Vacuolation in the Cytoplasm of Plant Cells

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There are two important aspects of cell elongation in plants: one is the lengthening of the cell membrane, both in the plasmalemma and in the cell walls and the other is the formation and development of the vacuoles which tend to occupy most of the cell's volume. The data we have obtained indicate that the growth which takes place from the final level, in meristematic cells, to the first level of the differentiated cells reaches a proportion of five times the size of the former in the first 24 hours of differentiation at 15°C; and this involves a marked increase in the surface area and volume of the cell.

Elongation of the cell membrane takes place, like the formation of the cell plate during cytokinesis (Whaley and Mollenhauer 1963; Frey-Wyssling, López-Sáez and Mühlethaler 1964) by the supply of vesicles from the Golgi apparatus, the membranes of which coalesce with the plasmalemma while the content is deposited as part of the cell wall. (Risueño, Giménez-Martín and López-Sáez 1968).

The process of vacuolation in plant cells, however, has been explained by various theories as the result of investigation carried out both with the light microscope and with the electron microscope. De Vries (1885) was of the opinion that the origin of the vacuoles was to be found in certain organules similar to plastidia (tonoplasts) passed on from cell to cell, while Pfeffer (1888) attributed their formation to the separation of a fluid phase within the cytoplasm, and not to any sort of organule. Both theories were re-stated when Guilliermond (1934) attributed vacuolation to the same origin as Pfeffer and Dangeard (1927) envisaged the existence of an original vacuole which was divisible "pari passu" with successive cell divisions.

The introduction of the electron microscope again raised the problem of the origin of these vacuoles, and a great variety of theories were proposed.

The results of the former authors were based, almost specifically, on observations of meristematic cells, or cells from the neighbouring areas within the meristems. The study here presented, on the other hand, was carried out during the development of cells from the exothecium and endothecium of *Allium cepa* anthers.

Materials and methods

Anthers from *Allium cepa* were obtained at different stages of development and studied under the light microscope. Of the six anthers from each bud, one was stained with acetic orcein to determine the stage of development, while the rest were fixed in 2% potassium permanganate, dehydrated in an acetone series, stained by the uranyl-lead-acetate technique (Giménez-Martín *et al.* 1967), and embedded in Durcupan. The sectioning was done with an LKB Ultratom and the observations were carried out with a Siemens Elmiskop II electron microscope.

Observations

First signs of vacuoles: In cells from very young anthers, in which no mother-cells of pollen grain in meiosis could be observed as yet, taken from the three or four outermost layers, it was possible to study the origin, formation and development of the vacuoles.

A first stage shows the cytoplasm of both exothecium and endothecium entirely devoid of vacuoles, with a relatively small number of Golgi bodies, and ER consisting of long cisternae widely dispersed throughout it.

In later stages, when slight elongation has begun as the cell grows, the beginnings of vacuolated areas can also be observed in parallel formation, invariably found on patterns of ER (Fig. 1), their appearance and development following an inward direction, from the outermost layer (exothecium) towards the inner layers of the endothecium.

The areas in which the vacuolation begins appear as slight local dilatations of the cisternae in the ER, the ends of which open up in angular fashion to form the widening (Fig. 2). A single cisterna may possess several initial vacuole areas connected by ER without dilatation while others may (Figs. 3b–9) at the same time, show bifurcations, with vacuoles beginning to form, usually, at the junctions (Fig. 3a).

The ER that forms the double nuclear membrane occasionally appears dilated in the perinuclear space, constituting formations similar to vacuoles (Fig. 4), while the wall of the vacuole, also occasionally, forms the actual limit of the nucleus (Fig. 5).

Young star-shaped vacuoles: As the dilatations increase, and the size of the vacuoles along with them, the latter become noticable star-like in shape, extending from the angles formed by the ER, varying in length. In the same ER formation where we find star-shaped vacuoles, other vacuoles can be observed in the incipient stage (Fig. 3a). When several vacuoles can be observed in the same cytoplasm, some of them can be connected by ER patterns, and most of this system is linked up with the vacuole formations.

The star-like vacuoles appear to be relatively early formations in relatively young cells (Fig. 6). As the cells grows, the number of vacuoles decreases

as larger vacuoles appear, and these gradually take up positions at the two opposite poles of the cell, while the number of corners decreases at the same time, and the outline become more regular (Fig. 7).

Spherical vacuoles: When the cell is growing fast, fully developed spherical vacuoles can be observed (Fig. 3c). The angular formations have

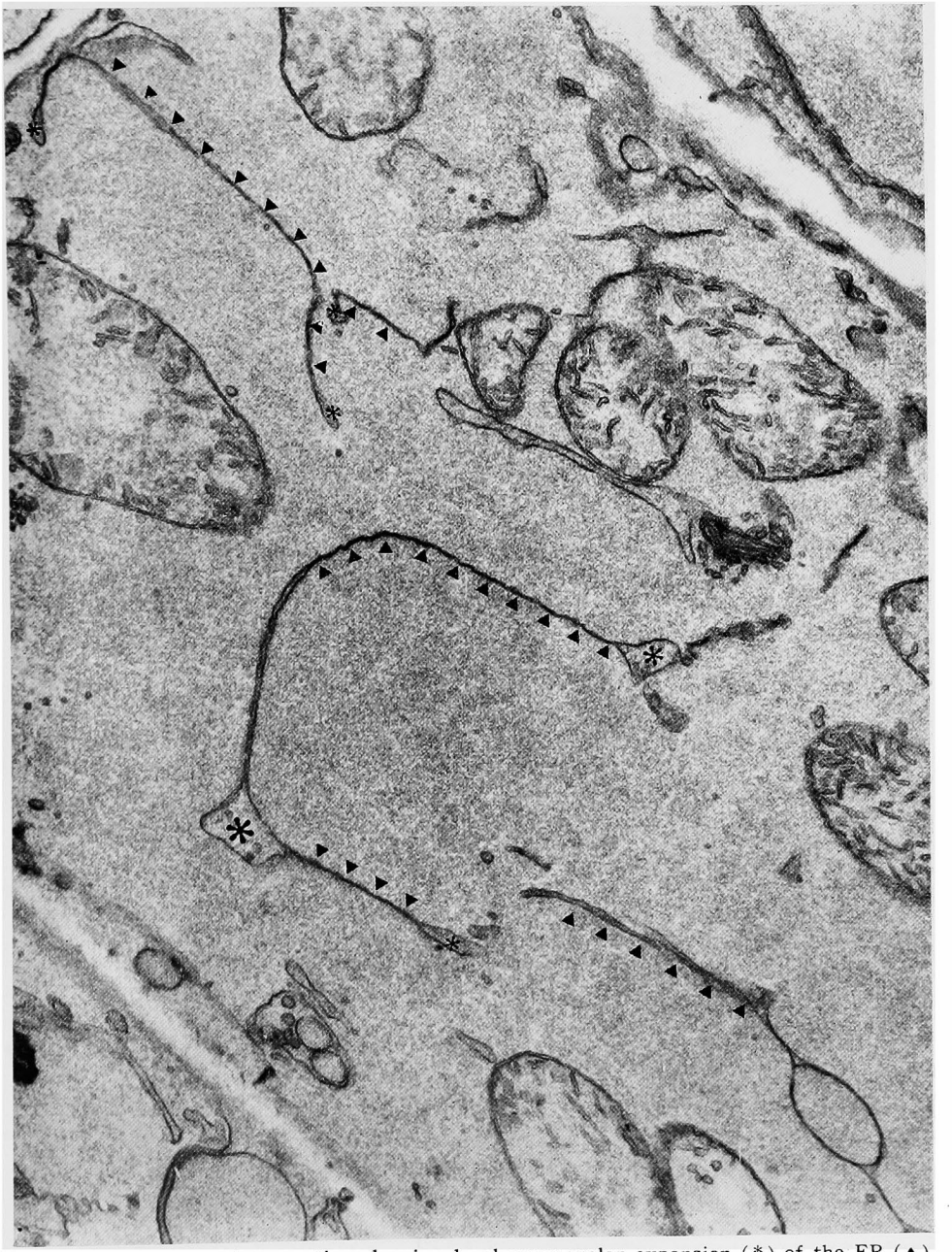

Fig. 1. Young cell of connective showing local prevacuolar expansion (*) of the ER (▲).

disappeared, and no more ER patterns are seen in connection with the vacuoles. The vacuoles grow during this period and occupy most of the cell, with the cytoplasm in the border area between the cell wall and the vacuole. Finally the vacuoles are reduced to one occupying most of the cell's

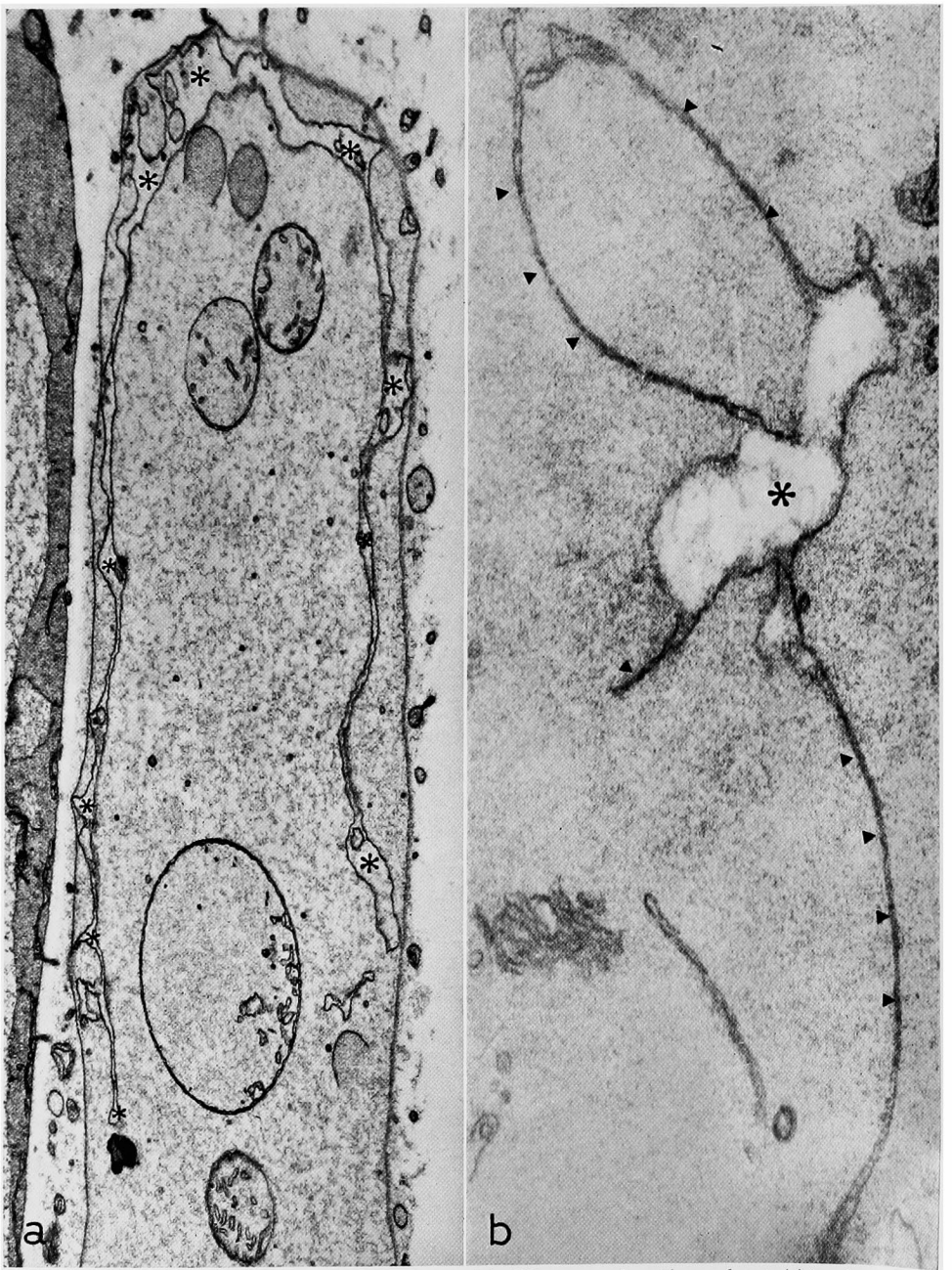

Fig. 2. a, cell belonging to the exothecium with ER expansion throughout (*). b, young vacuole (*) in endothecium cell with a number of connected ER (▲) patterns crossing it.

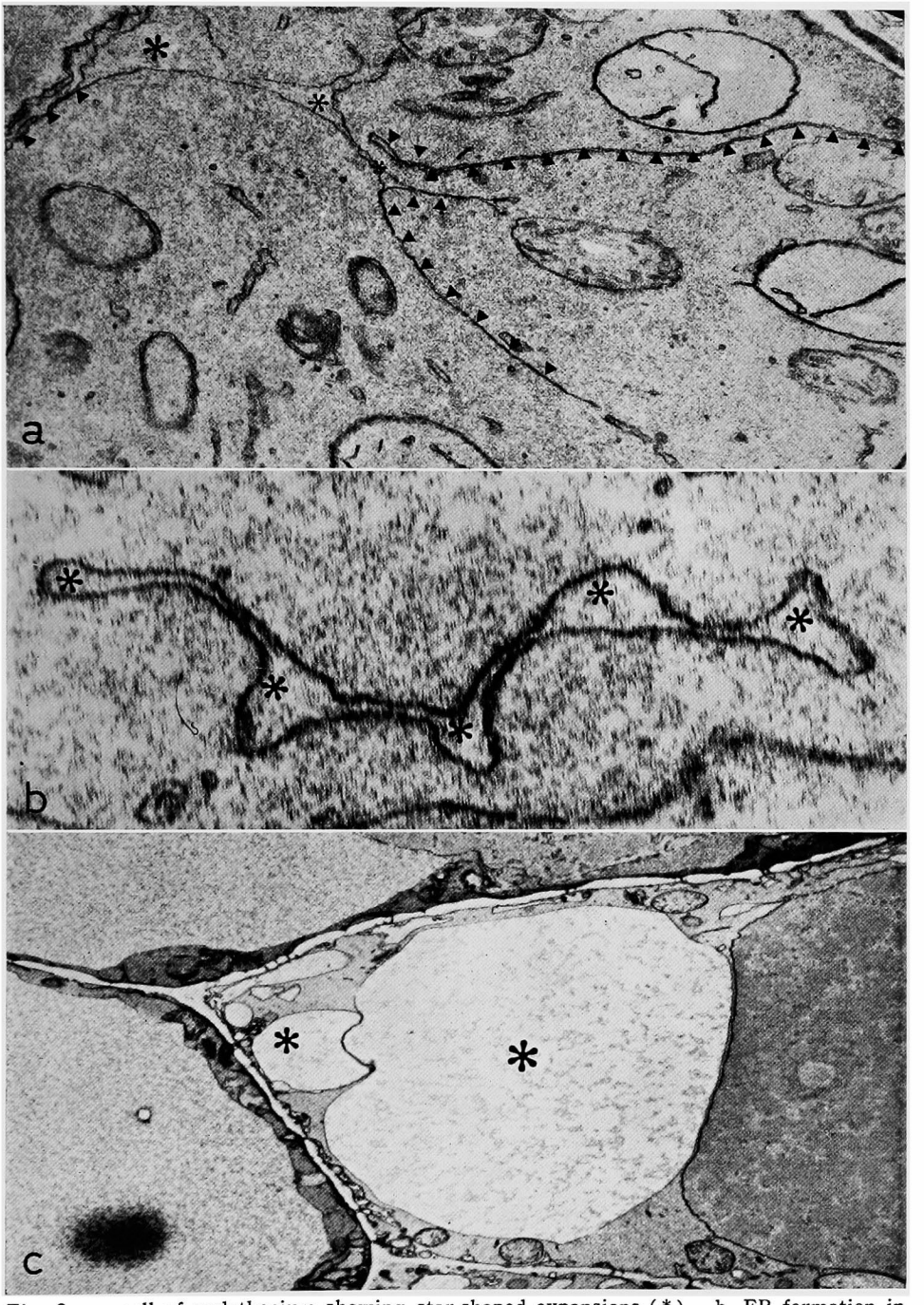

Fig. 3. a, cell of endothecium showing star-shaped expansions (*). b, ER formation in a parenchyma cell, in which we observe various provacuoles in chain formation (*). c, fully developed vacuole in a parenchyma cell showing the circular outline (*).

volume with its outline following the contours of the cell wall and nucleus (Fig. 8).

Tonoplast: The origin of the vacuoles inside the ER, and the distension

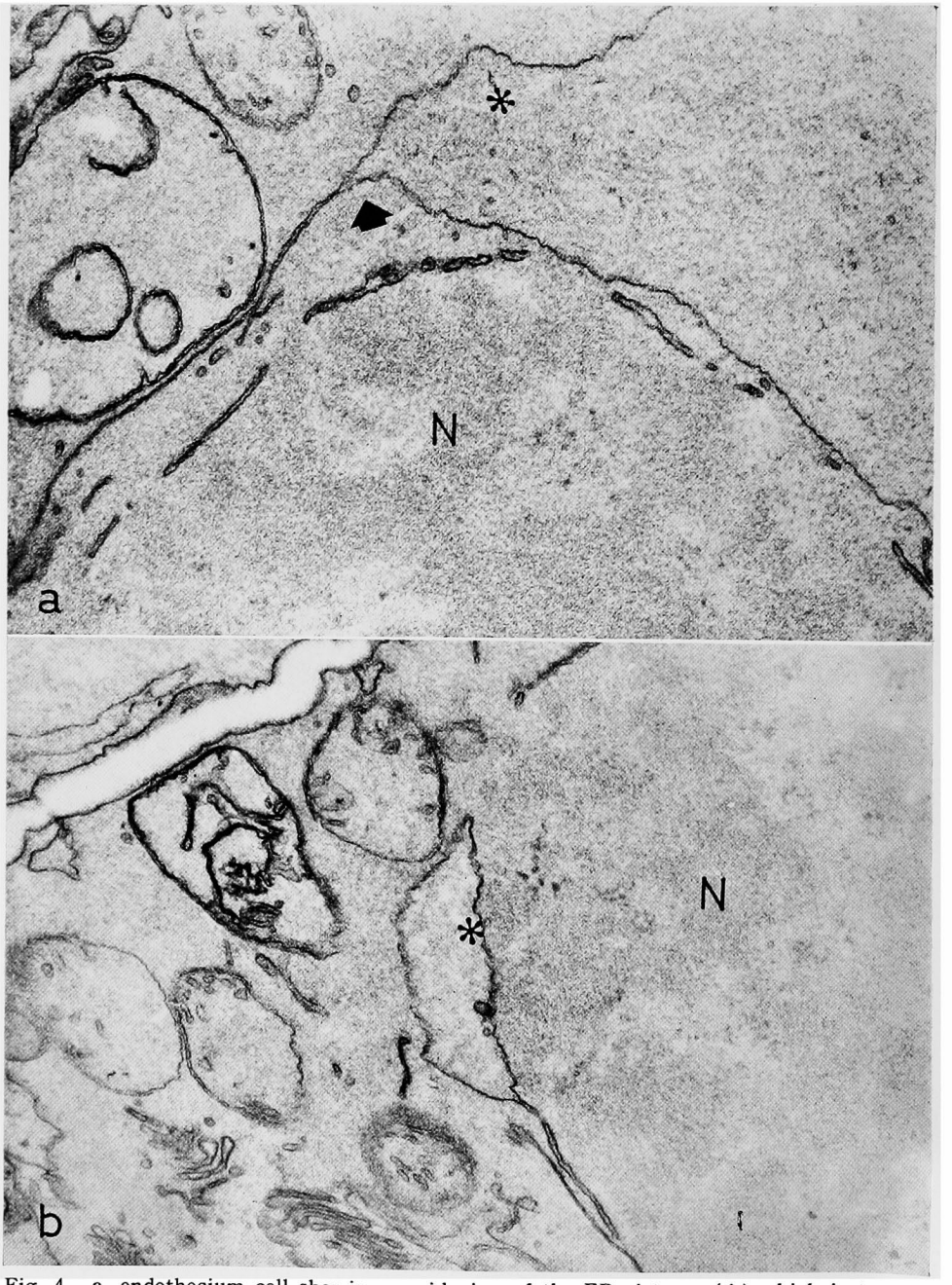

Fig. 4. a, endothecium cell showing a widening of the ER cisterna (⬆) which is to give rise to a vacuole (*). b, perinuclear expansion resembling vacuoles in course of formation (*).

of its walls, show us the limiting membrane of the ER as forming the borderline of the vacuole membrane itself. The tonoplast, consequently, appears as

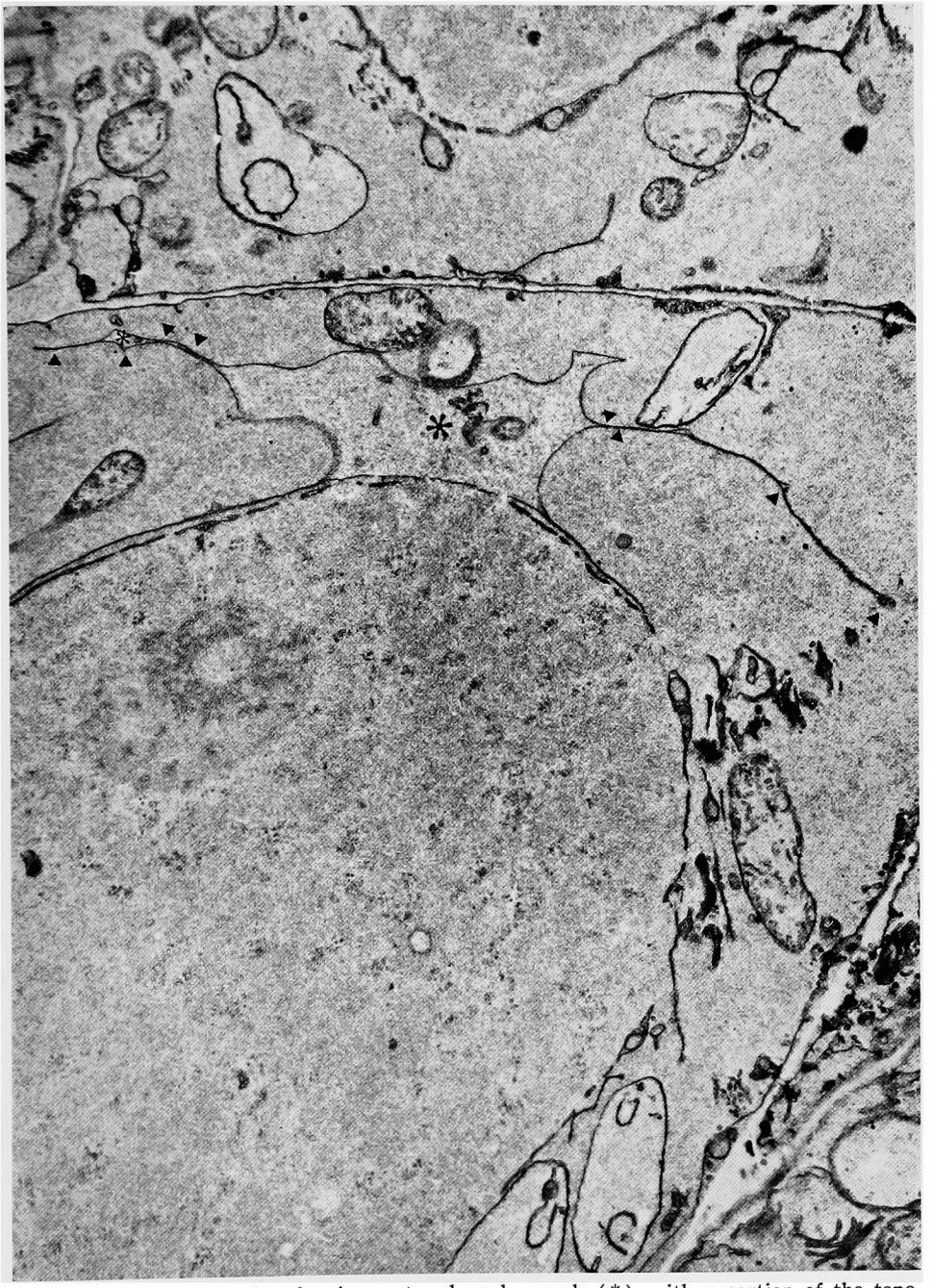

Fig. 5. Cell of connective showing a star-shaped vacuole (*), with a portion of the tonoplast forming part of the nuclear envelope, in connexion with ER patterns (▲) and provacuoles (*).

ontogenically originating in the actual membrane of the ER which it resembles in dimensions and characteristics.

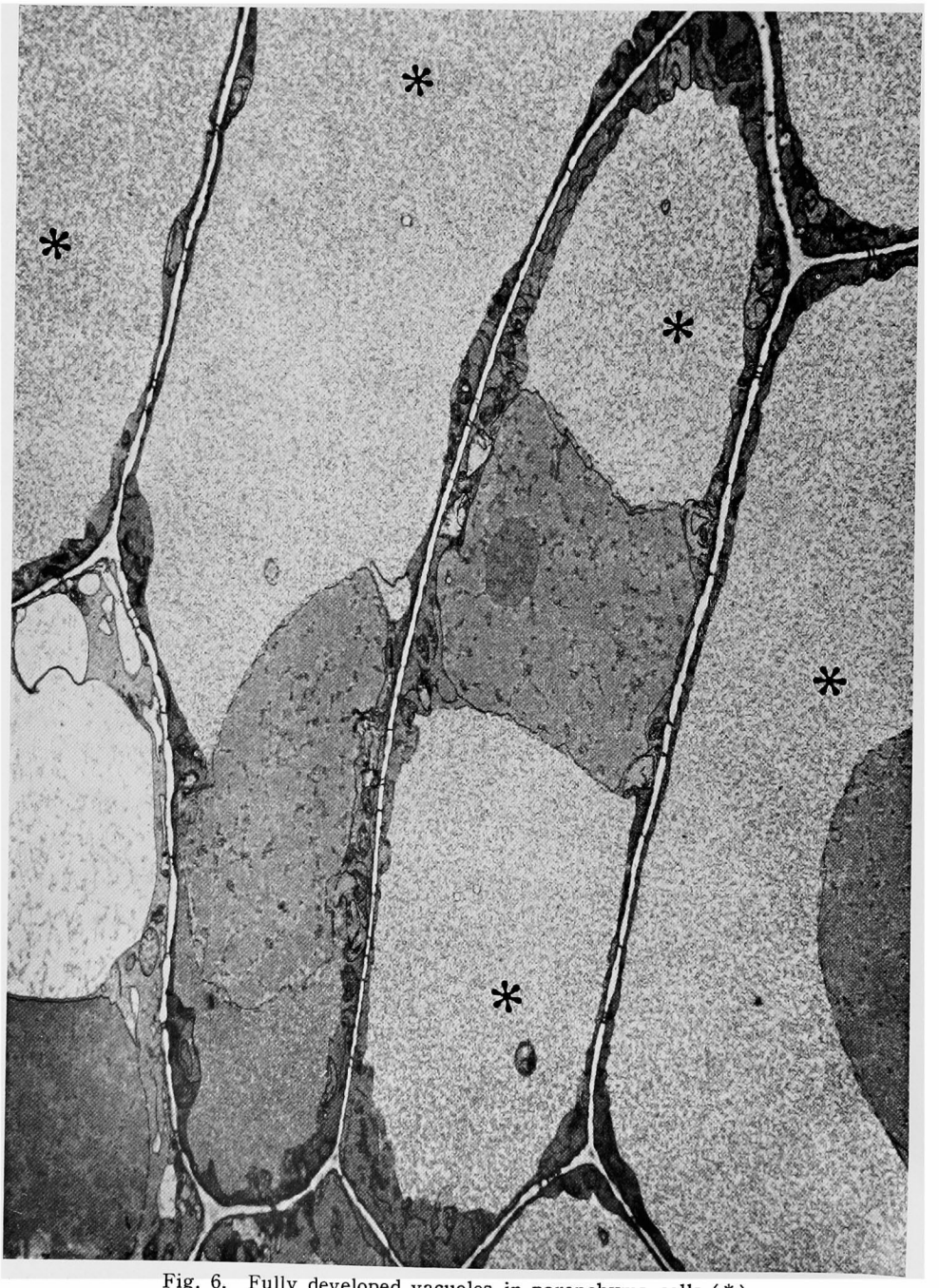

Fig. 6. Fully developed vacuoles in parenchyma cells (*).

Discussion

Various hypotheses were put forward when the origin of the vacuoles began to be studied with the aid of the electron microscope. Those of De

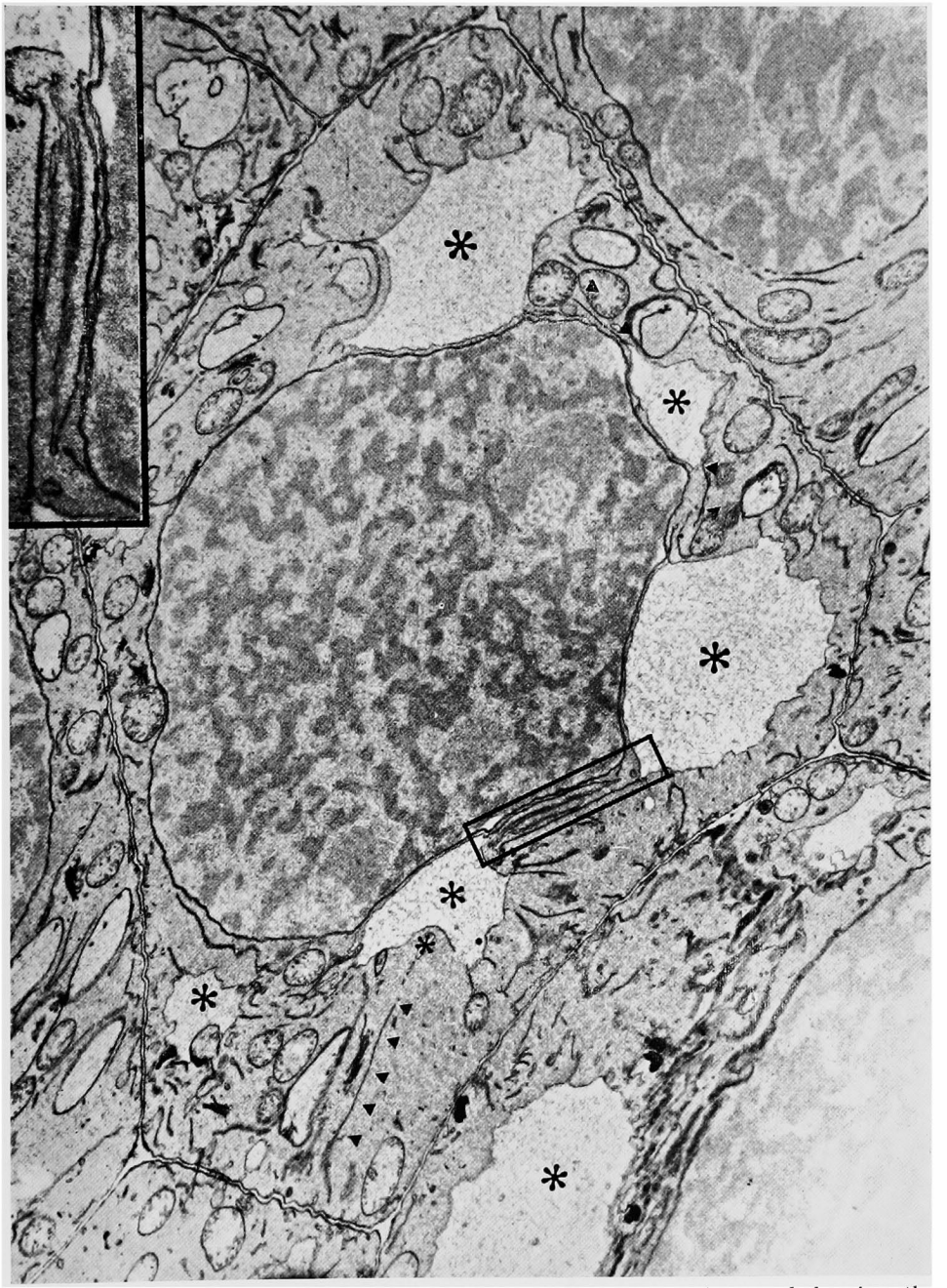

Fig. 7. Star-shaped vacuoles (*) showing connexions between them and forming the greater part of the cell's ER (▲).

Vries (1885) and Pfeffer (1888) were re-stated and various authors have worked out different hypotheses to explain the vacuolation. Mühlethaler (1958), accepting Pfeffer's theory, considered that vacuolation is not due to any organule or cytoplasmic system, but to fluid separation within the cytoplasm, a plasmic membrane being formed all round with the characteristics of the

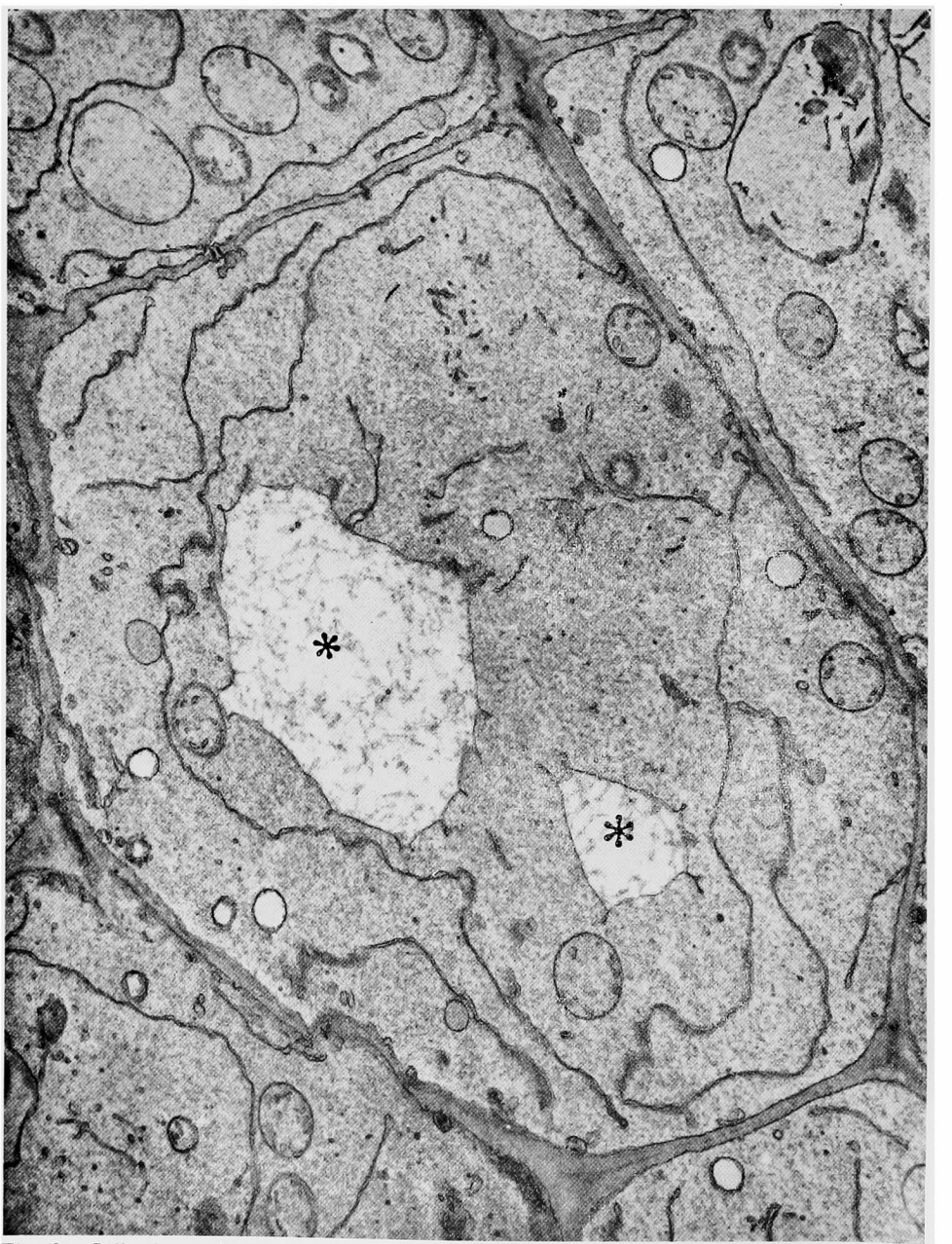

Fig. 8. Cells from the postmeristematic region of an *Allium cepa* root showing their characteristic vacuoles (*) like those of the anther cells.

membrane unit mentioned by Robertson. A number of authors have suggested the influence of various cytoplasmic membrane systems or a system specifically designed to produce vacuolation. Buvat (1957–1958–1962) is of the opinion that the provacuoles arise from the ER and might be formed by invaginations of the plasmalemma through pinocytosis (Poux 1962) followed by separation and isolation of the vacuole within the cytoplasm. The so-called ER in meristematic cells represents, in opinion of Gifford and Stewart (1967) the tonoplast already associated with a definable vacuole. Marinos (1963) suggested origin of the vacuoles is the Golgi apparatus, by dilatation of the sacs and subsequent coalescence among them to form larger vacuoles. According to others, again, the vacuoles might be produced by modifications of organelles such as plastidia or mitochondria.

There is a study by Barton (1965) of the meristem of *Phaseolus*, in complete accord with De Vries's suggestion that some specific organule was responsible for the organization of vacuoles, showing that one or more provacuoles remain during the process of cell division and ensure their structural continuity in the passage from cell to cell.

The simultaneous activity of several cytoplasmic system in the production of vacuoles (ER and Golgi apparatus) has been pointed by Ueda (1966) and by Matile and Moor (1968). The latter authors, experimenting with freeze-etched on corn meristems, were led to attribute the genesis of vacuoles to the formation of small vesicles from the ER (provacuoles) which may fuse together to form vacuoles, while these, in their turn, are joined by vesicles from the Golgi apparatus by invagination of the tonoplast.

The great diversity of opinions mentioned above will serve to give an idea of the present state of the problem. The existence of a limiting membrane for the vacuole, with structural dimensions like those of the membrane unit, makes it reasonable to suppose that it might be derived from one of the cytoplasmic systems with an already existing membrane unit, and not organized "de novo". It is difficult to study the origin, development and evolution of vacuoles in meristematic and postmeristematic cells on account of their intense elongation and the consequent rapid formation of vacuoles along with a notably high proportion of membrane systems, whereas when elongation takes place more slowly, with fewer membrane and organule systems, as in cells from the exothecium and endothecium, it may be deemed more feasible to study the origin of vacuoles.

Our own observations show that the vacuoles appear and develop against a pattern of ER. However, we have not found any formation of the type mentioned by Buvat and Poux, in which invaginations of the plasmalemma might play a part, in connexion with the ER as the source of the vacuoles. These seem to arise basically from local expansions of the ER which become greater and greater as differentiation proceeds until they occupy most of the space taken up by the cell. These expansions have occasionally been observed

in the perinuclear space itself, but we have not been able to observe any spatial relationship between such vacuoles and the cisternae of the Golgi apparatus.

Our observations therefore agree with those of other authors with regard to the locus of vacuole formation, but differ as to their further mode of development. In our opinion, the growth of the vacuole, and consequently of the tonoplast, takes place by gradual dilatation of the ER canal and transformation of the membrane into a tonoplast. Thus there would be no need for active growth of the vacuole membrane since, as the canal of the ER gradually dilates, its membrane continues to surround the contents. Buvat's, and our own, observations of chains of vacuoles within the pattern of the ER itself appear to suggest that the gradual dilatation of several vacuoles might form larger vacuoles by fusion of their contents, in which case the membranes would not need to coalesce since they would belong to the same membrane system. The star-like vacuoles would arise from dilatations in areas of the ER where a number of cisternae converge and the protrusions or tentacles would be formed by cisternae coming together and not, as has been suggested, the result of collapsed vacuoles. The star-like shape is observed only in the early stages of vacuole formation whereas, when the vacuoles are fully developed, they still become round, or fill most of the cell volume as before, which would not be the case if one of the effects of fixing were to cause the vacuoles to collapse (Manton 1962).

If the ER is a continuous formation within the cytoplasm of the cell, the formation of the provacuoles, the young vacuoles and, finally, the single vacuole might be conceived as a differentiation of the ER which takes place locally, then over a wide area and, finally, throughout, with consequent expansion of its contents until they come to occupy most of the volume possessed by the cell. On this hypothesis, the tonoplast would be the actual membrane of the ER itself.

Summary

By examining the cells of the endothecium and exothecium of *Allium cepa* anthers we have been able to study the formation and development of the vacuoles. These appear to arise from local expansions of the ER which, in the course of cell differentiation, increase in size until they form one single vacuole occupying most of the cell volume.

The three phases in the development of the vacuoles, local expansions of the ER provacuoles, young or star-shaped vacuoles, and fully developed, or spherical vacuoles, take "pari passu" with the differentiation and elongation of the parenchyma of the anther.

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