ON A RETICULAR DERIVATIVE FROM GOLGI BODIES IN THE MERISTEM OF *Anthoceros*

IRENE MANTON, Sc.D.

From the Botany Department, The University, Leeds, England

ABSTRACT

Micrographs of dictyosomes in face view and in profile, together with serial sections representing both these planes, are reproduced from three sample cells at different developmental stages in the meristem of *Anthoceros*. The stages are: a vegetative cell at anaphase of a mitotic division, a vegetative cell in an early stage of postmitotic extension growth, and a young spore mother cell in the act of rounding up before the onset of meiosis. The observations suggest that proliferation of tubules from the edges of the dictyosomal cisternae into the cytoplasm is occurring with varying intensity and with slightly different morphological expression in all three cells. In all, the tubules are joined into a reticulum which exhibits local swellings at varying distances from the unfenestrated part of the subtending cisterna. A comparison is suggested between the observed reticulum and "smooth" endoplasmic reticulum of animals but it is not claimed that all the cytoplasmic tubules detectable in *Anthoceros* need have arisen in this way. Morphological differences discernible between tubules near their point of attachment to dictyosomes and others apparently involved in the formation of the new nuclear membrane at the end of a cell division could mean that more than one category of tube may exist in these cells. A plea is registered for restraint in the formulation of far reaching theories until more facts are available on unequivocal evidence.

INTRODUCTION

The close resemblance when seen in section between the Golgi bodies (dictyosomes) of animals and of plants is so striking that it is one of the first observations to be forced on the attention of any botanical cytologist examining thin sections of even moderately well fixed plant cytoplasm for the first time. It is therefore not surprising that there is already a considerable literature and that the comparison has been made for almost all the major plant groups. Beyond this our knowledge is minimal. Moreover, since plant cytology is much less advanced than animal cytology in this field, some considerable mistakes could arise if it were assumed that a structural resemblance would justify carrying over intact all the concepts and conclusions which have laboriously been pieced together for the one kingdom into the other. An independent establishment of basic facts for each new type of material is therefore an essential requirement for effective progress.

The *Anthoceros* meristem has several special advantages as a source of material for the study of fine structure. The plant is a thalloid liverwort and therefore a member of a different and more primitive group (Bryophyta) than the higher plants which are usually preferred (see however,

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references 5 and 9). The meristem itself, at the base of the capsule, is cylindrical and is therefore convenient in shape as well as in size. Being embedded in soft tissue and further protected by copious mucilage it is easily dissected out without risk of drying. Finally the cell contents are a little more simply arranged than in many other small-celled meristems since the plastid primordia, which are often very numerous, are here replaced by only a single one in each cell which divides at each mitosis and which is large enough for its position in the cell to be easily ascertained; the remaining cytoplasm is thus less encumbered by objects than is often the case.

The only major disadvantage is the impossibility at present of raising capsules at will in cultivation. This is somewhat offset by the regularity and abundance with which fruiting material can be obtained in any normal year in a known locality. The quality of fixation with the usual methods is relatively good, and it is hoped that the present communication will be followed by several others as the many topics for which the material is suitable can be explored.

MATERIALS AND METHODS

The investigation was made possible by the discovery of a large colony of fertile material of *Anthoceros laevis* on a path near Leeds in October, 1957. Fixations were taken by dissecting out young unopened capsules under a binocular and removing the bottom half-millimeter with a razor blade. The fixative was 2 per cent osmium tetroxide buffered to pH 7 and used for 1 hour. In most specimens the haustorial base was also cut off leaving the material as a small cylinder of tissue open at each end. This cylinder contained young meristematic cells in various stages of vacuolation but no mature cells.

In order to obtain somewhat older tissues for comparison, a second half-millimeter slice was cut with a razor from similar capsules at a level just above the top of the involucre which envelops the base of the capsule outside the calyptra. These slices contained highly vacuolated cells, long past the meristematic condition though still not fully mature, and young spore mother cells in the fertile region.

In the following year (1958) a close watch was kept on the locality at intervals throughout the summer and the first signs of capsules were encountered in late August and early September. Fixations were repeated on the same lines as in the previous year using, however, rather younger capsules. Only those that had not yet burst through the extended calyptra were selected and in this material many division stages were encountered in the meristematic bases.

The remaining technical methods, including methacrylate embedding and sectioning on a Porter-Blum microtome with a glass knife, are standard. The observations were carried out on a Siemens Elmiskop I in the Leeds Botany Department.

OBSERVATIONS

The observations reproduced in the plates refer to three selected cells from the 1958 material. Fig. 1 illustrates a vegetative cell at anaphase from the innermost layer of the capsule wall immediately bordering on the fertile tissue. The plastid has already divided and its two halves (*p*, *p*) are at opposite ends of the cell. The two groups of chromosomes (*chr*, *chr'*) have moved apart though they are not yet separated by a new cell plate.

Two special fields in the cell of Fig. 1 are reproduced at higher magnifications in Figs. 2 to 4. Fig. 2 is from the region of cytoplasm indicated by the arrow on the left of Fig. 1. It shows a dictyosome in full face view at a magnification of

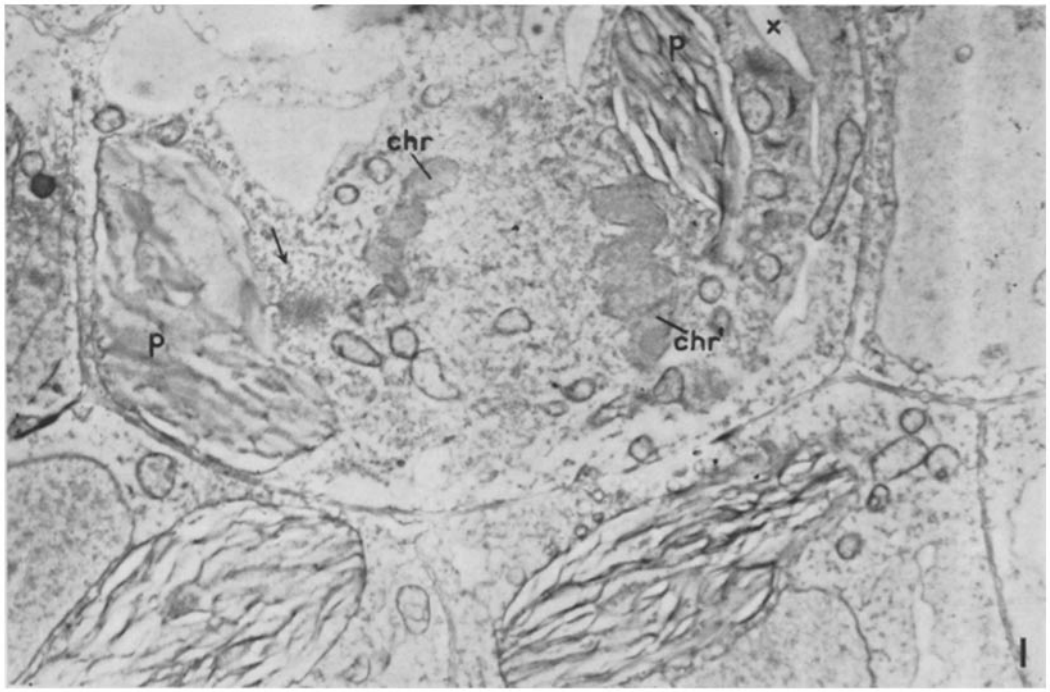
Meristem of *Anthoceros laevis*

FIGURE 1

A cell in anaphase from the innermost layer of the sporangial wall showing the two groups of chromosomes *chr*, *chr'* and the two halves *p*, *p* of the plastid at opposite ends of the cell. The other symbols denote fields reproduced at higher magnifications in later plates, the arrow indicating the field in Figs. 2 and 4 *a* to 4 *c*, the cross indicating the field of Fig. 3. Micrograph H3318. Magnification about 6,000.

FIGURE 2

Face view of a dictyosome from the field marked by the arrow in Fig. 1 with part of a mitochondrion *m* beside it; the edge of the dictyosome showing proliferation of tubules with local swellings and anastomoses. This field is reproduced again as part of a series of three sections at a lower magnification in Fig. 4 *b*. Micrograph H3332. Magnification 60,000.


P
x
chr
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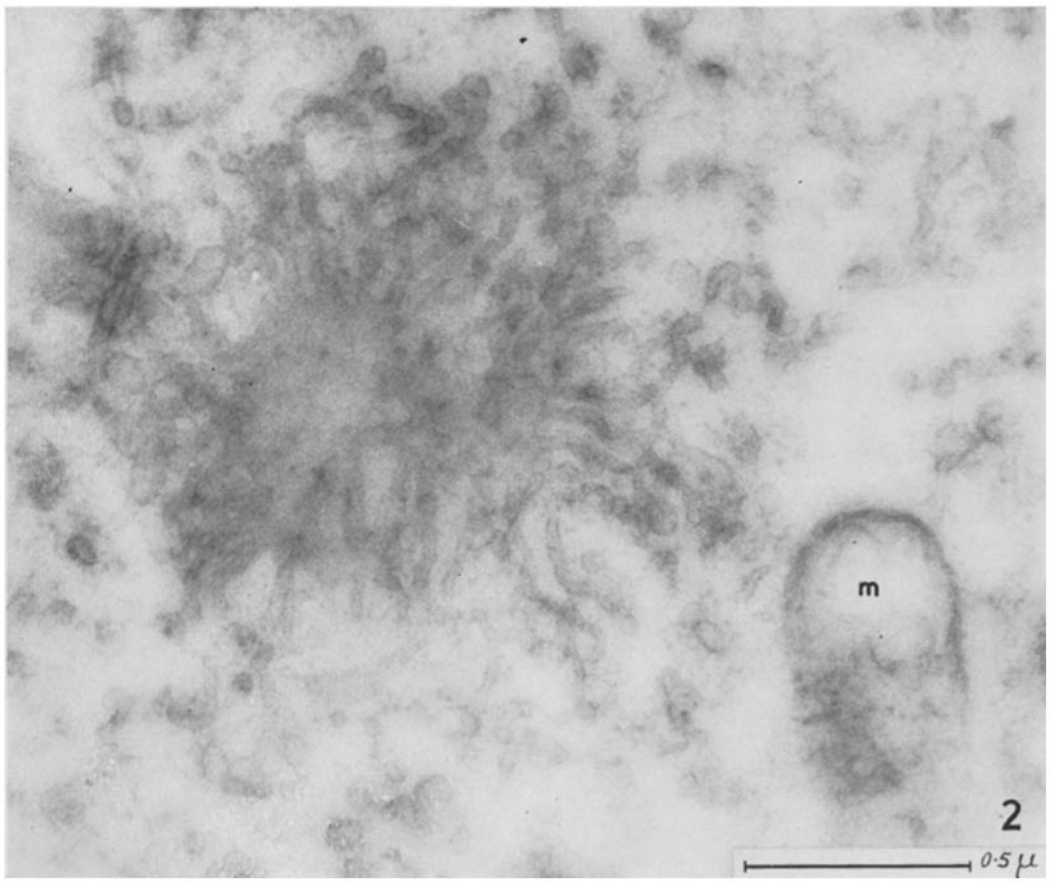
chr'
1


m
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0·5 μ

60,000, and the striking feature is the proliferation of very large numbers of apparently tubular extensions from the edge of the undivided surface of the centre of the organ. These tubules can be seen to be connected in a somewhat reticular manner here and there, and in a few places have swollen locally into subspherical blebs.

That the object in Fig. 2 is indeed a dictyosome in face view is better attested by comparison with Fig. 3 which illustrates, at the same magnification (× 60,000), a region of cytoplasm beside the cross in Fig. 1. In Fig. 3, a somewhat tangentially cut mitochondrion (*m*) lies beside three separate dictyosomes, the middle one of which is more or less in side view. This shows the normal array of stacked flattened cisternae characteristic of the Golgi complex, from the edges of which tubular proliferations, united at intervals into a reticulum, are also evident. The other two dictyosomes in this figure represent easily interpretable transitions toward the full face view of Fig. 2.

Figs. 4 *a* to 4 *c* amplify the information obtainable from Fig. 2 by showing three successive sections through this field at a lower magnification (× 40,000). Collectively this series indicates that tubular proliferations are occurring at the edges of all the dictyosomal cisternae throughout the organ and are not an accidental appearance depending on one peculiar plane of section. Further observations on Figs. 4 *a* to 4 *c* will be discussed below.

A sample of cytoplasm in a cell undergoing extension growth, though still at a relatively early stage, is contained in Figs. 5 *a* to 5 *c*. The cell in question is from one of the central layers of the capsule wall which has completed its growth by cell division. This series is reproduced at the same magnification as that of Figs. 4 *a* to 4 *c* (× 40,000) though it should be noted that one section is missing between Figs. 5 *b* and 5 *c*.

The field of Figs. 5 *a* to 5 *c* contains three dictyosomes which are separately numbered in Fig. 5 *a*, with traces of a fourth beginning to appear in Fig. 5 *c* in a position just above the last traces of dictyosome 3. When compared with the fields of Figs. 2 to 4 all the dictyosomes in Fig. 5 are smaller, the central area is more deeply fenestrated, and the reticular connections of the related tubules are more conspicuous (see especially Fig. 5 *c*). In certain dictyosomes (notably numbers 1 and 3 in Figs. 5 *a* and 5 *b*), bleb-like swellings are present in parts of the tubular reticulum at a distance closer to the edge of the organ than was the case in the cell of Figs. 2 to 4.

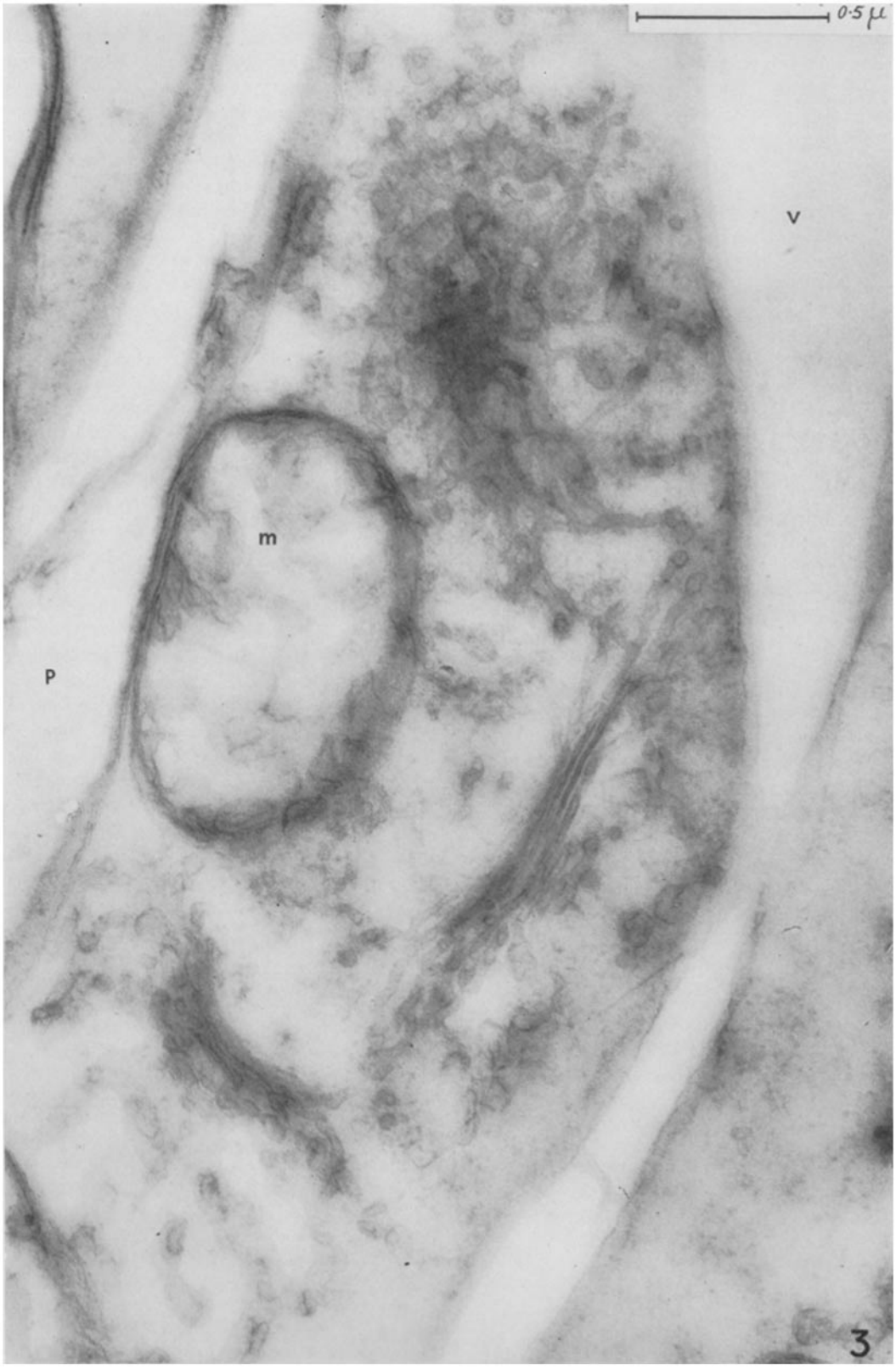
Finally, as a third example, Figs. 6 *a*, 6 *b*, and 7 represent profile and face views of two dictyosomes from one cell of a rather different type. This is a young spore mother cell in the act of rounding up in the condition preceding meiosis. The nucleus (*N*) is still in the premeiotic "resting" state though the cytoplasm is undoubtedly undergoing extensive changes, including vacuolation which cannot be discussed here. The magnification used for these figures is the same as that of Figs. 2 and 3; namely, 60,000. Proliferation of tubules seems to be substantially less than in either of the previous cases though signs of it are still present.

These are by no means the only appearances which dictyosomes can assume at different stages in this material, but to introduce others here would necessitate lengthy discussion of additional cytoplasmic components, which is better deferred. An experienced eye will nevertheless have no difficulty in detecting and recognising several categories of smaller and larger components in various parts of the cytoplasm depicted. One of these may usefully be selected for brief comment since to do so may assist in avoiding some misconceptions.

The field of the serial sections of Figs. 4 *a* to 4 *c* has been carefully selected to include part of the plate of anaphase chromosomes *chr*, from the cell of Fig. 1. The stage of development is in no possible doubt, as reference to the low power view will indicate, and though only a fraction of the chromosome plate can be included it is sufficient to

FIGURE 3

The field marked by a cross in Fig. 1 (right) showing parts of three dictyosomes in various planes for comparison with Fig. 2, all with tubular proliferations showing anastomoses and local swelling; the field also includes part of a mitochondrion *m*, the edge of the (immature) plastid *p* and a membrane-bounded true vacuole *v*. Micrograph H3351. Magnification 60,000.


0·5 μ
V
m
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3

show one important feature, namely, a stage in the formation of the nuclear membrane. As may be seen several times in Figs. 4 *a* to 4 *c* (clearest place marked with arrow, Fig. 4 *a*), the new nuclear membrane, which is still incomplete, is apparently being produced by apposition from the cytoplasm onto the surface of the compacted group of chromosomes (*chr*) of elements which, in the sections reproduced, cannot be distinguished from tubes. It should be noticed however that these apparently tubular elements are not identical morphologically with the undoubted tubes to be seen still attached to the dictyosome in the same three sections. The latter are narrower, more uniform in diameter, and thinner walled. It must therefore be supposed either that considerable developmental changes must occur in cytoplasmic tubules after their formation, or that more than one category of tube may have to be recognised.

INTERPRETATION

It is therefore clear that no hypothesis would at present be justified which presupposed either a common origin for all cytoplasmic tubules or complete homology between them. Without these assumptions and interpretating the observations in the most cautious manner possible, an attempt to summarise the situation encountered in *Anthoceros* may be suggested as follows:–

1. It has been shown in several types of cell from the meristem of *Anthoceros* that a reticulate system of tubes and local swellings is present in the cytoplasm in the immediate neighbourhood of the dictyosomes and in organic continuity with the edges of the dictyosomal cisternae.

2. The exact pattern varies according to the state or type of cell in a manner which becomes intelligible if thought of as depending on (*a*) the rate of production and (*b*) the rate of removal. Production appears to be most active in a cell undergoing mitosis. Removal is possibly most active in a cell undergoing rapid vacuolation.

3. Bleb-like swellings are always found at a distance from the unfenestrated edge of the parent cisterna; they are, therefore, likely to be later modifications. The tubules nearest to the edge of the cisterna are of more uniform width and sometimes narrower than those farther away.

4. It may be suggested that such swellings could represent the first of several types of change occurring in the tubular reticulum as it ages which might transform its appearance and properties in more distant parts of the cytoplasm.

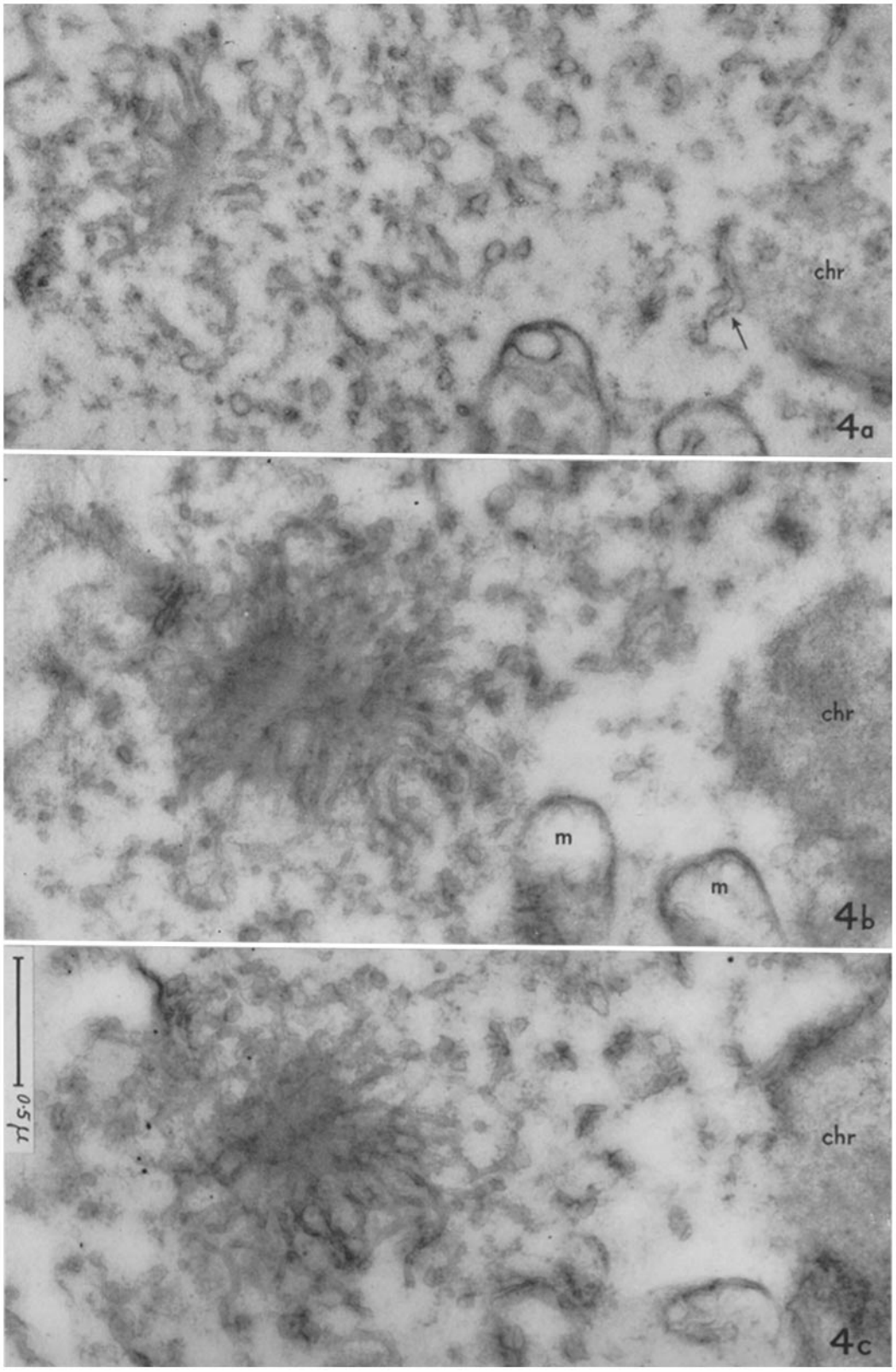
5. An ultimate continuity of all parts of the cytoplasmic tubular system is therefore not impossible, though it is still unproved. What is certain is that modification of several morphological attributes must take place before tubules of the type encountered in continuity with the edges of the dictyosomes can become similar to those involved in reconstruction of the nuclear membrane at the end of a mitosis.

6. A reticulate relationship has not yet been demonstrated in this material in any other type of cytoplasmic tubule though such may exist.

7. If the endoplasmic reticulum of animals is homologous in any way with that described here, the comparison on present evidence must be limited to the "smooth reticulum" of the Rockefeller school (*cf.* references 16, 2, etc.) but not yet to ergastoplasm in any more comprehensive sense. With this limitation, the comparison may be regarded as a reasonable working hypothesis.

FIGURES 4 *a* to 4 *c*

Three successive sections from a series through the field marked by the arrow in Fig. 1; section Fig. 4 *b* containing the field of Fig. 2. Parts of two mitochondria *m*, *m* present in Fig. 4 *b*, while part of one of the groups of anaphase chromosomes *chr* is included in all three sections. The arrow in Fig. 4 *a* points to a place where the cytoplasmic origin of the new nuclear membrane is particularly distinct. By showing successive levels through one dictyosome cut parallel to its surface, the series demonstrates the presence of tubular proliferations from the edges of dictyosomal cisternae throughout the organelle; reticular connections and local swellings are detectable in many places, notably in Figs. 4 *b* and 4 *c*, at a considerable distance from the centre of the dictyosome. Micrographs H3343, H3332, H3336. Magnification 40,000.


chr
4a
chr
m
m
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chr
4c

DISCUSSION

The risk of introducing serious misconceptions by pressing analogies with animal cells too far has been kept clearly in mind in the interpretation offered above. These are however by no means the only possible sources of error. Premature comparisons with other plants can be at least as misleading if carried out without a sufficient basis of fully authenticated facts, and this greatly limits the usefulness of any detailed comparisons at the present time since there is no organism which has been studied sufficiently to permit of a full elucidation of all the necessary facts in spite of considerable attention and considerable progress made at several evolutionary levels notably *Nitella* (6, 7), *Vaucheria* (3, 4), *Chlamydomonas* (18), other unicells (10, 11, 13, 14, 17), other Bryophytes (5, 9), and an array of flowering plants (1, 7, 15, 12, 19).

It should not be forgotten that genuine and great differences do exist between one organism and another, especially in the lower plants. That marked differences of appearance can be encountered in one and the same plant at different stages of growth or in different parts has been recognised by several recent investigators, notably Perner, 1958, Heitz, 1958, Greenwood, 1959, Whaley *et al.*, 1959. This also introduces a series of variables which must be separately studied before comparisons are made.

Therefore the most useful comment which may perhaps be added by way of discussion is to stress the great importance at present of exercising care and restraint to keep separate things which are potentially dissimilar, until adequate facts are available to provide a unifying theory with less uncertainty.

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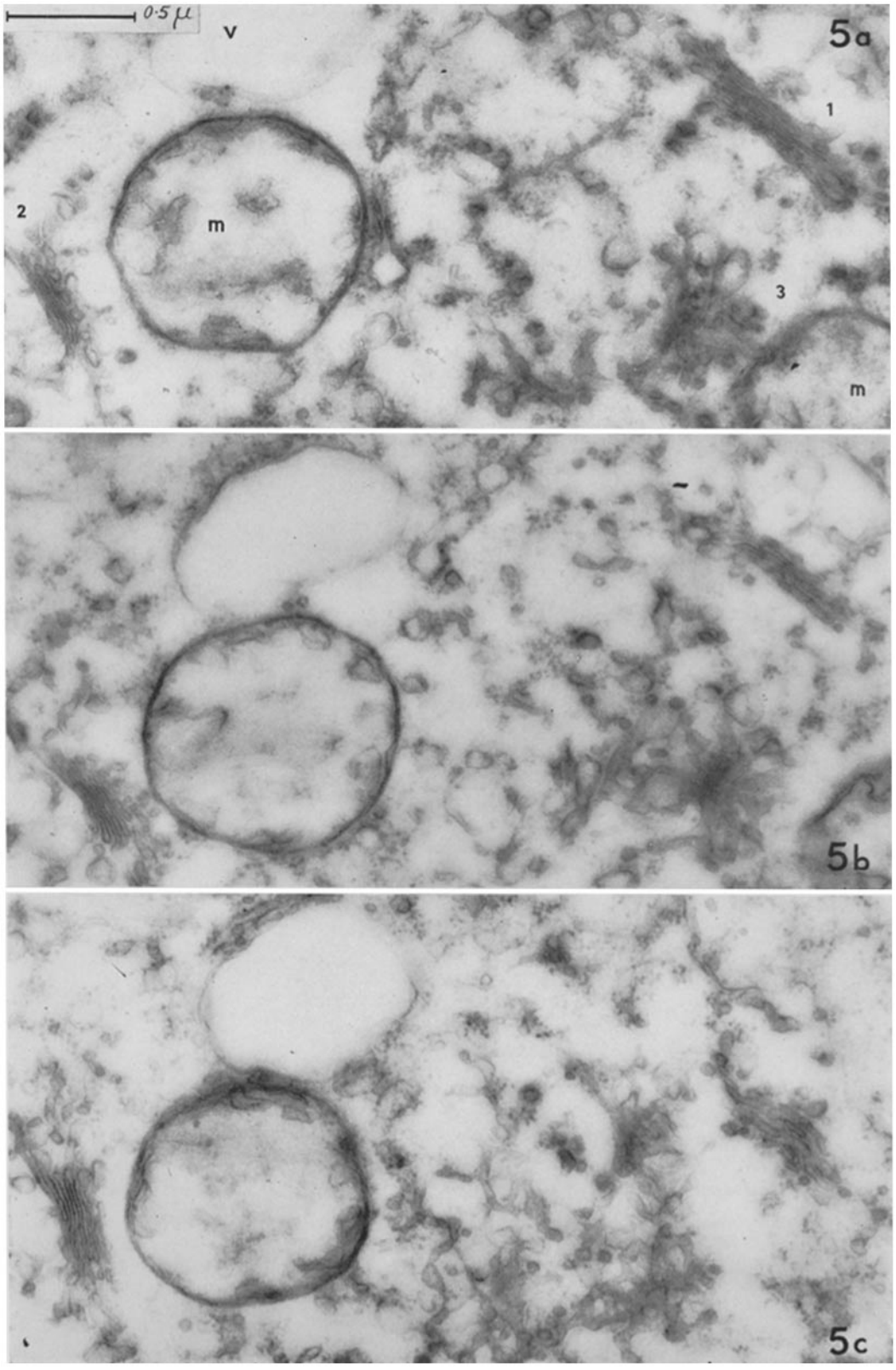
Meristem of *Anthoceros laevis*

Figures 5 *a* to 5 *c*

Three serial sections through the cytoplasm of a cell from one of the middle layers of the wall at a level otherwise comparable with that of Fig. 1. Extension growth is beginning, cell division having stopped; two mitochondria *m*, *m*, a membrane lined vesicle probably not a vacuole *v*, three dictyosomes labeled 1, 2, 3 in Fig. 5 *a*. The first two in side view, the third in face view. Reticular proliferations from the edges of the dictyosomal cisternae visible in all three dictyosomes and in all three sections, the field near dictyosome 3 being the most informative. Micrographs H5896, H5899, H5906. Magnification 40,000. Note that a section is missing between Figs. 5 *b* and 5 *c*.


0·5 μ
V
5a
1
2
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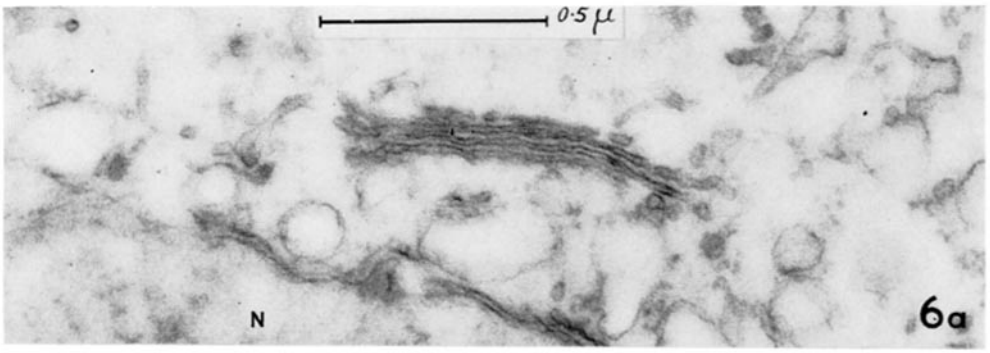
Young spore mother cell of *Anthoceros laevis*

FIGURES 6 *a* and 6 *b*

Two sections through the same dictyosome seen in profile near the nucleus *N* of a young spore mother cell recently rounded up and still undergoing cytoplasmic changes preceding meiosis. Micrographs H6676 and H6697. Magnification 60,000.

FIGURE 7

A dictyosome in face view from the same cell as Figs. 6 *a* and 6 *b*, showing the undivided centre and fenestrated edge, with traces of tubular proliferations less numerous but more conspicuously reticulate than in the dictyosome of Fig. 2, which is otherwise comparable in aspect and magnification. Micrograph H6710. Magnification 60,000.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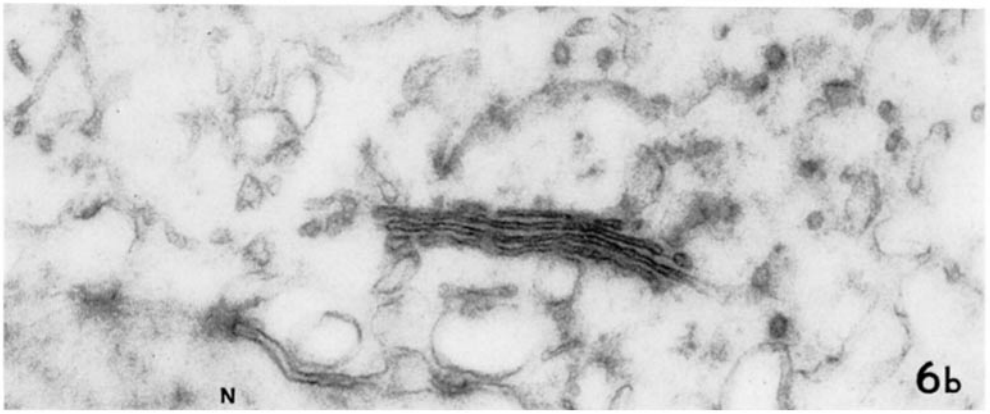
6a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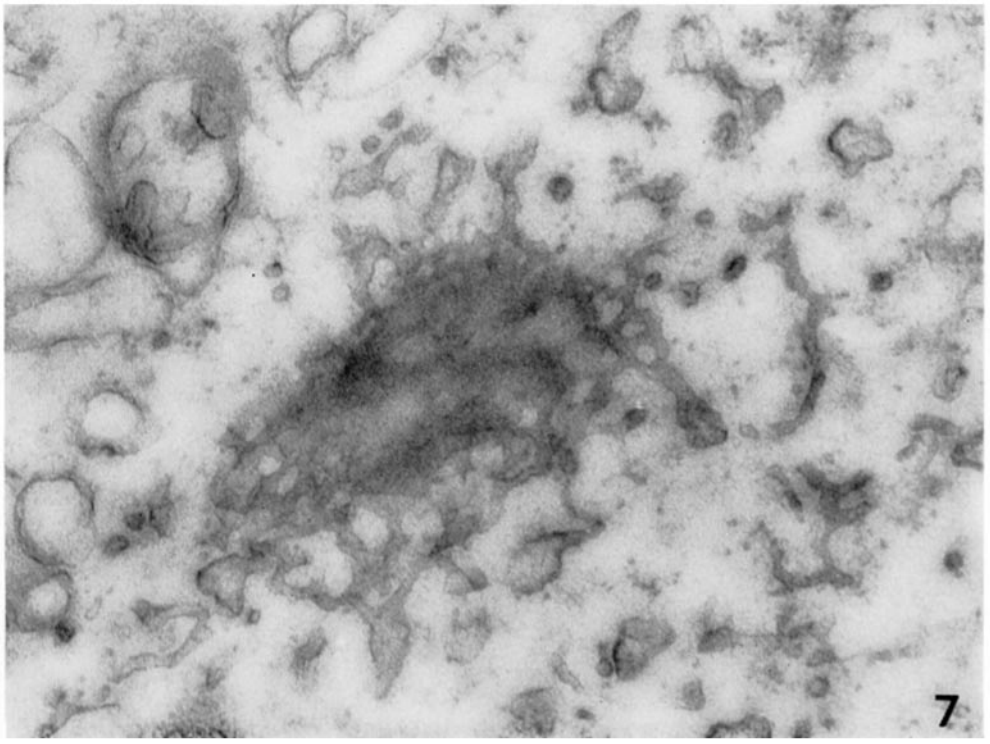
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