium tetroxide for 1 hr. Dehydration in an ethanol series graded in 10% steps, and a four-step graded transfer through propylene oxide were followed by embedment in Epon. Thin sections were stained with uranyl acetate and basic lead citrate, and all observations were made with an Hitachi HU-11a electron microscope.

OBSERVATIONS

Shortly before the *Blasia* antheridium completes its development, it contains many thousands of androgonial cells. These are small, measuring 4–6 μ on a side, and are cuboidal or slightly elongated in shape. Each bears a prominent, nearly spherical nucleus about 3.5 μ in diameter. Thin sections of an androgonial cell reveal a complement of cytoplasmic organelles that typically includes a few dictyosomes and undifferentiated plastids in addition to microtubules, mitochondria, and abundant ribosomes (Fig. 1). At this stage in spermatogenesis the system of cytoplasmic membranes is sparsely developed.

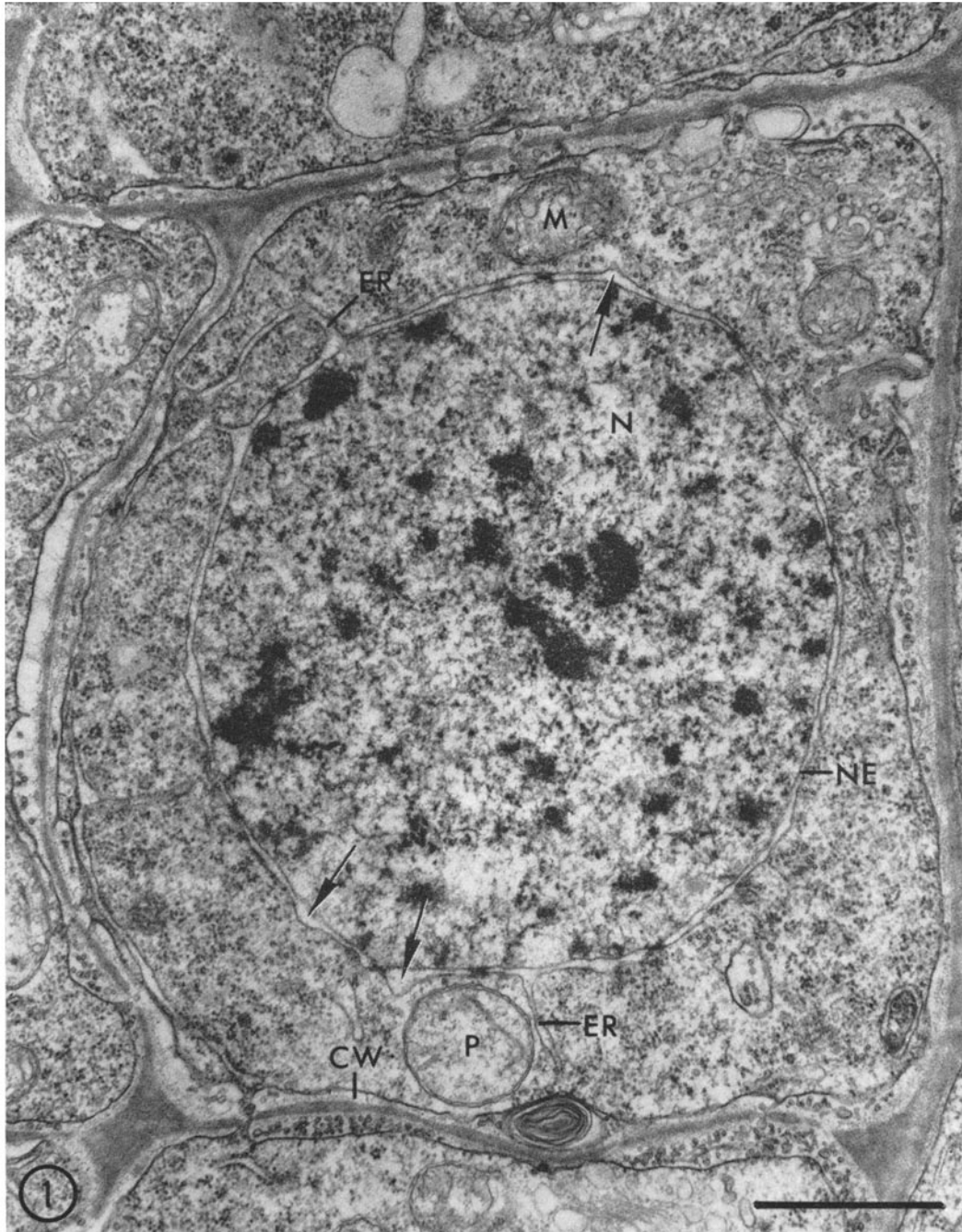
The nucleus usually appears located in the center of the cell where it occupies a substantial portion of the total cell volume. In other androgonial cells of the same antheridium, nuclei were observed to lie in particularly close proximity to the wall. Further examination revealed that the two membranes comprising the nuclear envelope become widely separated near the wall, and with surprising effect: while the inner membrane continues as a limiting boundary of the nucleus, the membrane on the cytoplasmic side flares outward

and continues as the plasma membrane (Fig. 2). In this and similar cells, one commonly sees what appear to be numerous, small vesicular bodies lying between the "exposed" portion of inner membrane and the wall, along which they often extend in limited local aggregations to the extreme corners of the cell. In addition to the vesicular matter, one occasionally sees small areas of ribosome-containing cytoplasmic ground substance and, sometimes, what appear to be small vacuoles essentially devoid of structured content. In some androgonial cells the nucleus is so closely appressed to the cell wall that a portion of the nuclear envelope's inner membrane lies in flat contact with the wall, without any intervening cytoplasmic material. Such a condition is illustrated in Fig. 3. There the nuclei of two adjacent cells are seen to lie against a thicker but more nearly homogeneous region of the cell wall, and are thus separated from each other only by their respective inner membranes, primary cell wall layers, and the common middle lamella. Fig. 3 also shows nuclear contact along three of the four visible sides of the cell wall.

Some of these spermatogenous cells cut in various planes of section reveal what appear to be small membrane-bounded vesicles or other inclusions in the so-called perinuclear space between the membranes of the nuclear envelope (Figs. 4, 5). The possible significance of these features will be discussed in the next section. In the micrographs presented here, both plasma and nuclear membranes appear alike in thickness and staining characteristics, and there is no evident change through their regions of continuity. Detection of possible structural differences will require higher resolution micrographs, as will the determination of the effect that nuclear envelope separation has upon the integrity of nuclear pores and plasmodesmata. In no case has any contact between the cytoplasmic membranes and either mitochondria or plastids been observed.

DISCUSSION

The demonstration of relationship between plasma and nuclear membranes depicted in this report appears to be unique. It seems appropriate, therefore, to consider the likelihood that the tissue exhibiting this condition might be other than normal. At the time of their collection the plants appeared healthy and robust. The probability that the antheridia had been subjected to a period of drying so early in the growing season is strongly



Bar in each figure represents 1 μ .

FIGURE 1 Section of androgonial cell showing sparse development of cytoplasmic membranes. Arrows denote possible blebbing of nuclear envelope (NE). CW, cell wall; ER, endoplasmic reticulum; M, mitochondrion; N, nucleus; P, plastid. $\times 27,000$.

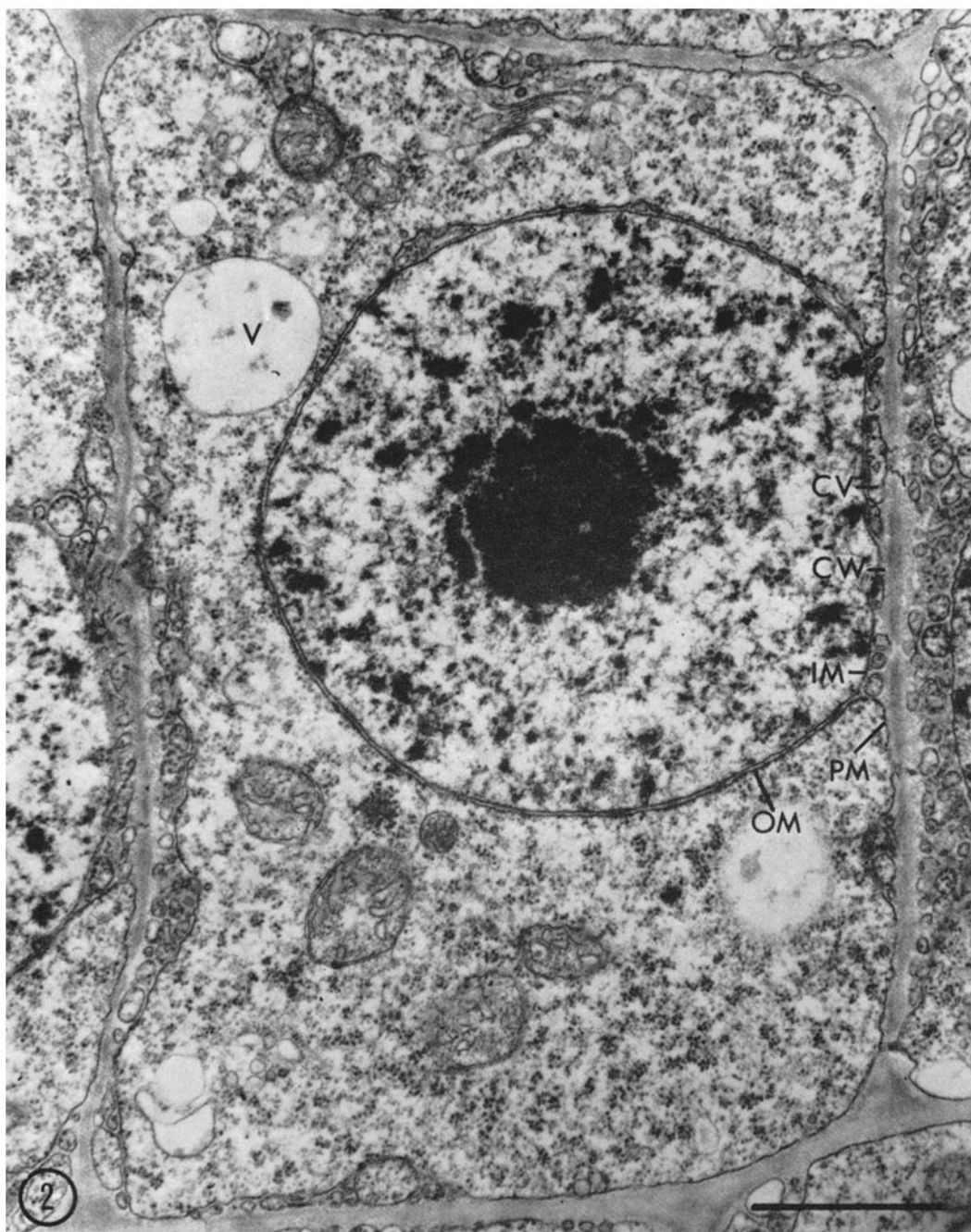


FIGURE 2 Nucleus with a conspicuous nucleolus is appressed to the cell wall (*CW*). At right, the inner membrane (*IM*) of the nuclear envelope contacts the cell wall and cytoplasmic vesicles (*CV*). The outer membrane (*OM*) is continuous with the plasma membrane (*PM*). *V*, vacuole. $\times 27,500$.

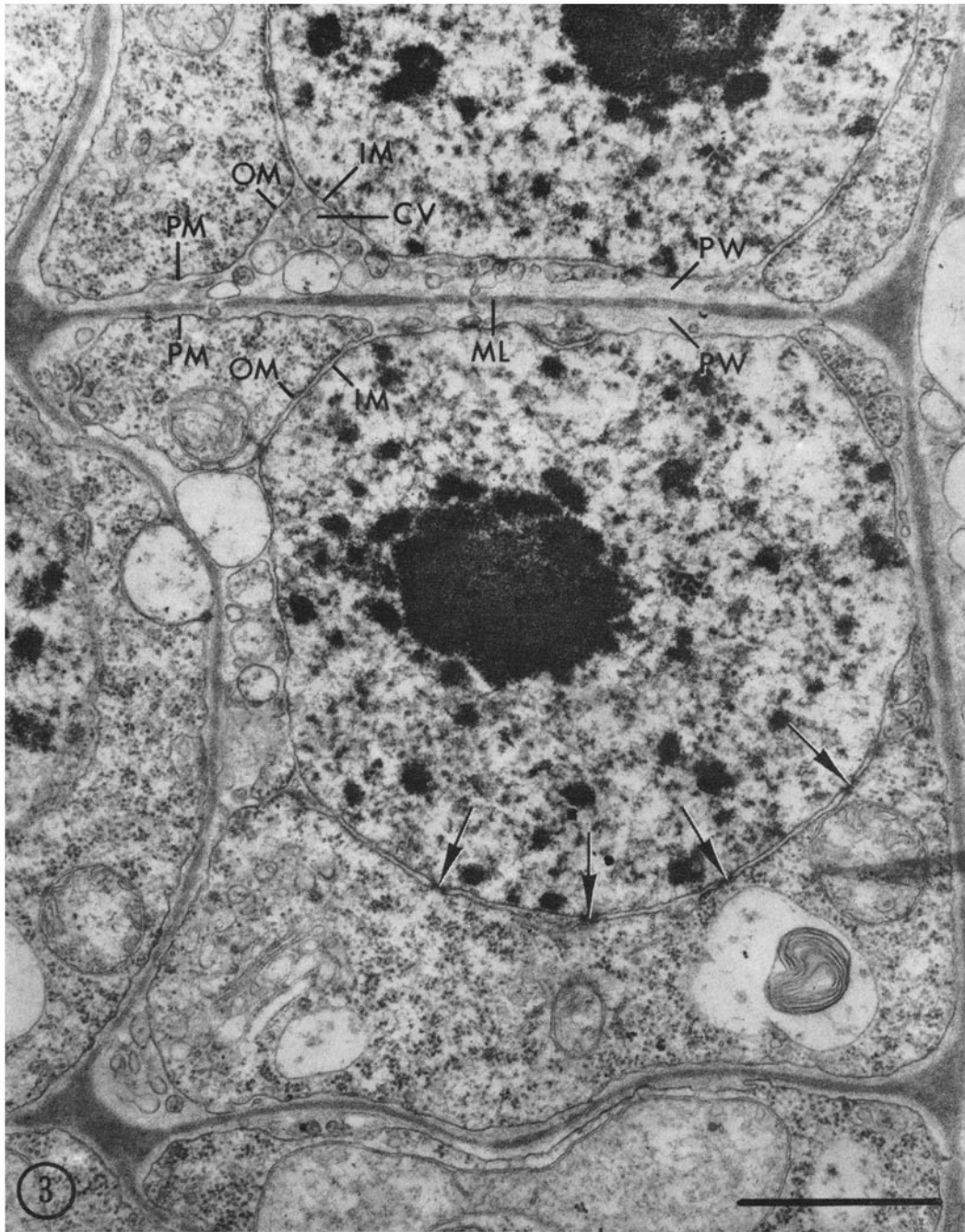


FIGURE 3 Nuclei of adjacent cells are appressed to the primary cell walls (PW). The outer membrane (OM) of the nuclear envelope is continuous with the plasma membrane (PM). Arrows denote nuclear pores. CV, cytoplasmic vesicle; IM, inner membrane; ML, middle lamella. $\times 29,000$.

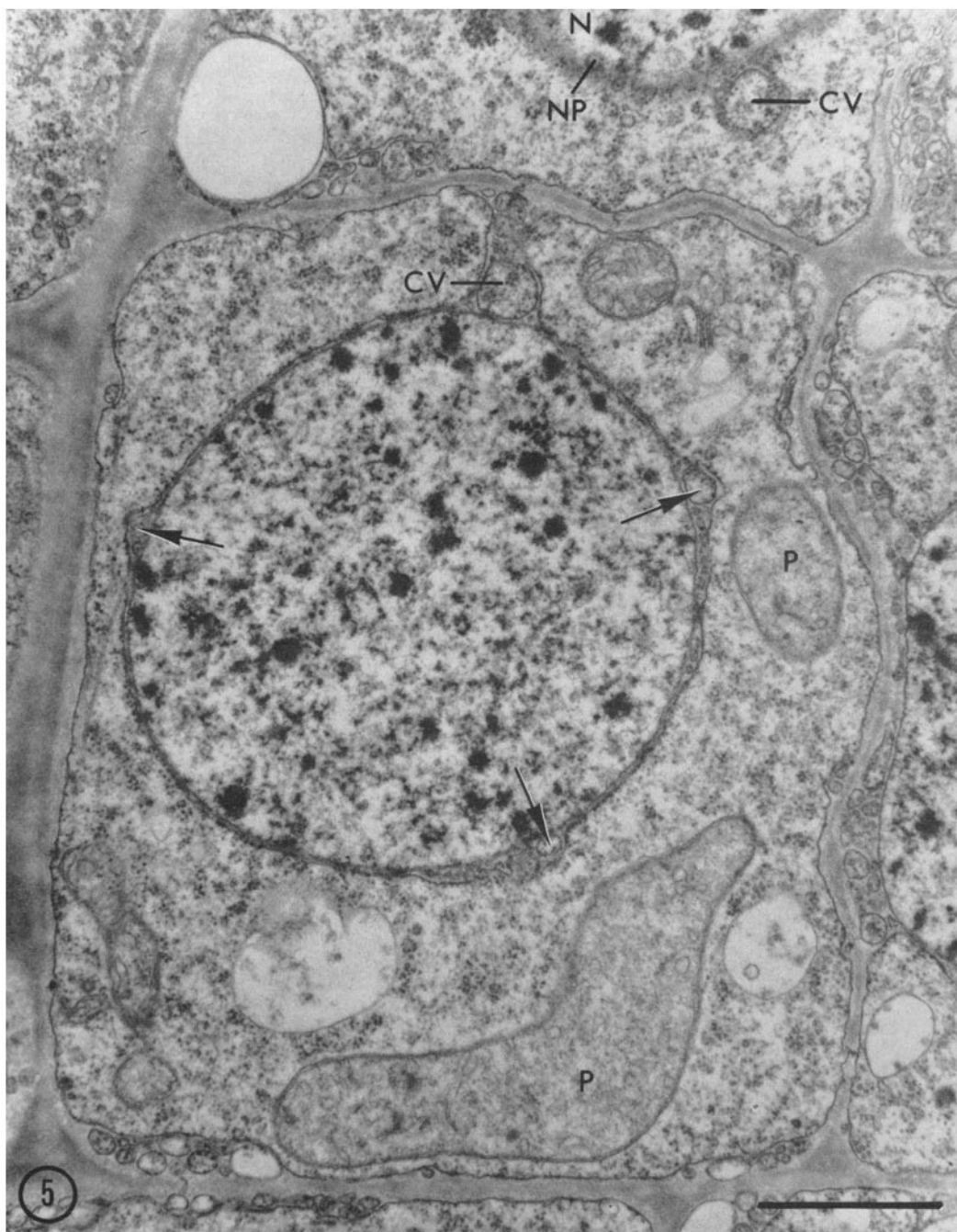


FIGURE 4 Small cytoplasmic vesicles (arrows) appear to be in the perinuclear space separating the membranes of the nuclear envelope. A possible fenestration (*F*) is shown in the endoplasmic reticulum (*ER*) close to the nucleus. *P*, plastid. $\times 26,000$.

minimized by the wet, shady natural habitat of these plants. Each *Blasia* antheridium is enclosed in a small, mucilaginous chamber within the vegetative thallus and is thereby further protected

against dessication and osmotic shock. Close examination in the laboratory revealed no lesions, discolorations, or hyphae that might signify a pathological state. Also, mature antheridia on the same

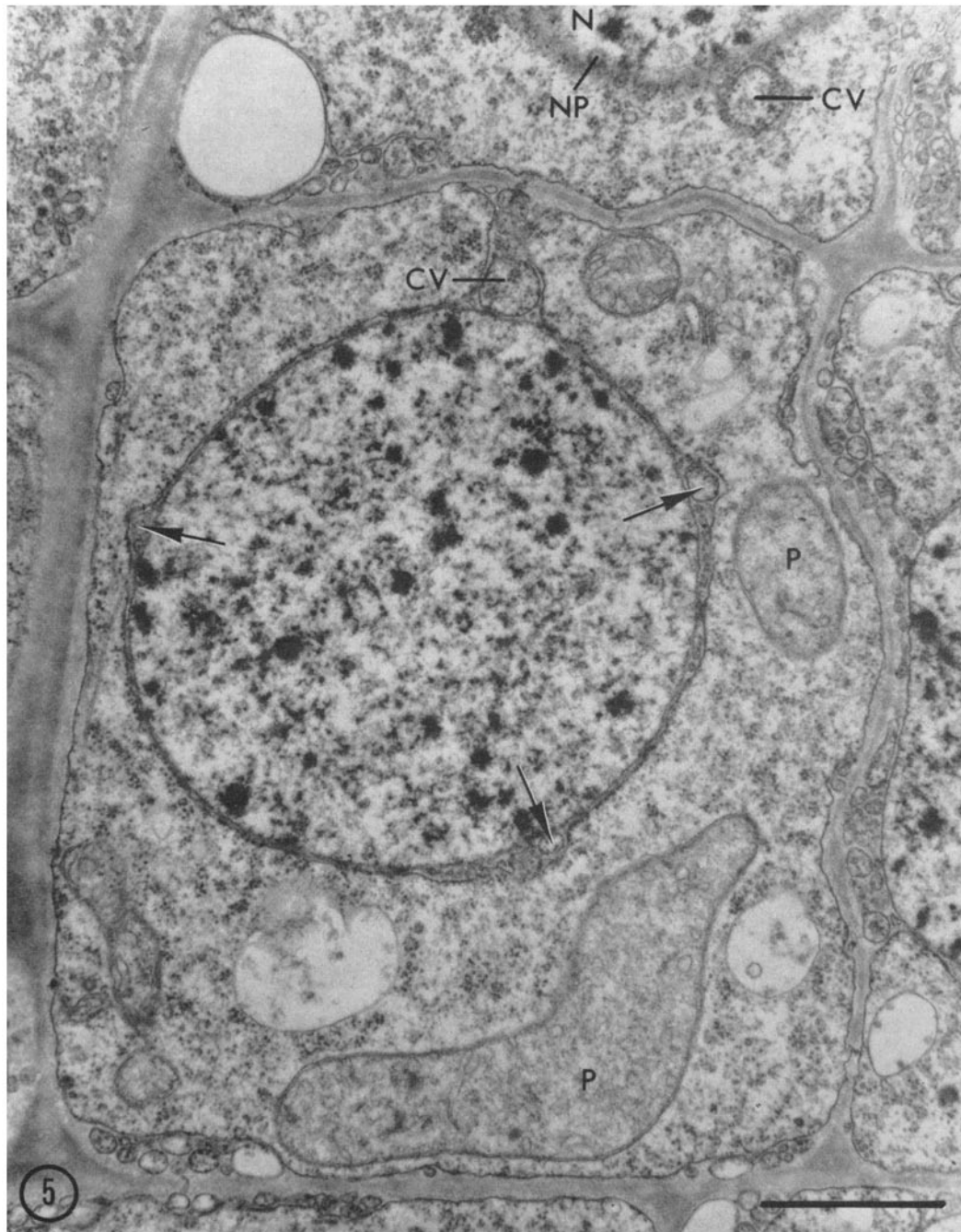


FIGURE 5 Cytoplasmic vesicles (CV, and arrows) are delimited by cytoplasmic membranes, some of which are continuous with the plasma membrane. N, nucleus; NP, nuclear pore; P, plastid. $\times 26,000$.

specimens produced many swimming spermatozooids on the same day the plants were collected and thereafter over a period of several weeks. All this is taken as indirect evidence that the plants were in normally good condition. One might also suspect that the unusual condition of the membranes could be a consequence of technical procedures for electron microscopy. However, fixation, dehydration, and embedment appear to have been uniformly good when judged by the usual morphological criteria. These same methods have been employed extensively in this laboratory for a variety of plant tissues including scores of antheridia from many liverwort species; nevertheless, this is the first material in which such a membrane relationship has been observed. It seems reasonable to assume that the condition is not an artifact of tissue preparation.

It has not yet been determined whether membrane continuity is a regularly occurring characteristic of this phase of male gamete formation. Only a few of the less mature antheridia have been examined, and continuity has been observed in just one of these. In fact, one can only estimate what that exact stage is. The cubical shape, small cell size, high nucleocytoplasmic ratio, relative scarcity of endoplasmic reticulum, and absence of centrosomes collectively indicate late-stage androgonial cells, probably within one cell generation of the penultimate spermatid mother cell stage. If membrane continuity occurs only occasionally, and only at this stage, and if this stage is of short duration, it might be necessary to assay a great many individual antheridia in search of confirmation. Ultrastructural studies of spermatogenesis in other bryophytes offer virtually no help because they generally concern the succession of cytological transformations beginning with spermatid mother cells. Androgonial cell structure is considered by Suire (19) in his extensive study of spermatogenesis in the thallose liverwort *Pellia epiphylla* and by Bonnot (3) in a moss, *Pogonatum aloides*, but neither investigator reports the occurrence of membrane continuity.

Some light microscope studies have included the development of androgonial tissue, and these generally picture the nucleus lying in the center of the cell. Occasionally a nucleus is shown in near contact with the cell wall as, for example, in the liverwort *Marchantia* (Motte, reference 12, Pl. 5, Fig. 8) and the moss *Polytrichum juniperinum* (Allen, reference 1, Pl. 6, Fig. 11). The interphase nucleus

has been shown also to lie appressed to the wall in a few cases, including the liverwort *Fossombronia* (Chaloud, reference 5, Pl. 5, Fig. 2), *Polytrichum commune* (Weier, reference 21, Pl. 2, Fig. 45), and *Sphagnum* (Eymé, reference 8, Pl. 18, Fig. 16). Light microscopy has also revealed substantial contact of the enlarged prophase nucleus with the wall, to the extent that the nucleus became flattened along its contacting sides in *Marchantia* (Gavaudan, reference 9, Pl. 4, Figs. 1–15), in *Fossombronia* (Chaloud, reference 5, Pl. 5, Fig. 3), in the liverwort *Riccia* (Black, reference 2, Pl. 38, Fig. 42). In none of the cited cases can membranes actually be resolved, and the apparent contact between nucleus and cell wall is mentioned only as a possible incidence of membrane continuity. It seems reasonable to conclude that this unusual relationship of the membranes is only occasionally manifest and that its observation in *Blasia* was fortuitous. If such is the case, it may be considered an anomalous (though not necessarily pathological) expression.

Androgonial cells bear a generally strong resemblance to the better known meristematic cells of the vegetative thallus. The similarities include many details of structure, and the mutual roles of cell duplication. They differ in several important ways, however. Unlike the vegetative meristem whose derivative cells mature to become the various tissues of the thallus, androgonial cells ultimately differentiate into motile male gametes exclusively, and are thus uniform and highly specific in their morphological expression. Further, as antheridial development continues, the daughter androgonial cells of each succeeding cell duplication are smaller than before—with the net result, of course, that each antheridium produces a limited but large number of small spermatozooids. Early in the development of the gametangium, this diminution in cell size is accompanied by a reduction in the number of plastids and mitochondria (17, 8). Nevertheless, the diminution undoubtedly imposes intracellular spatial restrictions that affect the nucleus and reduced complement of cytoplasmic organelles. The parietal position of the nucleus is correlated with the general reduction in cell size, to the degree that some nuclei contact (or appear to contact) two or more of the sides of the wall. With continued development through spermatid maturation, there occurs a characteristic loss of nearly all the cytoplasmic ground substance and dissolution of the cell walls.

Although the available evidence is still quite limited, one can suggest a possible sequence of events leading to the unusual relationship between nucleus and cytoplasm. The outer membrane of the nuclear envelope undergoes local proliferation, beginning with the appearance of small blebs (Figs. 1, 4). Extension outward of the inflated regions has the effect of delimiting small masses of adjacent cytoplasm. As seen in thin sections, some local inflations of the envelope appear to contain small, membrane-bounded vesicles which in turn apparently contain ribosomes (Figs. 4, 5). These vesicles are tentatively interpreted as cytoplasmic ground substance seemingly isolated in sectional profile by convolution of the membranes, rather than as some type of discrete particle having *de novo* origin in the perinuclear space. Sections also suggest that membranes of the endoplasmic reticulum may occasionally be fenestrated (Fig. 4). In time, the proliferating membrane contacts the plasma membrane in one or more places and fuses with it. At this stage a portion of the nucleus is separated from the cell wall only by vesicular cytoplasm (Fig. 2). As the nucleus moves closer to the wall some of that same cytoplasm is displaced laterally, thus bringing the inner membrane of the nuclear envelope into contact with the cell wall (Figs. 2, 3). Through the establishment of membrane continuity between nucleus and ectoplast, much of the vesicular cytoplasm apparently becomes isolated outside the cell, and the vesicles soon lose their resemblance to cytoplasmic ground substance. Some of the vesicular matter evidently becomes incorporated into the wall which then appears thicker in the area of nuclear contact (Fig. 3). It is noteworthy that the aforementioned blebbing and proliferation affects only the outer membrane of the nuclear envelope. In contrast, nuclear blebbing in response to cell injury involves both membranes (22). Whaley et al. (23) have reported proliferation of endoplasmic reticulum in response to various forms of injury, but their micrographs bear little or no resemblance to those showing the vesicular condition manifest in *Blasia*. Further, there is no evidence that the plasma membrane undergoes either proliferation or withdrawal from the cell wall as has been recorded for injured cells (23). Nevertheless, injury may not yet be excluded as a possible causal factor in the present case.

Listing a few of the interesting problems posed by the foregoing observations, one might consider whether this condition of membrane continuity is

reversible and, indeed, whether these cells can survive. It is questionable whether mitosis could occur when the nucleus is not completely invested by cytoplasm. One might consider the effects of the restraint imposed on the protoplast by the surrounding wall, and the effects of wall breakdown. How would the loss of its usual permeability barrier affect the cell under conditions of plasmolysis? One might also consider the significance of membrane continuity to virology. It has been shown that newly synthesized virions of potato yellow dwarf virus are released into the perinuclear space of infected cells of both plant host (10) and insect vector (6). It follows that an open system of cytoplasmic membranes, even if intermittent, would be important in viral transmission.

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