

Melzer's, Lugol's or Iodine for Identification of White-spored Agaricales?

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Abstract

Melzer's reagent is an iodine solution producing a blue-black "amyloid" reaction in some spores and parts of fungi. However, Melzer's reagent contains chloral hydrate, a medically controlled substance and therefore it has been hard to get. The history of iodine use for identification of fungi dates back to the mid 1800s; its use for white spore identification was described by Melzer in 1924. The production of the positive amyloid reaction is due to an amylose-iodine complex. In some cases a reddish "dextrinoid" color may occur due presumably to a glycine-betaine complex. The spores of 35 species of fungi were tested with Melzer's, Lugol's, and iodine solutions. All 35 species reacted as predicted from authoritative sources with Melzer's but results were inconsistent with Lugol's and iodine. Melzer's reagent should be used instead of Lugol's or iodine in spore identification. An easy source of Melzer's reagent is given.

Keywords: Fungi spores; amyloid; dextrinoid; Melzer's reagent source

Introduction

Melzer's reagent is an iodine solution used in the study of the white spored Hymenomycetes, the asci of Ascomycetes, and other parts of fungi. However, Melzer's reagent contains chloral hydrate, a medically controlled substance, and therefore is hard to get. I have often heard the question, "Is it OK to use Lugol's solution or iodine instead of Melzer's?" It seemed possible that perhaps just iodine or Lugol's solution which contains iodine and also potassium iodide could be used in place of Melzer's to produce the bluing which we call the "amyloid" reaction.

This paper will review the history of iodine solutions; try to show the mechanism of action in the color change with white fungal spores; review my experience during the summer of 2003 testing spores of 35 different species with Melzer's reagent, Lugol's, and tincture of iodine; give my conclusions; and finally, explain how you can now fairly easily get Melzer's reagent.

History

The earliest reference to using the bluing of fungi by iodine to identify fungi I could find was a report of the bluing of lichens in 1852 noted by the Tulasne brothers (1861) in their great three-volume work on Fungi in a chapter on the *Seminis Fungini*, "The Fungus Seed." They also noted the report of the bluing of an ascomycete *Amylocarpus encephaloides* Curr., by Currey in 1858 and they discussed bluing with sulfuric acid and iodine in young asci and ascospores. However they did not discuss the use of iodine on white spores of Hymenomycetes.

Berkeley (1860) did not mention iodine in his work although he noted that spore deposits were of a different color in different species and "they afford a ready test for grouping the species."

Nylander (1865) described iodine bluing in lichens and ascomycetes as did Rolland (1887) but then in his last sentence noted that Patouillard discussed violet coloration of the spores of a Hymenomycete "*Cyphella vitellina*, N. Sp. Du Chili et du Cap Horn." Patouillard described,

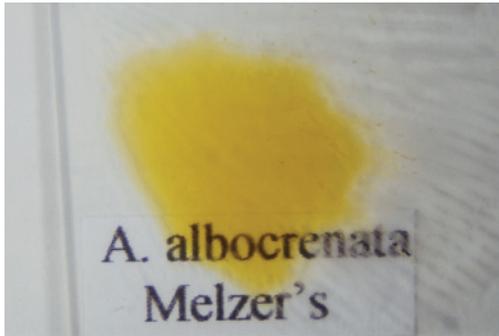


Figure 1. *Amanita albocrenata* 1.2x mag.; negative amyloid reaction with Melzer's.



Figure 2. *Amanita flavorubescens* 1.2x mag.; positive amyloid reaction with Melzer's.

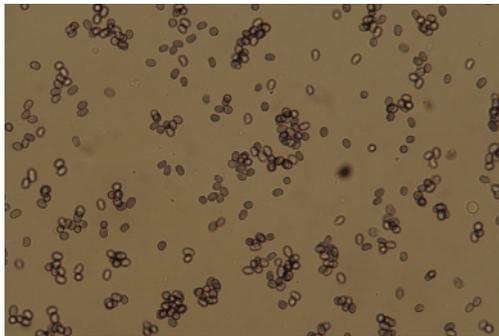


Figure 3. *Lactarius piperatus* 100x mag.; positive amyloid reaction with Melzer's.

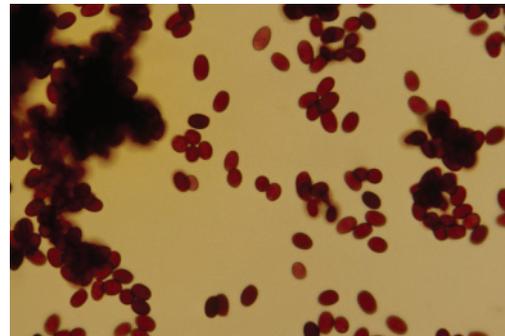


Figure 4. *Macrolepiota procera* 100x mag.; dextrinoid reaction.

Tome III, 2e Fascicule (1887) a violet reaction with "iode" and the spores. However, it is interesting that apparently spore color changes were not considered that important since the next year Patouillard (1888) published a 4 page article "Quelques Points de la Classification des Agaricinées" in which he discussed spore color but did not mention the use of iodine.

In 1900, McIlwaine did not use iodine solutions in his descriptions of fungi. Boudier (1905-1910) described and illustrated the use of iodine for Ascomycetes but still there was no note of its use in white spore identification.

So it appears that although iodine was used in the mid-1800s in lichen and asci evaluation, it was not until Melzer (1924) more thoroughly documented the use of iodine for white spore identification. He described the use of an iodine solution with chloral hydrate (formula noted Table 1) to help show the spore ornamentation in russulas. Baral (1987) believed that Melzer was "probably inspired by Meyer" (a botanist who

introduced the use of chloral hydrate in 1883). Dennis (1981) and others noted that chloral hydrate clears the cell contents so the color reaction is more obvious.

In reference to Melzer's reagent, Singer wrote (1975, pg 95), "Its composition though slightly altered in one sense or another (without much difference in effect) by some mycologists is still the original one indicated by Melzer (1924)." However, Singer used 22 g. of chloral hydrate instead of Melzer's 20 g. It is not clear to me if this was the slight alteration he mentioned or if he was referring to Langeron's modification (formula noted Table 1) which is common today. This change in the proportions of Melzer's is now known as "Langeron's modification" (Ainsworth and Bisby, 1961). Langeron (1945) had this formula in his book but there was no discussion as to why he changed the proportions. (personal communication Christian Volbracht, Mykolibri).

Singer emphasized "that any deviation from the formula . . . may (not must) cause a discrepancy

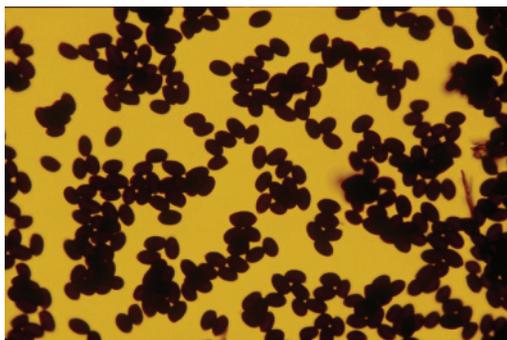


Figure 5. *Macrolepiota procera* 100x mag.; positive amyloid reaction with Lugol's solution.



Figure 6. *Amanita frostiana* 1.2x mag.; negative amyloid reaction with Melzer's.



Figure 7. *Amanita frostiana* 1.2x mag.; more or less dextrinoid reaction with Lugol's solution.

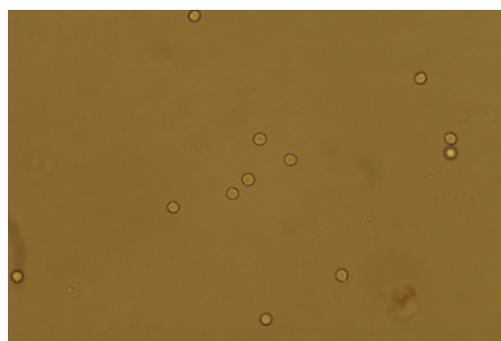


Figure 8. *Amanita frostiana* 100x mag.; negative amyloid reaction with Melzer's.

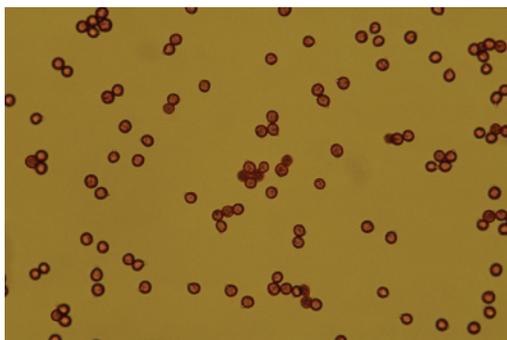


Figure 9. *Amanita frostiana* 100x mag.; dextrinoid reaction with Lugol's.

between the results obtained and those described by the authors [*sic*]. So what to use? Kohn and Korf (1975) Dennis, (1981), Baral (1987), and Breitenbach (1984) used Melzer's original formula; Gilbertson and Ryvardeen (1986) and Jenkins (1987) used the slight modification noted by Singer; but Rossman (1980), Gams (1987), Watling (? Date), Phillips 1991, Ainsworth and

Bisby (1991), Blackwell, (2001) and the International Culture Collection of VA Mycorrhizal Fungi, 2003 (<http://invam.caf.wvu.edu/methods/recipes.htm>) used Langeron's modification.

Kohn and Korf (1975) noted variation in asci reaction with Melzer's depending on whether the mount was in water or potassium hydroxide. Baral (1987) also emphasized that in Ascomycetes "strikingly different results" were obtained when different iodine-potassium iodine or Melzer's reagents were used and were different if pretreated with KOH. Neither of these papers reported on the study of these reagents with white spored Hymenomycete spores.

Mechanism of Action and Definitions

Iodine: "Tincture Iodine" a common first aid antiseptic is easily available as a non-prescription IKI alcoholic solution.

Lugol's solution (also known as Aqueous Iodine Solution or Strong Iodine Solution at times writ-

ten as IKI) is used medically to disinfect water and to protect the thyroid when using iodine for imaging. The formula provided by the Maine Medical Center Pharmacy is 5 g of iodine crystals and 10 g of potassium iodide in 100 ml water. Warning: it should be labeled “poisonous, for external use only” and contact with it should be avoided by people with iodine allergies or thyroid disease.

Melzer’s reagent: Table 1 shows Melzer’s formula as originally published in 1924 for a 20 g of water. It also contains iodine and potassium iodide, as does Lugol’s but in addition it has chloral hydrate. In the next column I have shown this formula in 100 ml water so it can be contrasted with Langeron’s modification. Chloral hydrate is a medically controlled sedative and hypnotic, so a licensed physician has to prescribe it. Interesting, it was first used medically in 1869, about the same time iodine was used as a plant/fungus reagent. Poisonings were reported in 1890, and the therapeutic range—that is, the range of effectiveness to poisoning, is small; and so though effective as a sedative, it is not used much these days (Mass General Hospital, 1998), though I do remember it being used there in 1959 when I was a resident physician. The adult oral dose is $\frac{1}{2}$ to 1 g, so your 1-oz bottle of Melzer’s reagent has about 33 g of chloral hydrate and thus is potentially very dangerous; adult deaths have been reported with 35 g. It is the drug used in “knockout drops” or the “Mickey Finn.”

Amyloid reaction: Fungal tissue that turns blue or black with Melzer’s reagent is an amyloid positive reaction, sometimes written as I+ or J+.

In high school chemistry we learned that starch and iodine produces a blue-black color, thus the “starch-reaction.” Starch, found in plants, is a carbohydrate and a polysaccharide, a polymer of alpha-glucose, and is a mixture of 25% amylose and 75% amylopectin. Starch and iodine form helical coils with the iodine atoms fitting into the coils forming an amylose-iodine complex (www.park.edu/bhoffman.com) and hence an “amyloid” reaction. The reaction with starch is blue-black and a brown-blue color with glycogen. Starch in plants is found in plastids (amyloplasts and chloroplasts). Dodd and McCracken (1972) noted that fungal “starch” is different from plant

starch in that it is not produced in plastids, is not in granular form, is mainly a cell-wall component (rather than a energy source), and is made up of “only short-chain amylose molecules.” He hypothesized that the amylose in the spore cell helped the spore stay viable until conditions were good for germination.

Dextrinoid reaction: This is a red to red-brown reaction with Melzer’s reagent.

Blackwell et al (2001) suggested that the red reaction does not involve starch or amylose, but is a reaction with glycine betaine, an “osmolyte” (an organic osmotic solute) which they found in high concentrations in the Basidiomycetes they studied. Betaine functions to attract water to the rapidly enlarging and differentiating basidiomata. The addition of iodine presumably results in a “glycine betaine-IKI complex.” Baral (1987; personal communication, 2004) noted that the dextrinoid reaction is “strongly enhanced” by chloral hydrate.

Inamyloid reaction: There is no color change with Melzer’s reagent, sometimes written as I- or J-.

Hemiamyloid: This is a red reaction with Lugol’s reagent.

Baral (1987) defined hemiamyloidity as the red change in the apical tip of an ascus or the outer layer of the ascus wall with Lugol’s solution (IKI, not Melzer’s). He and Rossman (1980) discussed the different reactions of the tips of asci with Melzer’s and other iodine solutions. Red changes occurred with IKI, but a blue reaction was the result if the specimen was mounted previously in KOH. Rossman (1980) noted that this was clearly discussed by Dobbeler (1978). Apparently chloral hydrate in Melzer’s also masks the hemiamyloid reaction in asci, and therefore Lugol’s solution should be used to show asci hemiamyloidity without KOH. Baral (personal communication, 2004) noted that it is “a frequent mistake to use Melzer’s instead of Lugol’s as iodine reagent for recognizing ascus amyloidity,” and both he and Korf (2003) stressed that KOH not only prevents this red hemiamyloid reaction, but produces an enhanced blue reaction that is a false amyloid reaction. Baral stated that KOH “transforms the iodine molecules into non-reactive iodide ions” and hemiamyloidity will not occur.

Method

Fresh spore deposits of 35 different specimens were tested with 1) Melzer's reagent, (Langeron's modification), 2) Lugol's solution, and 3) tincture of iodine. Many specimens were also examined in just water. The spores initially were deposited on paper; a portion of spores was carefully scraped off, placed on a slide with the test solution, and then examined without magnification. Some of the spore deposits were made directly on a glass slide, and these are noted in Table 2 (gl). Many of the gross examinations were photographed at 1.2x on outdoor positive color film using balanced outdoor electronic flashes. The specimens were examined microscopically at 100x and 400x was then many were photographed.

Results

Table 2 shows what the expected reaction with Melzer's should be using authoritative sources (Bessette (1997); Jenkins (1986). There were no inconsistencies between my Melzer's reagent (Langeron's modification) reactions and reports from the authoritative sources. Notations in red indicate a difference in what was found and what was expected.

Generally the amyloid positive reaction was quite easy to see under gross examination. Figure 1, *Amanita albocrenata* 1.2x, shows a white spore deposit directly on glass and photographed so that the white deposit would look a bit gray and show up in the photograph. The drop of Melzer's is yellow and clearly there is no positive black amyloid reaction. Figure 2, *A. flavorubescens*, 1.2x shows a clear positive black amyloid reaction with Melzer's. However microscopic evaluation of amyloidity is at times a bit difficult. If so I then made a water mount for comparison or I could compare

mature spores with immature spores which do not react and look clear as in Figure 3, *Lactarius piperatus*, mag. 100x, in Melzer's. Figure 4, *Macrolepiota procera*, 100x shows a clear dextrinoid reaction with Melzer's. It also shows what can be a problem: when the spores overlap, they look black or amyloid positive, and so a thick spore deposit can be deceiving. Rather than scrape a thick spore deposit from paper, Sam Ristich (personal communication) suggested making the spore deposits directly on a slide. This helps somewhat, but you can still have a thick deposit and be fooled if the spores are clumped. Figure 5, *M. procera*, 100x shows an inconsistent black positive with Lugol's solution. Figures 6–9 are *Amanita frostiana*: Figure 6 1.2x in Melzer's shows no reaction, that is amyloid negative; Figure 7 1.2x in Lugol's shows some reaction which is more or less dextrinoid; Figure 8 is in Melzer's, 100x and is amyloid negative; but Figure 9 is in Lugol's, 100x, and shows a clear (inconsistent) dextrinoid reaction.

The color reactions with Lugol's and iodine were inconsistent at times as can be seen from Table 2. Though Baral (1987) noted that the dextrinoid reaction was enhanced by chloral hydrate, at times I found a dextrinoid reaction with Lugol's when the reaction with Melzer's was positive or negative. There is a problem with the alcoholic solution of the drug-store iodine quickly spreading out on the slide and evaporating. An aqueous solution might be better, but I did not try it.

I have only begun to examine asci for their reactions; there seemed to be some inconsistencies but I cannot comment definitely on them at this time.

A Solution to a Problem Solution

The reason for attempting to substitute Lugol's or iodine for Melzer's is that there a problem obtaining Melzer's as the chloral hydrate in it is a con-

Table 1. Formulas for Melzer's Reagents

Components	Melzer, 1924	Melzer, 1924/100ml	Langeron's Melzer's
Potassium iodide	1.5 gms	7.5 gms	5.0 gms
Iodine	.05 gms	2.5 gms	2.5 gms
Chloral hydrate	20.0 gms	100 gms	100 gms
Water	20.0 gms	100 ml	100 ml

Table 2. Melzer's reaction from authoritative sources (reference) and results of author's testing with Melzer's reagent, Lugol's solution and Iodine.

	REFERENCE ¹	MELZER'S	LUGOL'S	IODINE
<i>Amanita albocrenata</i> (Atk.) Gilbert	neg	neg	neg	neg
<i>Amanita albocrenata</i> (Atk.) Gilbert (gl) ²	neg	neg	dextr	dextr; neg (micro) ³
<i>Amanita brunescens</i> Krieger	pos	pos	pos	pos
<i>A. brunescens</i> var. <i>pallida</i> Krieger	pos	pos	pos	+/- brownish
<i>Amanita flavoconia</i> Atkinson	pos	pos	pos	neg / pos ⁴
<i>Amanita flavorubescens</i> Atkinson	pos	pos	pos	neg
<i>Amanita flavorubescens</i> Atkinson (gl)	pos	pos	brown-dextr(micro-dextr)	brown (micro-pos)
<i>Amanita frostiana</i> (Peck) Saccardo (gl)	neg	neg	dextr	neg
<i>Amanita frositana</i> #2 (Peck) Saccardo (gl)	neg	neg	dextr	neg
<i>Amanita fulva</i> (Schaeff.) per Pers.	neg	neg	neg	neg
<i>Amanita rubusens</i> (Persoon:Fries) S.F. Gray	pos	pos	pos	neg
<i>Amanita sinicoflava</i> (Tullios)	neg	neg	neg	neg
<i>Amanita</i> sp. (non-striate)	pos ⁵	pos	pos	neg
<i>Collybia dryophyla</i> (Bulliard:Fries) Kummer	neg	neg	pos	neg
<i>Collybia dryophyla</i> #2 (Bulliard:Fries) Kummer	neg	neg	pos	neg
<i>Lactarius corrugis</i> Peck	pos	pos	pos	pos
<i>Lactarius piperatus</i> (Fries) S.F. Gray (gl)	pos	pos	brown(micro-dextr)	brown (micro-pos)
<i>Lepiota lutea</i> (Bolton) Quelet (gl)	dextr	dextr	dextr	dextr
<i>Lyophyllum semitale</i> (Fries) Kuhner (gl)	neg	neg	dextr	dextr

Macrolepiota procera (Scopoli) Singer	dextr	dextr(bl mass)	neg	pos in mass
Macrolepiota procera (Scopoli) Singer #2 (gl)	dextr	dextr	pos	dextr(pos in mass)
Megacollybia platyphyla (Persoon:Fries) Kotlaba & Pouzar	neg	neg	pos	red/brown
Megacollybia platyphyla (Persoon :Fries) Kotlaba & Pouzar #2	neg	neg	pos	pos
Melanoleuca melaleuca (Persoon:Fries) Murrill	pos	pos	pos	pos
Mycena leatiana (Berkeley) Saccardo (gl)	pos	pos	dextr	dextr
Omphalotus olearius (De Candolle:Fries) Singer (gl)	neg	neg	dextr	neg
Porpoloma umbrosum (Smith & Wallroth) Singer (gl)	pos	pos	pos	pos (slowly)
Russula adusta (Fries)	pos	pos	neg	neg
Russula barlae (Quelet) (gl)	pos	pos	dextr	dextr
Russula densifolia (Secretan) Gillet	pos	pos	pos—5 min	pos, slowly
Russula emetica (Schaeffer) Persoon	pos	pos	+/- brown	pos
Russula sp	pos ⁶	pos	+/-	neg
Russula sp #2	pos ⁶	pos	pos (slowly)	pos (slowly)
Tricholomopsis rutilans (Schaeffer:Fries) Singer (gl)	neg	neg	pos	neg
Zerula radicata (Rehlan:Fries) Dorfelt	neg	neg	pos	neg

NOTES

1. Results of Meizer's reagent reaction from Besette (1997), Jenkins (1986)
2. (gl) means spores were deposited directly on glass.
3. All observations were macro (without magnification) unless "micro" is noted; micro=400x magnification.
4. This was positive after a few minutes.
5. Species not identified; it was a non-striate Amanita and so (probably) should be positive.
6. All Russula spp. are amyloid positive.

trolled substance. Here is a way to get Melzer's:

Have a doctor friend, family practitioner, or some licensed physician in your group write a prescription for "Melzer's reagent." You then need to find a druggist who can mix or "compound" chemicals (a real old-fashioned druggist), known now as a "compounder." You can call (800) 331-2498, the Professional Compounding Center of America, and they will give you the names of compounders in your state (if there are any). Then call the compounder and explain the situation, send them the Melzer's prescription, choosing one of the formulas from Table 1. Have them dispense it in a 1-oz brown dropper bottle labeled "poison." Many compounders probably will have to obtain the chemicals and will want to make a minimum of 100 ml.

If you cannot find a compounder in your state, I have made arrangements with one in Maine who is willing to send out a single 1-oz bottle. Contact Gerry Bouchard, Medical Center Pharmacy, 121 Medical Center Drive, G-500, Brunswick, ME 04011; tel. (207) 729-3642; fax (207) 729-2704. He will still need a valid prescription from a physician in your state. Have the physician write "Melzer's Reagent, Disp: (# of 1-oz bottles you want), Sig: for Fungi ID only; Label: For Fungi ID only; Poison." He will send Langeron's modification. Cost, including postage, will be approximately \$12 for 1 oz in a brown dropper bottle.

Conclusions

1. There are two slightly different sets of "Melzer's reagents."
2. When reporting results with Melzer's, it should be clear which one is being used.
3. The positive "amyloid" reaction involves short-chain amylose molecules; the "dextrinoid" reaction does not, but presumably reacts with glycine betaine.
4. I do not feel that Lugol's reagent (IKI) or tincture of iodine can be substituted for Melzer's reagent when testing white spores for amyloidity.
5. Melzer's reagent can now easily be obtained as noted above.

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