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Studies on Fungal Membranes

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This study represents an attempt to apply established methods of detection for cellulose and chitin as structural materials in the membranes of a group of organisms consisting, for the greater part, of higher fungi. These methods have been combined with purification procedures resulting in a technique which has not been used previously on so broadly representative a group of higher fungi.

In the latter part of the nineteenth century, a number of workers began to publish their findings regarding the chemical composition of fungal membranes. Anton de Bary (1884) and others attempted with some success to identify the membrane or wall constituents.

Some confusion in analysis of hyphal walls resulted from an equation of fungus structures with those of higher plants and a transfer of terms, as well as testing methods, from the latter to the former. Perhaps the prime example of this type of confusion was the introduction of the persistent term "pilze cellulose" or fungus cellulose, by de Bary. Using macrochemical analyses, he found that the walls of certain fungi appeared to contain cellulose. Histochemical tests, on the other hand, yielded results which deviated from reactions obtained with cellulose from higher plants. To this type of wall material he applied the term "fungus cellulose". He was undecided as to whether the peculiar qualities of this material were due to foreign deposits or to other causes. He further pointed out that there were fungi which exhibited perfectly good histochemical reactions for cellulose.

De Bary (1884) reported his fungus cellulose from six species of Basidiomycetes. Winterstein (1895) examined several members of this class and of the Ascomycetes and stated that fungus cellulose was a chitin-like substance. Molisch (1921) felt, on the basis of work done by Gilson, Winterstein, Iwanoff, and Wisselingh, that those fungi which do not give the usual cellulose tests have chitin in their membranes.

Investigations of hyphal walls have, more recently, been occupied with analyses of the entire wall including sheathing and impregnating substances, and analyses of the alkali resistant fraction of the walls. Thomas in 1928 began a series of studies on four genera, *Fusarium, Sclerotinia, Pythium,* and *Phytophthora,* involving the detection, isolation and identification of the membrane constituents of

these fungi. Using macro- and histochemical tests, solubility and optical activity he found cellulose and chitin and in *Fusarium* found the two occuring together. He pointed out that there is no good reason to believe that the cellulose found here is different from that found in higher plants. It does, however, occur in different association. Instead of being mixed with lignin, the cellulose of *Fusarium* is highly impregnated with fatty materials and proteins. The work of Thomas more than that of any other has served to verify and elaborate the earlier conclusions as to the complexity and impregnation of fungal membranes.

Hopkins (1929) tested the walls of a number of fungi and showed that in the case of *Mucor rouxii* the composition of the wall changed during the growth of this fungus in culture. Early samples showed neither cellulose nor chitin, later, chitin was found to be present and still later both cellulose and chitin were found.

May and Ward (1934) and others have investigated the similarities between fungus and animal chitin. The results of these investigations, though not altogether consistent, indicate lower nitrogen percentages in fungus than in animal chitin. It has not been determined whether the chitinous materials occurring in widely separated groups of fungi are structurally identical; the same, no doubt could be said of chitinous materials in widely separated animal groups.

Von Wettstein (1921) discussed the usefulness of membrane studies in certain phases of botanical work. The occurence of chitin in the plant kingdom, he pointed out, can to a certain extent be correlated with the heterotrophic mode of nutrition and cellulose with the autotrophic type. This view was implicitly coupled with the assumption that at least some of the fungi have algal ancestry. From this it was inferred that those fungi which have cellulose in their membranes are more recent derivatives from the algal stock than those which have chitin.

Later work (Wurdack, 1923; Tiffany, 1924) showed that contrary to Wettstein's belief, chitin is a membrane component in some of the autotrophic green algae. It must be added that Wettstein did not overlook the absence of chitin in the heterotrophic higher plants as a drawback to that part of his phylogenetic assertion which dealt with heterotrophism and chitin formation.

Nabel (1939) elaborated further on the phylogenetic significance of membrane composition. Histochemical studies on a number of the lower fungi led him to conclude that the Chytridiaceae and Blastocladiaceae have chitinous membranes while the Oomycetes

(his separation) have cellulose. Nevertheless, a phylogenetic series involving these groups is not considered unlikely if the chytrid, *Rhizidiomyces bivellatus* Nabel is taken into account as some kind of link. As the specific epithet implies, the wall of this fungus is two layered and according to Nabel the inner layer is cellulose and the outer chitin.

The use of membrane reactions with cellulose reagents for purposes of classification in the Oomycetes is well known (Coker, 1937).

Cultural conditions and age have been found by several workers to influence the relative amounts of chitin and cellulose in the membranes of fungi (Foster, 1949). Chitin-containing fungi grown on physiologically alkaline media for a long period of time were found to suffer a decline in dry weight percentage of chitin, while a percentage increase occurred on acid media. Autolysis, occurring to a greater extent in old alkaline cultures than in acid cultures of an equivalent age, was held responsible for the loss of chitin.

Materials and Methods

The schedule for the detection of cellulose and chitin in the fungi concerned in this study was first established using known material. Validity of the testing methods and reagents was checked with non-fungus sources of the substances in question. The chitinous inner skeleton of the squid and the exoskeleton of several insects yielded the positive red-violet color with the chitosan test and produced characteristic chitosan sphaerites.

Cotton fibers provided the cellulose source and gave the expected results with the cellulose tests described below. The insect chitin and the cotton fibers were used later to check the reagents for deterioration before each series of tests.

Using fungi which have been reliably reported to have chitin or cellulose in their membranes, purification methods as well as testing procedures were standardized. As gelatinous, pectin and fatty substances have been shown to be the main impregnating and sheathing materials, the purification procedures were designed mainly to remove these.

The mycelial mats, having been killed and collected were thoroughly washed in hot 0.5 per cent ammonium oxalate followed by hot distilled water for the removal of pectin and gelatinous materials. Saponification in hot alcoholic potassium hydroxide was next used to remove fatty materials and this was followed by extraction under continuously dripping ether.

With the exception of an observation with the polarizing microscope for doubly refractive material after the initial ammonium oxalate treatment, all tests were carried out on purified mycelia.

Histochemical tests used for the detection of cellulose were, (1) solubility in Schweitzer's reagent (cuprammonium), (Molisch, 1921), (2) hydrocellulose reaction, IKI—75% H_2SO_4 (Johansen, 1940), (3) chlorozinc-iodide (Artschwager, 1921). For chitin, a red-violet chitosan test (Johansen, 1940) and the production of chitosan sphaerites (Molisch, 1921) were regarded as positive evidence.

The fungi used in this investigation were so selected that they fall into two major groupings. The first group represents a cross section of the major divisions of the fungi and the second group consisted of a number of woodrotting Basidiomycetes.

Mycelial mats for testing were grown in 25X200 mm. cotton-stoppered glass culture tubes containing approximately 30 cc. liquid malt extract medium. The first group of fungi were allowed to grow for 15 days, the second for 25 days as growth was generally slower among the woodrotters. The cultures were killed in flowing steam for 60 minutes and washed.

The following fungi were used in this study:

Conidiobolus villosus
Emericella variecolor
Chaetomium aureum
Sordaria bombardioides
Ascobolus saccoboloides
Cyphella sp.
Ptychogaster rubescens
Vararia effuscata
Stereum gausapatum
Coniophora cerebella
Corticium galactinum
Corticium conigenum
Odontia bicolor
Hericium erinaceus
Poria oleracia
Poria cocos
Polyporus versicolor
Polyporus distortus
Polyporus gutulatus
Polyporus gilvus
Ganoderma applanatum
Fomes everhartii
Fistulina hepatica
Lenzites trabea
Schizophyllum commune
Pholiota adiposa
Penicillium claviformum
Curvularia lunata
Troposporella fumosa
Papularia sp.
Helicoma morgani
Sclerotium rolfsii

Results

Of these fungi, only *Sordaria bombardioides* and *Emericella variecolor* reacted in ways which would indicate the presence of cellulose. Upon examination with the polarizing microscope, after ammonium oxalate treatment, the massed hyphae of *S. bombardioides* were seen to be faintly doubly refractive. Ammonium oxalate treatment was followed by saponification and ether extraction after which the double refraction of *S. bombardioides* was seen to persist and doubly refractive material in the cleistothecial fragments of *E. variecolor* was exposed. No doubly refractive material was noted

in any of the other fungi, before or after purification. Anisotropy in the two species mentioned was taken as presumptive evidence for the presence of cellulose.

A faint blue coloration was detected in the hyphae of *S. bombardioides* and in the cleistothecial fragments of *E. variecolor* when the chlorozinc-iodide and hydrocellulose tests were applied. The faintness of the color as compared to the deep blue obtained with cotton fibers may have been due to the persistence of masking materials or the very small amount of cellulose present or to both.

No fungus sample placed in the cuprammonium cellulose solvent dissolved completely. Complete dissolution was not to be expected even in the case of the cellulose containing fungi as only a small part of these membranes was shown to be doubly refractive. Only with *E. variecolor* was regenerated cellulose recovered from the neutralized cuprammonium.

All fungi excepting *Vararia effuscata* and *Schizophyllum commune*, but including the two which gave positive cellulose tests, showed the red-violet color with the chitosan test indicating the presence of chitin. Due to the drastic nature of the chitosan test, negative results are not always reliable. The production of chitosan sphaerites was successful with a number of the fungi but was never reproducable with the consistency of the color tests.

For comparative purposes, the walls of five green algae were tested for the presence of cellulose and chitin. *Vaucheria* sp., *Stigeoclonium* sp., *Oedogonium* sp., *Cladophora* sp., and *Spirogyra* sp. all were found to have cellulose walls. *Chladophora*, in addition to an inner layer of cellulose, was shown to be enveloped by a continuous sheath of chitin which was external to the cellulose and did not follow the septa of the filament.

The fruiting bodies of four Myxomycetes were also tested for cellulose and chitin. Dried herbarium specimens were used and were prepared for testing by saponification. The cellulose tests were clear in the cellular bodies of the stalks of *Arcyria cinerea* and in the spores of that slime-mold. The peridial elements of *Lycogala flavofuscum* gave unmistakable cellulose reactions but the pseudocapillitium was non cellulose in composition, this is interesting in view of the supposedly identical origin of these two structures. The fructification of *Ceratiomyxa fruticulosa* and *Hemitrichia stipitata* did not appear to contain cellulose. None of the Myxomycetes tested were found to contain chitin.

Conclusions

It is evident that a basic membrane of a chitinous nature is characteristic of the higher fungi and that cellulose may occur infrequently in conjunction with this material. The difficulty encountered in staining fungal membranes may be traced not to the individual components, but to the close combination of these components which tend to mask the activity of one another. It may be possible with further work to discover a relationship between the resistance of many fungi to toxic chemical agents and the structure of their walls. The membrane composition of different structures can be utilized in clarifying developmental questions.

If phylogenetic significance is to be ascribed to the results of membrane studies, further work must be done to clarify the influence of substrate and other factors on the occurrence and relative amounts of membrane materials.

On the basis of experimental reports in the literature and the results of the studies reported here, the following is offered as a general summary of the composition of the basic fungal membrane. Exceptions have been reported for several of the groups listed.

Myxomycetes	(fructifications) proteinaceous materials, cellulose, no chitin.
Phycomycetes	
Oomycetes	Plasmodiophorales; chitin
	Chytridiales; mostly chitin
	Monoblepharidales; cellulose
	Blastocladiales; chitin
	Saprolegniales; cellulose
	Peronosporales; cellulose
Zygomycetes	Entomophthorales; chitin
	Mucorales; chitin
Ascomycetes	Mostly chitin
	Laboulbeniales; cellulose
	Saccharomycetaceae; neither cellulose nor chitin
Basidiomycetes	Mostly chitin

Bibliography

1. Artschwager, E. 1921. Use of Chlorozinc-iodide in Plant Histology. Bot. Gaz., Vol. 71:400.
2. Bary, Anton de, Comparative Morphology and Biology of Fungi, Mycetozoa and Bacteria. Oxford, 1887.
3. Castle, E. S. 1945. Structure of Cell Walls of Aspergillus. Am. Jour. Bot. 32:148-151.
4. Coker, W. C. 1937. Blastocladiales, Monoblepharidales, Saprolegniales. North Am. Flora. 1(2).
5. Foster, J. W. 1949. Chemical Activities of Fungi. Academic Press. New York.

6. Haas, P. and T. G. Hill. 1921. Chemistry of Plant Products. New York.
7. Hopkins, E. W. 1929. Microchemical Tests on the Cell Walls of Certain Fungi. Trans. Wisc. Acad. Sci., Arts, Letters. 26:187-196.
8. Johansen, D. A. 1940. Plant Microtechnique. McGraw-Hill. New York.
9. May, O. F. and C. E. Ward, 1934. Hydrolysis of the Chitinous Complex of Lower Fungi. Jour. Am. Chem. Soc. 56:1597-1615. 1921.
10. Molisch, H. 1921. Mikrochemie der Pflanze. Jena.
11. Nabel, K. 1939. Archiv. fur. Mikrobiol. 10:515-541.
12. Norman, A. G., and Peterson, W. H. 1932. The Chemistry of Mold Tissue. Biochem. Jour. 26:1946-1953.
13. Peterson, W. H. et. al. 1933. Nitrogen Constituents of Mold Mycelium. Jour. Biol. Chem. 100:lxxviii.
14. Thomas, R. C. 1928. Composition of Fungus Hyphae, I. Am. Jour. Bot. 15:537-547.
15. Thomas, R. C. 1930. Composition of Fungus Hyphae, II. Am. Jour. Bot. 17:779-788.
16. Thomas, R. C. 1940. Composition of Fungus Hyphae, III. Ohio Jour. Sci. 42:60-62.
17. Thomas, R. C. 1943. Composition of Fungus Hyphae, IV. Ohio Jour. Sci. 43:135-138.
18. Tiffany, L. H. 1924. Ohio Jour. Sci. 24:65-98.
19. Wettstein, F. v. 1921. Das vorkommen von Chitin . . . Vien K. Akad. der Wiss. Sitzungsber. Math. Nature. Abt. 1: 130:3-20.
20. Wisselingh, C. v. 1897. Mik. Untersuch. die Zellwande der Fungi. Jarv. fur Wiss Bot. XXXI.
21. Wurdack, M. E. 1923. Ohio Jour. Sci. 23:181-191.

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