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A CONSIDERATION OF THE TERM GLOEOCYSTIDIUM

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Structures termed "gloeocystidia" occur in diverse genera throughout the major groups of Homobasidiomycetes and have been variously defined: Ainsworth et al. (1971) state that it is a cystidium "that is thin-walled, usually irregular and with highly refractive hyaline or yellowish contents." Snell and Dick (197 1) list the variant spelling "gleocystidium" with the definition, "A special form of cystidium in Hymenomycetes, of gelatinous or horny consistency and with oily, resinous, or granular contents."

Talbot (1954) and Price (1975) have provided comprehensive statements on the concept of gloeocystidia. We present a condensed version here of Talbot's (1954, p. 288) definition.

> Sterile organs, with thin walls; lack of sculpturing and encrustation; contents hyaline to brownish, highly refractive, homogeneous, granular, or oily; arising from subhymenial and contextual tissues; staining deeply in phloxine and eosine in KOH mounts and becoming brown in iodine solutions.

In 1944, Romagnesi proposed the term "macrocystide" for a cystidial form in the "Lactario-russulés," and these cystidia (macrocystidia) were described as "trés longue . . . fusiform ou claviforme, souvent terminée par une pointe ou un appendice variable; son pédicule est très long et souvent en connexion avec laticifères de la trame," and secondly "très souvent, mais non toujours, devient gris-bleu ou noirâtre . . . au contact de la sulfovanilline. . . ."

Romagnesi's (1944) interpretation of macrocystidia is apparently based primarily on form, and secondarily on the chemical reaction with sulfovanillin. Donk's (1964) interpretation is somewhat contrary to this, as is Singer's (1962), for both have interpreted Romagnesi's statement or definition as one that explicitly includes the blueing of gloeocystidia (macrocystidia) in sulfovanillin. This, apparently, is not the case. Boidin (1951, 1958) substituted sulfuric benzaldehyde for sulfovanillin, a modification subsequently used by numerous authors. Also, see Lentz (1954).

Singer (1962), in discussing pseudocystidia in the Russulaceae, *Lactocollybia, Lentinellus,* and *Linderomyces,* recognizes macrocystidia (of Romagnesi, 1944) and gloeocystidia as distinct structures. Macrocystidia in his sense are sulfo-aldehyde positive and in addition absorb Cresyl Blue weakly. In contrast, Singer's (1962) concept of gloeocystidia involves, primarily, "the deep blue color they [gloeocystidia] assume when stained with Cresyl Blue (excepting the walls which remain a pale violet color)." Singer (1962) then goes on to say that the observed "metachromatism" is an infallible sign that this pseudocystidial type is "part of the gloeo-system." Donk's (1964) opinion that we cannot unconditionally extend Singer's (1 962) definition to Aphyllophorales is worth noting here.

Other terms have also been used variously to designate gloeocystidia and apparently are interchangeable; namely "sulfocystidia" (Boidin, 1966), "oleo-

cystidia" (Corner, 1950), "pseudophyses" (Lemke, 1964), and others. It is apparent after reviewing the various concepts and proposed definitions that the term includes several types of structures. It appears necessary to ask the questions, "What are gloeocystidia?" and "How can we define them with a greater degree of precision?".

Several characteristics of gloeocystidia (sensu lato) stand out, perhaps because of constant reference to them by numerous authors. One characteristic is the shape (or shapes). Another is the interpretation of the contents of gloeocystidia as being oils or fats, observations which are, most likely, biased by the etymology of the term itself *gloia*-(Gr., glue) + *cystidium* (L.).

We have attempted to appraise the reactions of gloeocystidia (so-called) with various reagents, some of which are now in use as aids for the detection of these structures.

METHODS AND REAGENTS USED FOR TESTING

Cresyl Blue

Singer **(1962)** has advocated the use of this stain for a cystidial form whose contents absorb the stain strongly and become blue, but whose walls absorb (?adsorb) it weakly and appear pale violet. Apparently, Cresyl Blue (now called Brilliant Cresyl Blue) is not a stain that is normally used for the detection of specific classes or types of compounds. However, it has been used for the study of root tip chromosomes (Stewart and Schertiger, 1949), and is described as a "vital stain". After application of 95% ethanol and 2% KOH to swell tissues, we applied Cresyl Blue according to the formula given by Sharma and Sharma **(1965).** Lower concentrations of Cresyl Blue were also used. Interference of KOH and ethyl alcohol with the test was not evident.

Nile Blue

The procedures used in this study for detection of lipids using Nile Blue are those proposed by Cain (194'7) and summarized by Jensen (1962). The natural lipids (fats, oils, and waxes) stain red, whereas acidic lipids (fatty acids and phospholipids) stain blue. Similar methods as those cited for the test with Cresyl Blue were used with Nile Blue.

Sudan Black B

Sudan stains have been used widely for the detection of lipids. Their application to the study of fungi, however, has received little attention. In our use of Sudan Black B, we have, with minor modifications, followed the procedures outlined by Jensen (1962).

Sulfuric Benzaldehyde (Sulfuric Acid-Benzaldehyde)

This test reagent (concentrated sulfuric acid, benzaldehyde, and distilled water (10:9:3)) for gloeocystidia has been frequently referred to as the "sulfoaldehyde" or the "sulfobenzaldehyde" test. Feigl (1966) refers to the LeRosen test for aromatic compounds, in which the components are concentrated sulfuric acid and concentrated formaldehyde (1:50). Thus, we considered in our tests that benzaldehyde might be an appropriate substitute for formaldehyde in this reagent, and would provide similar test results.

Sulfuric benzaldehyde elicits a limited variety of dark colored reaction

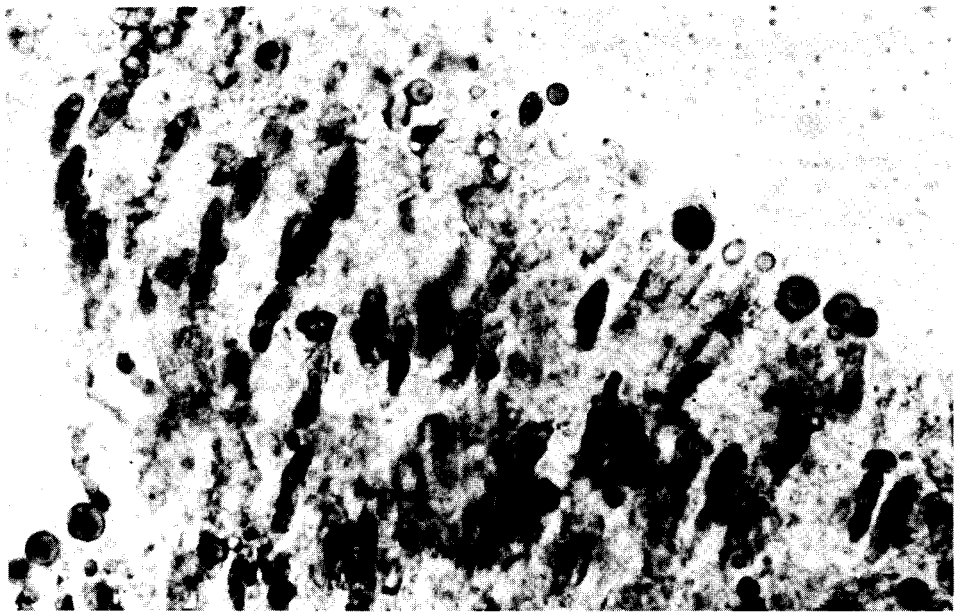

Fig. 1. Gloeocystidia of *Gloeodontia columbiensis* with dark colored contents. Section mounted in sulfuric benzaldehyde (×400, from HHB 7429).

products in gloeocystidia (Fig. 1), namely gray-blue, dark blue, and indigo, and in a few instances dark green and dark violet. Feigl (1966) lists several phenolic compounds that react with sulfuric formaldehyde, forming similarly colored reaction products. These are:

Diphenyl	blue-green
Nitronapthalene	green-blue
Hydroquinone	black
1,3,5-triphenyl benzene	blue

However, the reagents in the LeRosen test also form a variety of green, red and brown-red reaction products with many other phenolics.

A summary of our tests with the four reagents, as well as with sulfuric formaldehyde and sulfuric acid, are given in Table I, with the exceptions of Nile Blue and Cresyl Blue which did not provide meaningful results.

DISCUSSION

Interpreting the data from tests on gloeocystidial content was difficult due to inconsistency of results. A number of species possessed gloeocystidia that became blue, blue-gray, or black in sulfuric benzaldehyde. Some specimens, however, reacted positively in one test and negatively in a repeat of the same test (see *Auriscalpium vulgare, Gloeocystidiellum heimii, Gloeodontia discolor*.)

Another reagent which yielded positive results was Sudan Black B. In almost every specimen in which the gloeocystidial content stained black to indigo in Sudan Black B, staining also occurred with sulfuric benzaldehyde. *Gloeocystadiellum heimii* (FP 100802, FP 100812, HHB 6385) and *Peniophora duplex* (FP 133550) were the exceptions. Why gloeocystidia of so many species should react

Table I

Staining of Gloeocystidial Contents by Various Reagents in Basidiocarps of Aphyllophorales

Fungus Name and Test Specimen	Reagent[a]			
	Sulfuric Benzaldehyde	Sulfuric Formaldehyde	Sulfuric Acid	Sudan Black B
Auriscalpium vulgare S. F. Gray				
HHB-6078	+/+	–	–	++
HHB-6186	++/–	–	–	++
OKM-6487	++/–	–	–	++
Basidioradulum radulum (Fr. per Fr.) Nobles				
FP-125068	–	–	–	–
HHB-226	–	–	–	–
HHB-285	–	–	–	–
Chondrostereum purpureum (Pers. per Fr.) Pouz.				
HHB-8271	–	–	–	–
FP-104338	–	–	–	–
FP-71516	–	–	–	–
HHB-3198	–	–	–	–
Cryptochaete rufa (Fr.) Karst.				
FP-3825	++/++	–	–	++
FP-29181	++/++	–	–	++
FP-39654	++/+	–	–	++
Cystostereum murraii (Berk. & Curt.) Pouz.				
FP-105668	–	–	–	–
RLG-9637	–	–	–	–
FP-86035	–	–	–	–
Dacryobolus sudans (Fr.) Fr.				
HHB-5779	–	–	–	–
HHB-7350	–	–	–	–
ERC 71-321	–	–	–	–
Gloeocystidiellum citrinum (Pers.) Donk				
HHB-5682	–	–	–	–
HHB-5670	–	–	–	–
HHB-6151	–	–	–	–
G. heimii Boid.				
FP-100802	+/++	–	–	–
FP-100812	+/+	–	–	–
HHB 6385	++/+	–	–	–
FP 100808	+/–	–	–	++
G. heterogeneum (Bourd. & Galz.) Donk				
HHB-7798	–	–	–	–
HHB-1270	–	+ lt brown	–	–
HHB-7871	–	+ lt brown	–	–
Gloeocystidiellum leucoxanthum (Bres.) Boid.				
HHB-5733	–/–	–	–	++
HHB-5080	++/–	–	–	++
(?) HHB-5457	++/+	+ red brown	+ pale red-brown	++
G. porosum (Berk. & Curt.) Donk				
HHB-8138	++	–	–	++
HHB-2166	+	–	–	++
HHB-3441	+	–	–	+

Table I (Continued)

Fungus Name and Test Specimen	Reagent[a] Sulfuric Benzaldehyde	Sulfuric Formaldehyde	Sulfuric Acid	Sudan Black B
Gloeodontia columbiensis Burt ex Burds. & Lombard				
HHB-7422	++/++	–	–	++
HHB-7429	++/++	++ purple-red-brown	–	++
G. discolor (Berk. & Curt.) Boid.				
FP-105687	+/+	–	–	+
FP-90183	+/++	–	–	+
FP-105031	–/+	–	–	+
Hyphoderma argillaceum (Bres.) Donk				
HHB-7903	–	–	–	–
HHB-7631	–	–	–	–
HHB-1425	–	–	–	–
Hyphoderma puberum (Fr.) Wallr.				
HHB-5243	–	–	–	–
HHB-7351	–	–	–	–
HHB-7313	–	–	–	–
H. tenue (Pat.) Donk				
HHB-7294	–	–	–	–
HHB-1205	–	–	–	–
HHB-1376	–	–	–	–
Hypochnicium geogenium (Bres.) J. Erikss.				
HHB-4762	–	–	–	–
HHB-5981	–	–	–	–
H. punctulatum (Cke.) J. Erikss.				
HHB-6281	–	–	–	–
HHB-7771	–	–	–	–
KJM-271	–	–	–	–
H. sphaerosporum (Höhn. & Litsch.) J. Erikss.				
RLG-9027	–	–	–	–
HHB-2025	–	–	–	–
Laxitextum bicolor (Pers. ex Fr.) Lentz				
FP-12711	–	–	–	++
FP-21703	–	–	–	–
FP-110477	–	–	–	–
Laxitextum crassum (Lev.) Lentz				
FP-103844	–	–	–	–
FP-103938	–	–	–	–
HHB-6216	–	–	–	–
Peniophora albobadium (Schw. per Fr.) Boid.				
FP-12978	–	–	–	–
FP-18484	–	–	–	–
FP-18509	–	–	–	–
P. duplex Burt				
FP-133550	++/++	–	–	–
P. incarnata (Pers.) Karst.				
HHB-5534	+/++	+ red-brown	–	++
HHB-7259	+/++	–	–	++
HHB-5019	++/++	–	–	++

[a] ++ Strongly positive reaction
+ Weakly positive reaction
- No visible reaction
/ Separates results of two replications

Table 1 (Continued)

Fungus Name and Test Specimen	Reagent[a] Sulfuric Benzaldehyde	Sulfuric Formaldehyde	Sulfuric Acid	Sudan Black B
P. nuda (Fr.) Bres.				
FP-100966	++/+	–	–	++
FP-100964	–	–	–	–
FP-101246	–	–	–	–
P. violaceolivida (Sommerf.) Mass.				
HHB-6467	–	–	–	–
HHB-6629	–	–	–	–
Poria latitans Bourd. & Galz.				
HHB-1146	–	–	–	–
HHB-1766	–	–	–	–

[a] ++ Strongly positive reaction
+ Weakly positive reaction
- No visible reaction
/ Separates results of two replications

positively with both sulfuric benzaldehyde and Sudan Black B is of interest, but these coincident reactions do indicate that perhaps both aromatics and lipids are present simultaneously.

The cystidia reacted positively with the LeRosen test for aromatic compounds in specimens of only four species. These were *Gloeodontia columbiensis* (one specimen reacted negatively), *Peniophora incarnata, Gloeocystidiellum heterogeneum* (one specimen reacted negatively), and *G. leucoxanthum.* In *G. leucoxanthum,* however, the same reaction was also noted in the control: sulfuric acid applied alone. Therefore, the reaction apparently was not caused by aromatic compounds but rather by a reaction with the sulfuric acid. The only specimens that seem to possess gloeocystidia containing aromatic compounds are those of *Gloeodontia columbiensis* (HHB 7429), *Gloeocystidiellum heterogeneum* (HHB 1270 & 7871), and *P. incarnata.* Since the results from using the two sulfuric aldehyde reagents were not similar, we tentatively conclude that benzaldehyde is not an appropriate substitute for formaldehyde in the LeRosen reagent. As with most of the gloeocystidia of the other specimens that reacted positively with sulfuric benzaldehyde, those in HHB 7429 and *P. incarnata* also reacted positively with Sudan Black B.

An important inconsistency was encountered in the test with sulfuric benzaldehyde. In some instances, a single specimen tested several times would yield different results in these replications. Two specimens of the same species would sometimes react differently, one positively and one negatively, and in the same section some cystidia reacted positively and others negatively. The cause of this inconsistency is unknown. If the test detects the presence of aromatics, possibly all of the reacting sites are bound up through condensation reactions producing a negative result. This might explain the tendency for cystidia in older collections more than 40 years old (Boidin, 1966) to react negatively, although this is not always the case (Burdsall and Lombard, 1976).

Our tests using Nile Blue and Brilliant Cresyl Blue were inconclusive. All tissues were stained with these two reagents when used as directed (Jensen 1962; Sharma and Sharma, 1975). Much lower concentrations (0.5% w/v) of Brilliant Cresyl Blue were also used, but similar results were obtained. Cresyl Blue, advocated by Singer (1962) for detection of gloeocystidia in Agaricales, does not appear to be applicable for similar purposes in Aphyllophorales.

The original questions then remain, "What are gloeocystidia?" and "How can we define them with a greater degree of precision?" Supposedly, those reacting positively with Sudan Black B could be called gloeocystidia since they contain lipids. But what about those gloeocystidia that do not react in the same thin section as those reacting positively? In all probability they are not different structures, but merely representatives of developmental stages.

We view the term "gloeocystidium" as vague and suggest that it be dropped from use until a more precise interpretation and explanation for the variability of the observed chemical reaction is available. It appears more appropriate at present to term the sterile structures "cystidia" (or when applicable, "pseudocystidia"), describe them accordingly, and include their reactions with sulfuric benzaldehyde, Sudan Black B, or other suitable reagents.

SUMMARY

The term "gloeocystidium" is discussed and various definitions reviewed. Since structures called gloeocystidia are often indicated as having "oily" contents, many species with so-called gloeocystidia were tested for oil content by using Sudan Black B and Nile Blue. Tests for reactions with sulfuric formaldehyde (LeRosen test), sulfuric benzaldehyde, and the control (sulfuric acid) were also carried out. Cresyl Blue (or Brilliant Cresyl Blue), indicated to be a test for gloeocystidia, was also used.

With Brilliant Cresyl Blue and Nile Blue all tissues stained darkly and no evaluation of gloeocystidial content could be made. The LeRosen test proved positive for only four examples, whereas sulfuric benzaldehyde and Sudan Black B reacted positively with gloeocystidia of a larger number of species. An unexplained relationship apparently exists between positive reactions of individual species with both sulfuric benzaldehyde and Sudan Black B. Sulfuric benzaldehyde was found to be extremely erratic in reacting with gloeocystidial contents, making this a character, in our opinion, of questionable taxonomic value.

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