

FINE STRUCTURE OF MYCOTA

4. The Occurrence of the Golgi Dictyosome in the Fungus *Neobulgaria pura* (Fr.) Petrak

ROYALL T. MOORE, Ph.D., and JAMES H. McALEAR, Ph.D.

From the Department of Plant Pathology, Cornell University, Ithaca, New York, and the Division of Laboratories and Research, New York State Department of Health, Albany, New York. The authors' present address is Electron Microscope Laboratory, University of California, Berkeley

ABSTRACT

Though the dictyosome of the Golgi apparatus appears to be generally distributed in plant and animal cells, it is here described for the first time in the fungi. The present report illustrates, in electron micrographs of thin sections, the characteristic structure of the Golgi dictyosome in a special cell type of a supporting pseudo-tissue (the inner ectal excipulum) of a highly evolved Ascomycete, *Neobulgaria pura* (Fr.) Petrak, a monotypic discomycete. This organelle may secrete the gelatinous matrix filling the cup formed by the inner ectal excipulum. All the other cells in this species appear more typical of fungal cells; *i.e.*, they have no dictyosome and, unlike the cup-forming cells, they show characteristic continuities of the plasma membrane with the perinuclear cisternae. The dictyosome, in those cells in which it appears in this fungus, is formed by a series of vesiculations of the outer component of the nuclear envelope that align to form a stack of sacs. The sacs near the nucleus are flattened (by what appears to be an intermembrane cement) while those near the plasma membrane are more distended. These observations suggest three possibilities: first, fungi may be more closely related to other eukaryotic cells than previously suspected from electron microscopic studies; second, the outer nuclear membrane may have been the primitive precursor of the dictyosome; and third, the inverse relationship of the occurrence of the nuclear membrane plasma membrane continuities and the dictyosome suggests that the latter may have evolved as a means of removing from the cell the products of reactions occurring on a discontinuous membrane system.

INTRODUCTION

The Golgi dictyosome is most generally characterized by a stack of flattened sacs from the margins of which vesicles sometimes appear to be pinched off. In animal cells where this structure has been most frequently noted it is often involved in secretory processes (35, 42-45) and it very likely has a similar function in some higher plant cells (27). While a similar appearing structure has been described in a moss (19), in some algae (17, 18, 20-22, 36, 37), and protozoa (5, 10-14, 34, 38), its function here is less clear than in the aforementioned organisms. Generally speaking, the distribution of the dictyosome corresponds

closely to the distribution of organized nuclei and it is considered to be an ubiquitous organelle of advanced cell types. In a survey of nearly 50 genera of the Eumycota we have noted that while nuclei are consistently present they differ markedly from the nuclei of other major groups of nucleated cells. The limiting cisternae of fungal nuclei are often directly continuous with the plasma membrane (9, 23, 29). Reports of optical (1-4, 40, 41) and electron microscopic studies (7, 8, 15, 16, 46) suggest that the nuclear envelope commonly does not break down and reform during somatic karyokinesis, and that

only rarely are mitotic figures to be observed (24–26). At first glance the fungi, because of the simplicity of their membrane relationships, their lack of a Golgi complex, and their mode of cell division, would seem to represent an intermediate stage in the secondary evolution of cells. However, like other cells above the level of the Schizomycota, they possess nuclei, mitochondria, and well developed cilia in some Phycomycetes (6). Two previous reports (6, 9) tentatively identified vesicle aggregations as belonging to the Golgi complex; the present report, however, presents evidence for the presence of a Golgi system on the less controversial identification of the Golgi dictyosome. This organelle has been found to occur in an unusual fungous cell type in which the nuclear membrane is discontinuous and appears to break down during cell division in a manner comparable to that occurring in mitosis. This report also provides direct evidence for the origin of the dictyosome from the outer membrane of the nuclear envelope.

MATERIALS AND METHODS

Substrate

Apothecia (fruiting bodies) of the discomycete, *Neobulgaria pura* (Fr.) Petrak, were collected October, 1960, in the northern Adirondack Mountains, New York State.

Method

The apothecia were prefixed in 1 per cent unbuffered KMnO_4 for 9 minutes. (A discussion of this method has been presented in the first paper in this series (28).) Subsequently, they were fixed in 2 per cent OsO_4 for 3 hours at 4°C , washed in buffer, dehydrated in an ethanol series, and embedded in a mixture of butyl and methyl methacrylates (90:10). Sections were cut on a Porter-Blum microtome with a Fernández-Morán diamond knife and subsequently examined in a Siemens Elmiskop I.

OBSERVATIONS

The gross morphology of the fruiting body (apothecium) of *Neobulgaria pura* is shown semi-diagrammatically in Fig. 1. In general, the apothecium is a bowl-shaped structure filled with a mixture of gel and diffuse hyphae (the medullary excipulum). At the upper surface of this mixture the hyphae become concentrated into the relatively dense subhymenial layer. From the hyphae of this layer arises a stratum of vertically oriented

hyphae, the hymenium, composed of the spore-bearing asci and inter-ascal, sterile paraphyses. The uncomplicated micromorphology of the cells of these mycelial layers (Fig. 10) is typical for fungal cells when compared with observations made on over 50 genera of fungi (28–33). The apothecium is given its shape by the two-layered ectal excipulum. The outer layer is a gel-hyphae mixture comparable to the medullary excipulum in both composition and micromorphology (Fig. 10). However, the inner layer is composed of thick-walled hyphae (*textura prismatica*) laterally compacted into a firm pseudotissue. It is this inner layer of the ectal excipulum that gives the apothecia their shape and support.

A typical representation of cells from the inner ectal excipulum (*Ex*) is shown in Fig. 2. These cells display a number of unusual characteristics: 1) the prominent nuclei (*N*) are more homogeneously light in appearance than the nuclei of most fungal cells; 2) the endoplasmic reticulum (*ER*) in its most common appearance here is composed of disarticulated vesicular elements that seem to arise from the outer nuclear membrane; 3) the endoplasmic reticulum does not form continuities between the nuclear and plasma membranes as is common in fungal cells and typical in *N. pura* in cells not of the inner ectal excipulum (Fig. 10).

The most singular feature of the cells of the inner ectal excipulum is the presence of stacks of flattened vesicles (*D*) of the same appearance as the Golgi dictyosome (Figs. 2 to 8). The nuclear membrane (Figs. 2 to 5, 7, 8) shows some apparent activity adjacent to the dictyosome. In Figs. 5 and 7 the outer nuclear membrane appears to be continuous with the adjacent dictyosome. It would seem, therefore, that the dictyosome does arise from the nuclear membrane in these cells. The flattened sacs proximal to the nucleus (Fig. 6) are compressed by a structured cementing material within them. The peripheral sacs, however, are distended. This gives the impression of a dynamic process from the nuclear membrane to the plasma membrane. Since KMnO_4 fixation destroys ribosomes it is not possible to determine in this preparation if the dictyosomes shown here are agranular, as is characteristic of other dictyosomes. However, the membranes are so compacted on their adicisternal surfaces that there does not appear to be room between them for any significant number of granules of the diameter of 150 Å.

On occasion (Fig. 9) the nucleus is only partially delimited by cisternae, and these are represented by only a few isolated vesicles surrounding the nuclear phase. This configuration may represent a reformation of the nuclear membrane following division (39).

A variety of other inclusions are found in the cytoplasm of the inner ectal excipular cells. These

DISCUSSION

The occurrence of the Golgi apparatus was classically considered to be limited to animal cells. Electron microscopic studies, however, have now generally extended the existence of the dictyosome of the Golgi apparatus, its hallmark, to the higher plants, mosses, algae (except Cyanophyta), and

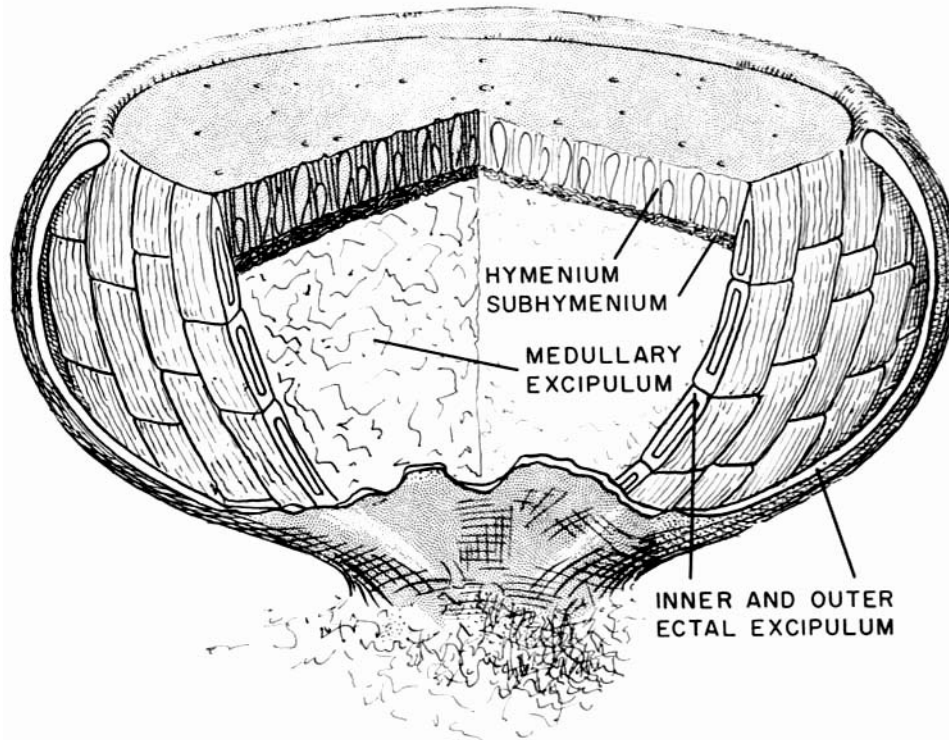


FIGURE 1

Representation of the fruiting body (apothecium) of the discomycete *Neobulgaria pura* showing the nature and relationship of the structures discussed in the text.¹

include: 1) a large single membrane-limited body (a) containing a light granular material (Fig. 3); 2) lomasomes (L), structures frequently found in other fungal cells (30) (Figs. 2 to 4, 8, 9); 3) dense globose granules (b) (Figs. 4 to 6) (one of these (Fig. 3) is found within an annulus of the nuclear membrane); and 4) inclusions of low electron opacity (with dense centers) (Figs. 2 to 8).

¹ We wish to express our sincere appreciation to Professor R. P. Korf for his assistance in the preparation of this drawing, and to Mr. F. C. Reed for his execution of the drawing.

protozoa. This establishment of the dictyosome as an ubiquitous organelle in higher cell types would seem to greatly enhance the probability that the structure which is described above is indeed a dictyosome and commonly derived with the dictyosomes of other higher cell types. It would, nevertheless, be tenuous on the basis of the above report to express a conviction that the described structure is an authentic dictyosome because it is described in only one cell type of one genus (a highly evolved Ascomycete, in fact). However, while this discussion was in its final draft, a similar

observation of a characteristic dictyosome in an undescribed monotypic flagellated Phycomycete² has been made and will be subsequently studied. This, then, provides one example from the carpomycetes (Ascomycetes, Basidiomycetes, and Deuteromycetes) and one from the Phycomycetes. The inclusion of the dictyosome in the Eumycota is significant in that fungi would seem to be as phylogenetically distinct from plants as plants are from animals and protozoa. This discovery would then establish the occurrence of the dictyosome in all major taxa of nucleated cells (except perhaps the Myxomycota).

The dictyosome might well come to be regarded as an organelle which was quite primitively derived from internal membrane systems. While its modern function seems most often to be linked to secretion, the understanding of its primitive function may depend on further phylogenetic studies at the electron microscopic level. A clue from the present report might deserve further study. That is the observation that the Golgi-

² Professor R. Emerson's Costa Rican collection, number 78.

bearing cells of *Neobulgaria* have nuclear membranes discontinuous with the plasma membrane while the non-Golgi-bearing cells show such continuities. As a working hypothesis,³ could the dictyosome have evolved as a means of eliminating the by-products of biochemical reactions occurring on discontinuous membranes? Surely, without some such mechanism, plasma membrane enzyme complexes if internalized would find their formerly externally facing surfaces looking in upon cisternae, and any products deposited within them would either be accumulated or would have to pass out of the cisternae to the plasma membrane to be excreted.

This work was supported in part by Grant No. H-3493 from the National Heart Institute, Public Health Service.

This work was also supported in part by a research fellowship 9197-C1 to the senior author from the National Institute of Allergy and Infectious Diseases, Professor R. P. Korf, sponsor.

Received for publication, February 16, 1961.

³ Junior author.

BIBLIOGRAPHY

1. BAKERSPIGEL, A., The structure and manner of division of the nuclei in the vegetative my-

celium of the Basidiomycete *Schizophyllum commune*, *Canad. J. Bot.*, 1959, 37, 835.

KEY TO LABELING OF FIGURES

a, large vacuolar body
b, dense bodies
c, smaller vacuolar bodies
D, dictyosome
ee, envelope elements
ER, endoplasmic reticulum
Ex, inner ectal excipulum

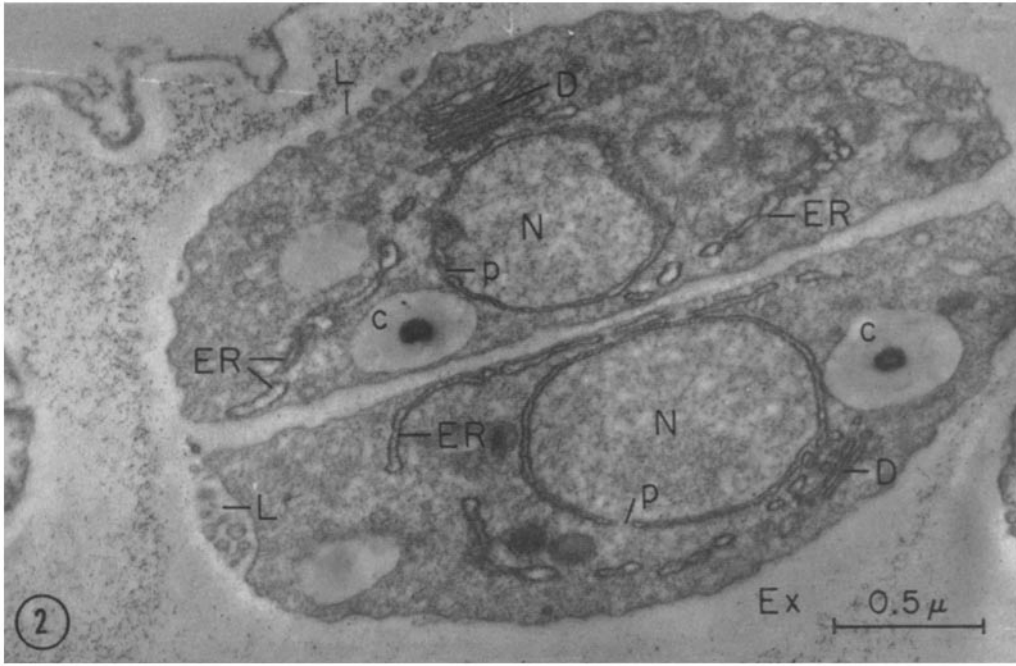
HW, hyphal wall
L, lomasomes
M, mitochondria
N, nucleus
NE, nuclear envelope
p, nuclear pores

FIGURE 2

Longitudinal section through a pair of cells from the inner ectal excipulum. Two dictyosomes (*D*) adjacent to the outer components of the two nuclear membranes are seen. Vacuolar structures (*c*), lomasomes (*L*), nuclear pores (*p*), and a vesicular endoplasmic reticulum (*ER*) are evident. (*Ex*) indicates the region of the cell walls of the inner ectal excipulum. $\times 40,000$.

FIGURE 3

Another pair of cells of the inner ectal excipulum showing two other types of inclusions (*a* and *b*), a spherical mitochondrion (*M*) with radially oriented tubular cristae, and another dictyosome (*D*) adjacent to the nucleus (*N*). Note the symmetrical pair of diverticula on the outer component of the nuclear envelope adjacent to the dictyosome. $\times 50,000$.



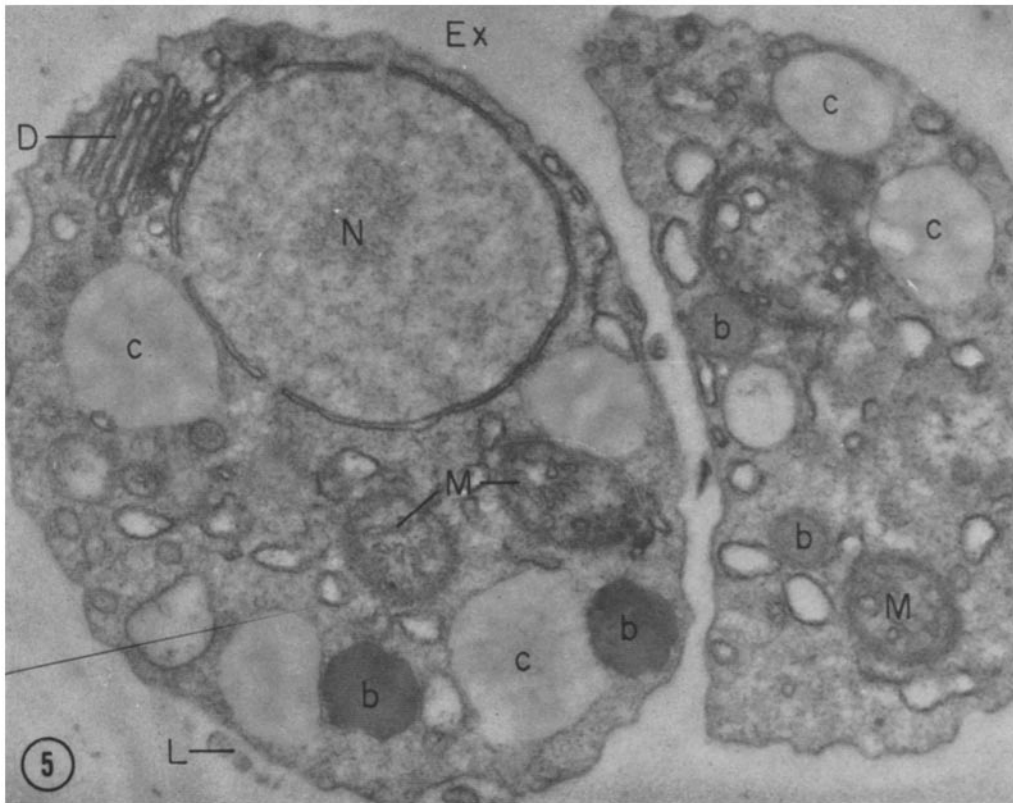
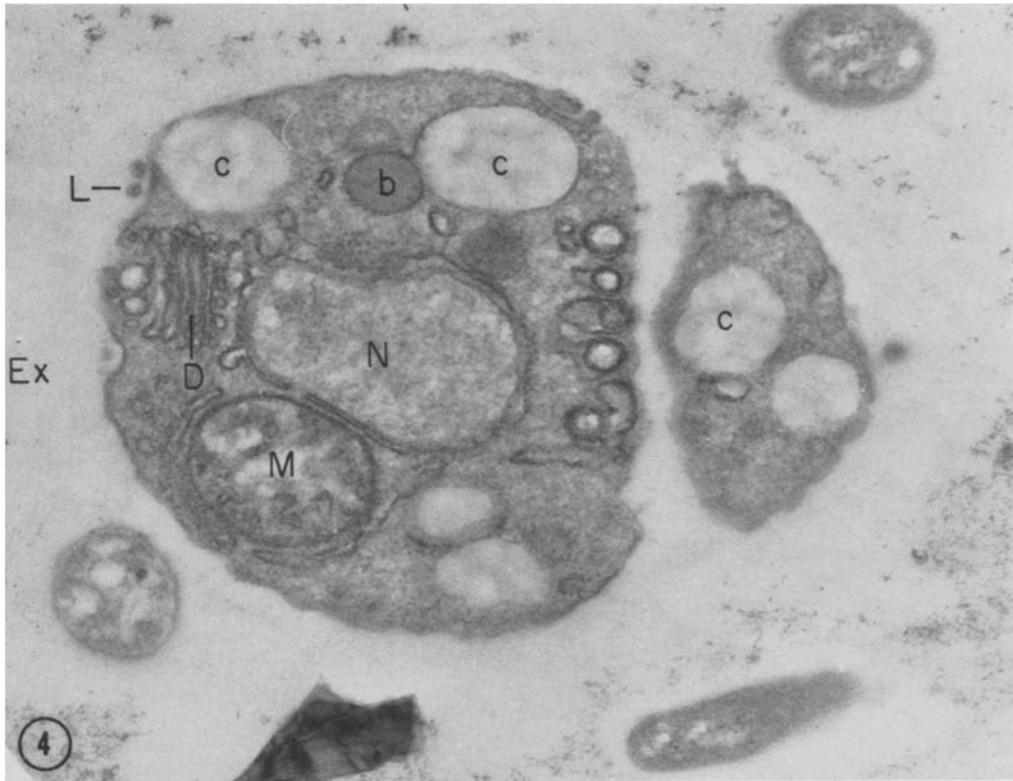
2. BAKERSPIGEL, A., The structure and manner of division of the nuclei in the vegetative mycelium of *Neurospora crassa*, *Am. J. Bot.*, 1959, **46**, 180.
3. BAKERSPIGEL, A., The structure and manner of division of the nuclei in the vegetative mycelium of *Gelasinospora tetrasperma* Dow., *Canad. J. Microbiol.*, 1959, **5**, 126.
4. BAKERSPIGEL, A., Nuclear structure and division in the vegetative mycelium of the Saprolegniaceae, *Am. J. Bot.*, 1960, **47**, 94.
5. BEAMS, H. W., and ANDERSON, E., Fine structure of protozoa, *Ann. Rev. Microbiol.*, 1961, **15**, 47.
6. BLONDEL, B., and TURIAN, G., Relation between basophilia and the fine structure of cytoplasm in the fungus *Allomyces macrogynus* Em., *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 127.
7. CONTI, S. F., and NAYLOR, H. B., Electron microscopy of ultrathin sections of *Schizosaccharomyces octosporus*. II. Morphological and cytological changes preceding ascospore formation, *J. Bact.*, 1960, **79**, 331.
8. CONTI, S. F., and NAYLOR, H. B., Study of life-cycle of *Schizosaccharomyces octosporus* by means of ultra-thin sectioning and electron microscopy, *Nature*, 1960, **185**, 633.
9. EDWARDS, G. A., and EDWARDS, M. R., The intracellular membranes of *Blastomyces dermatitidis*, *Am. J. Bot.*, 1960, **47**, 622.
10. GRASSÉ, P. -P., L'appareil parabasal et l'appareil de Golgi sont un même organite. Leur ultra-structure, leurs modes de sécrétion, *Compt. Rend. Acad. sc.*, 1956, **242**, 858.
11. GRASSÉ, P. -P., Ultrastructure of the Golgi apparatus in protozoa and metazoa (somatic and germinal cells), *Nature*, 1957, **179**, 31.
12. GRASSÉ, P. -P., and THÉODORIDES, J., Recherches sur l'ultrastructure de quelques grégarines, *Ann. Sc. Nat.*, 1959, **XII** 1, 237.
13. GRIMSTONE, A. V., Cytoplasmic membranes and the nuclear membrane in the flagellate *Trichonympha*, *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 369.
14. GRIMSTONE, A. V., Fine structure and morphogenesis in protozoa, *Biol. Revs.*, 1961, **36**, 97.
15. HASHIMOTO, T., CONTI, S. F., and NAYLOR, H. B., Nuclear changes occurring during bud-formation in *Saccharomyces cerevisiae* as revealed by ultrathin sectioning, *Nature*, 1958, **182**, 454.
16. HASHIMOTO, T., CONTI, S. F., and NAYLOR, H. B., Studies of the fine structure of microorganisms. IV. Observations on budding *Saccharomyces cerevisiae* by light and electron microscopy, *J. Bact.*, 1959, **77**, 344.
17. MANTON, I., Observations with the electron microscope on the internal structure of the zoospore of a brown alga, *J. Exp. Bot.*, 1957, **8**, 294.
18. MANTON, I., Observations on the internal structure of the spermatozoid of *Dictyota*, *J. Exp. Bot.*, 1959, **10**, 448.
19. MANTON, I., On a reticular derivative from Golgi bodies in the meristem of *Anthoceros*, *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 221.
20. MANTON, I., and LEEDALE, G. F., Observations on the fine structure of *Paraphysomonas vestita*, with special reference to the Golgi apparatus and the origin of scales, *Phycologia*, 1961, **1**, 37.
21. MANTON, I., and LEEDALE, G. F., Further observations on the fine structure of *Chrysochromulina ericina* Parke & Manton, *J. mar. biol. Assn. U. K.*, 1961, **41**, 145.
22. MANTON, I., and LEEDALE, G. F., Further observations on the fine structure of *Chrysochromulina minor* and *C. kappa* with special reference to the pyrenoids, *J. mar. biol. Assn. U. K.*, 1961, **41**, 519.
23. McALEAR, J. H., and EDWARDS, G. A., Continuity of plasma membrane and nuclear membrane, *Exp. Cell Research*, 1959, **16**, 689.
24. MCGINNIS, R. C., Cytological studies of chromosomes of rust fungi. I. The mitotic chromosomes of *Puccinia graminis*, *Canad. J. Bot.*, 1953, **31**, 522.
25. MCGINNIS, R. C., Cytological studies of chromosomes of rust fungi. II. The mitotic chromosomes of *Puccinia coronata*, *Canad. J. Bot.*, 1954, **32**, 213.
26. MCGINNIS, R. C., Cytological studies of chromosomes of rust fungi. III. The relationship of chromosome number to sexuality in *Puccinia*, *J. Heredity*, 1956, **47**, 255.

FIGURE 4

Another cell, similar to those in Figs. 2 and 3, which shows a further example of symmetrical diverticula on the outer component of the perinuclear cisterna adjacent to the dictyosome (D). $\times 50,000$.

FIGURE 5

In this micrograph the region of the dictyosome proximal to the nucleus appears to be in continuity with the outer nuclear membrane. $\times 50,000$.



27. MOLLENHAUER, H. H., WHALEY, W. G., and LEECH, J. H., A function of the Golgi apparatus in outer rootcap cells, *J. Ultrastruct. Research*, 1961, **5**, 193.
28. MOORE, R. T., Fine structure of mycota. I. Electron microscopy of the discomycete *Ascodesmis*, *Nova Hedwigia*, 1963, in press.
29. MOORE, R. T., and MCALEAR, J. H., Fine structure of mycota. 3. Reconstruction from skipped serial sections of the nuclear envelope and its continuity with the plasma membrane, *Exp. Cell Research*, 1961, **24**, 588.
30. MOORE, R. T., and MCALEAR, J. H., Fine structure of mycota. 5. Lomasomes—previously uncharacterized hyphal structures, *Mycologia*, 1961, **53**, 194.
31. MOORE, R. T., and MCALEAR, J. H., Observations on ascospore initiation in the discomycete *Dasyascyphus*, *J. Gen. Microbiol.*, 1962, **28**, 211.
32. MOORE, R. T., and MCALEAR, J. H., Fine structure of mycota. 7. Observations on septa of Ascomycetes and Basidiomycetes, *Am. J. Bot.*, 1962, **49**, 86.
33. MOORE, R. T., and MCALEAR, J. H., Fine structure of mycota. 8. On the aecidial stage of *Uromyces caladii*, *Phytopath. Z.*, 1961, **42**, 297.
34. NOIROT-TIMOTHEE, C., Recherches sur l'ultrastructure d'*Opalina ranarum*, *Ann. Sc. Nat. Zool.*, 1959, **XII** 1, 265.
35. PALADE, G. E., and SIEKEVITZ, P., Pancreatic microsomes, an integrated morphological and biochemical study, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 671.
36. PARKE, M., MANTON, I., and CLARKE, B., Studies on marine flagellates. IV. Morphology and microanatomy of a new species of *Chrysochromulina*, *J. mar. biol. Assn. U. K.*, 1958, **37**, 209.
37. PARKE, M., MANTON, I., and CLARKE, B., Studies on marine flagellates. V. Morphology and microanatomy of *Chrysochromulina strobilus* sp. nov., *J. mar. biol. Assn. U. K.*, 1959, **38**, 169.
38. PAPPAS, G. D., Electron microscope studies on amoebae, *New York Acad. Sc. Ann.*, 1959, **78**, 401.
39. PORTER, K. R., and MACHADO, R. D., Studies on the endoplasmic reticulum. IV. Its form and distribution during mitosis in cells of onion root tip, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 167.
40. ROBINOW, C. F., The structure and behavior of the nuclei in spores and growing hyphae of Mucorales. I. *Mucor hiemalis* and *Mucor fragilis*, *Canad. J. Microbiol.*, 1957, **3**, 771.
41. ROBINOW, C. F., The structure and behavior of the nuclei in spores and growing hyphae of Mucorales. II. *Phycomyces blakesleeanus*, *Canad. J. Microbiol.*, 1957, **3**, 791.
42. SIEKEVITZ, P., and PALADE, G. E., A cytochemical study on the pancreas of the guinea pig. I. Isolation and enzymatic activities of cell fractions, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 203.
43. SIEKEVITZ, P., and PALADE, G. E., A cytochemical study on the pancreas of the guinea pig. II. Functional variations in the enzymatic activity of microsomes, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 309.
44. SIEKEVITZ, P., and PALADE, G. E., A cytochemical study on the pancreas of the guinea pig. III. *In vivo* incorporation of leucine 1-C¹⁴ into the proteins of cell fractions, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 557.
45. SIEKEVITZ, P., and PALADE, G. E., A cytochemical study on the pancreas of the guinea pig. IV. Chemical and metabolic investigation of the ribonucleoprotein particles, *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 1.
46. THYAGARIJAN, T. R., CONTI, S. F., and NAYLOR, H. B., Electron microscopy of *Rhodotorula glutinis*, *J. Bact.*, 1962, **83**, 381.

FIGURE 6

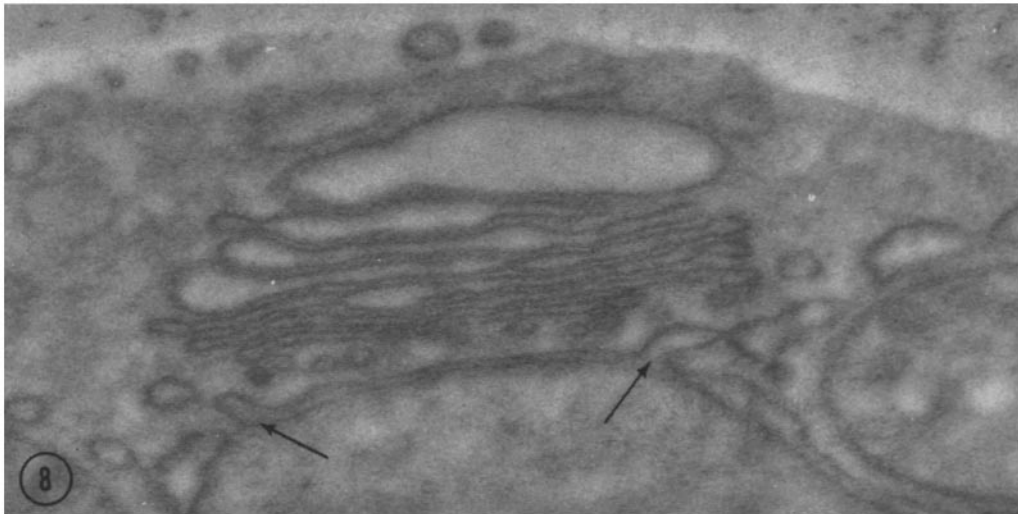
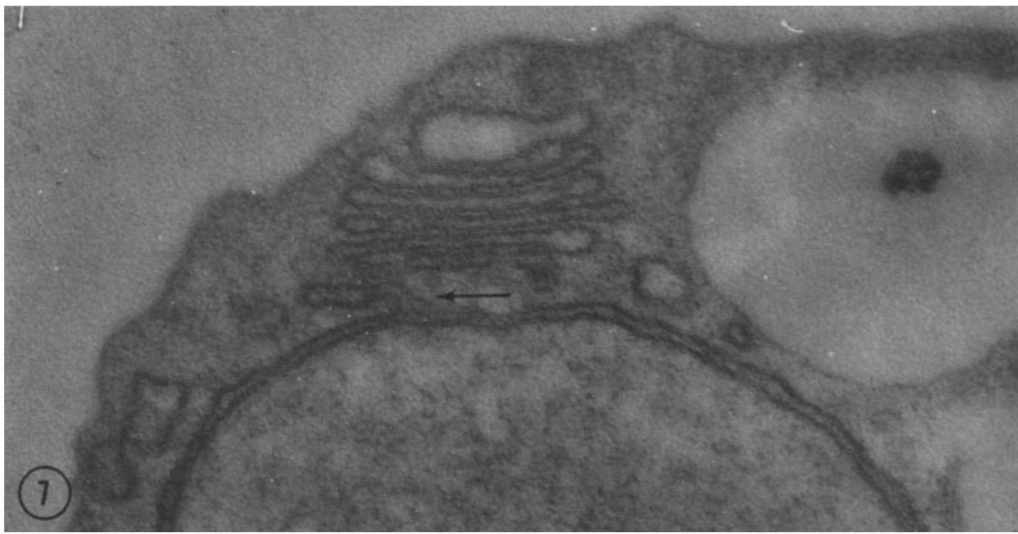
A dictyosome showing compact lamellae adjacent to the nucleus and distended ones adjacent to the plasma membrane. The innermost of these flattened cisternae shows fairly regularly spaced densities between its apposed membranes. $\times 100,000$.

FIGURE 7

A diverticulum of the outer nuclear membrane (*arrow*) appears to align in the plane of the cisternae of the dictyosome. $\times 100,000$.

FIGURE 8

Symmetrical diverticula of the outer nuclear membrane appear opposite the dictyosome (*arrows*) and seem to be related to vesicular elements of the endoplasmic reticulum. $\times 100,000$.



Note added in proof: Since the submission of this paper several complementary observations have come to our attention: (1) The Golgi complex has now been observed in three additional Phycomycete genera. In personal communications it has been noted to occur in *Peronospora* and *Albugo* by C. C. Bowen and in *Pythium* by Lilian Hawker. (E. C. Cantino, however, states that he has failed to find it in his studies of *Blastocladiella*.) (2) Zeigel and Dalton (*J. Cell Biol.*, 1962, 15, 45) in a recent paper on the micromorphology of the Golgi systems of several animal tissues associated with the secretion of various kinds of pro-

teins offer a number of micrographs to support the hypothesis that blebs from the nuclear envelope may be "one source of the small variety of Golgi vesicles." A similar relationship of the perinuclear cisterna to the Golgi dictyosome also has been previously noted by McAlear (unpublished) in the sixth abdominal ganglion of the crayfish and other micrographs of the material shown in the present contribution have appeared previously in an abstract (Moore and McAlear, *Electron Microscopy*, 1962, 2, UU-7, New York, Academic Press).

FIGURE 9

A nucleus showing a region of disarticulated nuclear envelope (*ee*) suggesting that the nuclear envelope (*NE*) in the cells of the inner ectal excipulum may break down and reform. $\times 100,000$.

FIGURE 10

A cell not of the inner ectal excipulum which shows a continuity of the nuclear membranes with the plasma membrane. This is frequently found in other fungal cells but not found thus far in the inner ectal excipulum of *Neobulgaria pura*. $\times 100,000$.

