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# SIDEROPHILOUS GRANULES IN THE BASIDIA OF HYMENOMYCETES

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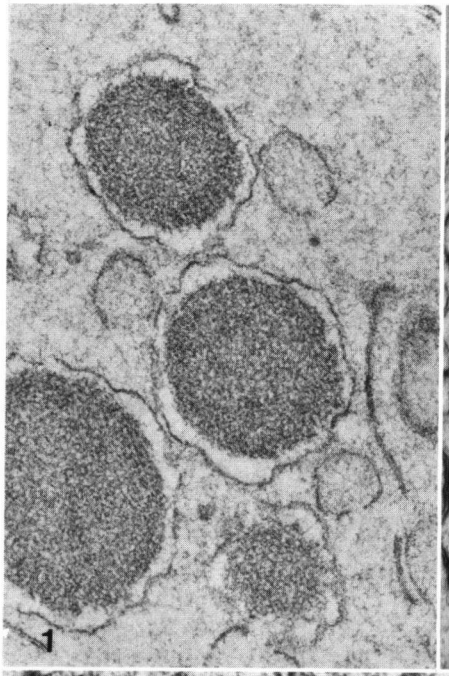
Siderophilous granules of postmeiotic basidia are protein filled vesicles derived from the endoplasmic reticulum capable of binding relative large amounts of metal ions thus standing out with high opacity in the electron microscope and becoming stainable with acetocarmine or hematoxylin for light microscopy. They range from very small, submicroscopic, single and rare particles to easily observable granules almost filling the basidium. The number and size of the granules can be used to describe five types of granulation, the crypto, micro, oligo, macro, and gigas type. The macrotype is taxonomically important for the Lyophylleae, and it seems that the micro type has taxonomic significance in the Rhodophyllaceae (Entolomataceae) and the Russulaceae.

Historically, the siderophilous granules are a key character of the genera *Lyophyllum* (including *Tephrocybe*) and *Calocybe*. Here they are easily observed with the light microscope. But investigation with the electron microscope indicated that siderophilous granules are more widely distributed in Hymenomycetes, but frequently are too small to be readily identified with the light microscope.

## LIGHT MICROSCOPY

When heated with iron-acetocarmine the mature basidia of several agarics belonging to different genera are seen to contain many small, intensely stained granules. This is the 'granulation carminophile' of Kühner (*1938*), described from species of the genera *Lyophyllum* and *Calocybe*. These granules contain basic proteins capable of binding large amounts of metal ions, such as iron, cobalt, osmium, copper, lead, hafnium, thorium, uranium, and others. The carmine only acts as an indicator for the presence of these metals, giving dark purple to blackish complexes when heated in acid solutions. It may be replaced by other relevators, such as sodium sulphide or hematoxylin. Based on the fact that it is the metal rather than the carmine that is responsible for the positive staining reaction the term 'siderophilous' has been proposed to replace 'carminophilous' (Clémençon, *1967*, *1969*).

To intensify the staining and to obtain clean preparations exempt of precipitations Clémençon (*1968a*) introduced a method which separates the application of metal ions from the staining with acetocarmine. The folowing is a simplified method avoiding the radioactive and dangerous thorium nitrate and the not always available zirconyl chloride. These two metals enhance somewhat the final staining, but for routine observations they are not necessary. It should be noted that this method gives good nuclear staining well suited for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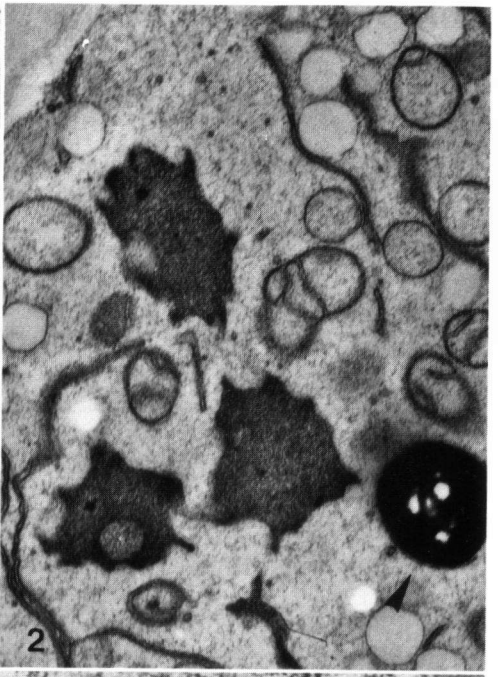
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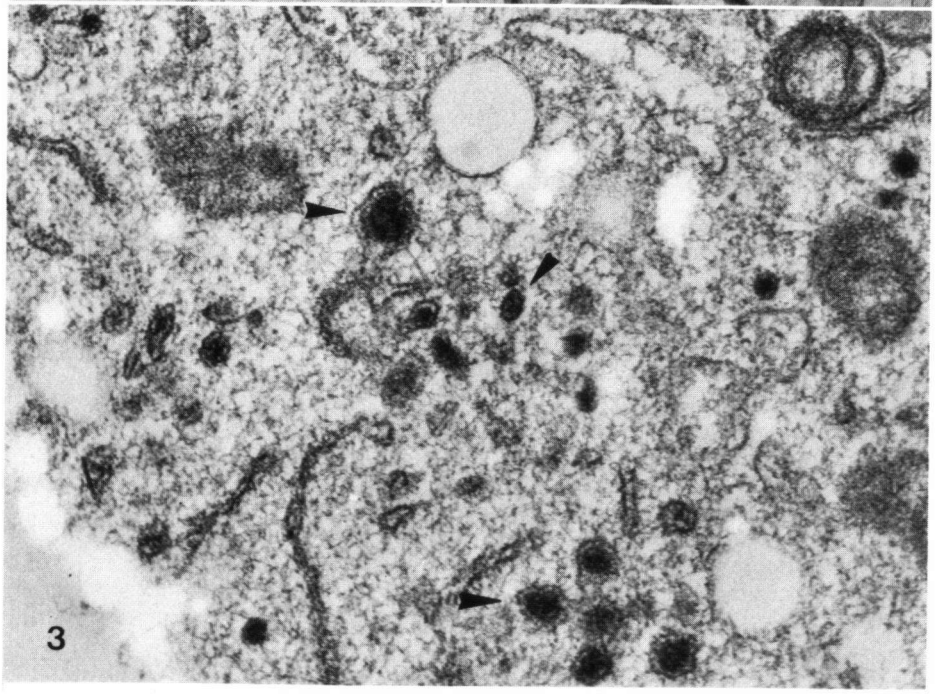
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phase microscopy. Other siderophilous structures, such as some spore walls (e.g. in *Rhodophyllus, Lyophyllum*) are equally well stained.

(1) A small fragment of a gill of living material or of a herbarium specimen is immersed in a few drops of the following fixing and mordanting solution:

| | |
|---|---|
| Ferric chloride, 10% sol. in acetic acid 50% | 5 ml |
| Copper acetate, 10% sol. in acetic acid 50% | 5 ml |
| Picric acid, saturated sol. in dist. water | 5 ml |
| Formaldehyde, saturated sol. in dist. water | 5 ml |
| Lead acetate, 1% sol. in acetic acid 50% | 1 ml |

The lead acetate solution is added last, drop by drop while stirring constantly. The final solution is stable for years.

Fix for a few minutes.

(2) Pick up the fragment, blot off the excess of the fixing and mordanting solution and transfer directly into acetocarmine. Add one or two small pieces of broken porcelain or brick to avoid explosive boiling, and heat gently over a small flame. Keep boiling for 1 or 2 minutes. The solution should turn dark from the metal-carmine complexes formed.

Small Pyrex beakers with a capacity of 1 ml are ideally suited for this step, but other small recipients may of course be used as well.

Commercial acetocarmine solutions frequently do not work well because of low carmine content. Prepare your own solutions by boiling under reflux a few grams of carmine (e.g. Merck 2233) with 200–300 ml of 50% acetic acid for 2–3 hours. Filter next day.

(3) After boiling, the entire content of the small beaker is poured onto a paper towel. The fungus fragment is picked up with a needle and transferred into a solution of chloral hydrate prepared from 70 g chloral hydrate and 30 ml of distilled water. Here the fragment is rinsed and infiltrated for a few minutes.

Avoid chloral hydrate solutions older than a few months. They will destain the granules and nuclei very rapidly.

(4) Mount in chloral hydrate solution between slide and coverglass, squash, and observe with high resolution optics. For permanents mounts use Hoyer's medium. Here the stain is durable for many years (in my collection preparations more than 10 years old did not fade appreciably), but do not expose them to frost.

For the preparation of Hoyer's medium see Cunningham (*1972*).

Figs. 1–3. Types of siderophilous granulation in maturing basidia, × 50.000. — 1. Macro type, *Asterophora lycoperdoides*. — 2. Gigas type at the very beginning of its growth, the granules still being irregular in shape. They will grow up to 2–3 μm and form hollow spheres. To the right of the three young granules there is a mature granule in very tangential section (arrow). — 3. Micro type, *Lactarius griseus*. Some granules indicated by arrows.

TABLE I

| | GIGAS | MACRO | OLIGO | MICRO | CRYPTO |
|---|---|---|---|---|---|
| Size range, μm*) | 2–3 | 0.2–0.6 | 0.2–0.6 | 0.05–0.18 | 0.05–0.15 |
| Extreme size (rare), μm | 5 | 1.4 | 1 | 0.3–0.4 | unknown |
| Frequency | low | very high | low | very high | extremely low |
| Distribution in the basidium | uniform | uniform | basal | uniform/peripheral | anywhere |
| Visibility, light microscope | striking | very good | very good | from none to good | none or overlooked |
| *Lindtneria* | *trachyspora* | | | | |
| *Tylopilus* | *felleus* | | *felleus* | | |
| *Lyophyllum* (incl. *Tephrocybe*) | | *leucophaeatum*<br>*favrei*<br>*fumosum*<br>*anthracophilum* | *connatum*<br>*ulmarium* | | |
| *Calocybe* | | *gambosa*<br>*ionides* | | | |
| *Asterophora* | | *lycoperdoides* | | | |
| *Rhodophyllus* (*Entoloma*) | | | | *bicolor*<br>*prunuloides*<br>*hirtipes*<br>*incanus*<br>*turci*<br>*viridulus* | |
| *Rhodocybe* | | *suburens*<br>*leucopaxilloides*<br>*Clémençon 77/73* | | *nitellina* | *parilis* |
| *Russula* | | | | *fragilis*<br>*sardonia*<br>*adusta* | |
| *Lactarius* | | | | *maculatus*<br>*griseus* | |
| *Melanoleuca* | | | | *alboflvida* | |
| *Agrocybe* | | | | | *praecox* |

Normal size range of full-grown granula. Since the formation of grana continues after kayryogamy, there are always very small granules present in a mature besides the ones that reached final size.

## ELECTRON MICROSCOPY

In ultrathin sections siderophilous granules can easily be identified. They are dense fibrillar bodies, mostly circular to elliptic, rarely irregular in outline, surrounded by a unit membrane, and of high electron opacity after staining with uranyl acetate and lead citrate. They are formed by the endoplasmic reticulum in connection with the nuclear cycle. The first granules can be seen at the time of karyogamy. Their number and size increase during the

ripening process of the basidium. The nature and significance of this correlation are still unknown (Clémençon, *1968b*).

Vesicles derived from the endoplasmic reticulum (called ERV) occur in every agaric so far studied (over 200 species) and seem to be a common feature of the meiotic and postmeiotic basidium. Siderophilous granules differ from common ERV in their ability to accumulate greater amounts of metal ions, thus standing out with high electron density and with their light microscopic stainability discussed above. Siderophilous granules are a special kind of ERV.

## TYPES OF SIDEROPHILOUS GRANULES

Using the light and electron microscope several types of siderophilous granules may be described, depending on the size, abundance, and distribution in the basidium. It is evident from the observed material and from Table I that the types do not represent isolated classes. They intergrade to some extent, but still proved usefull in my laboratory.

### (A) THE MACRO TYPE

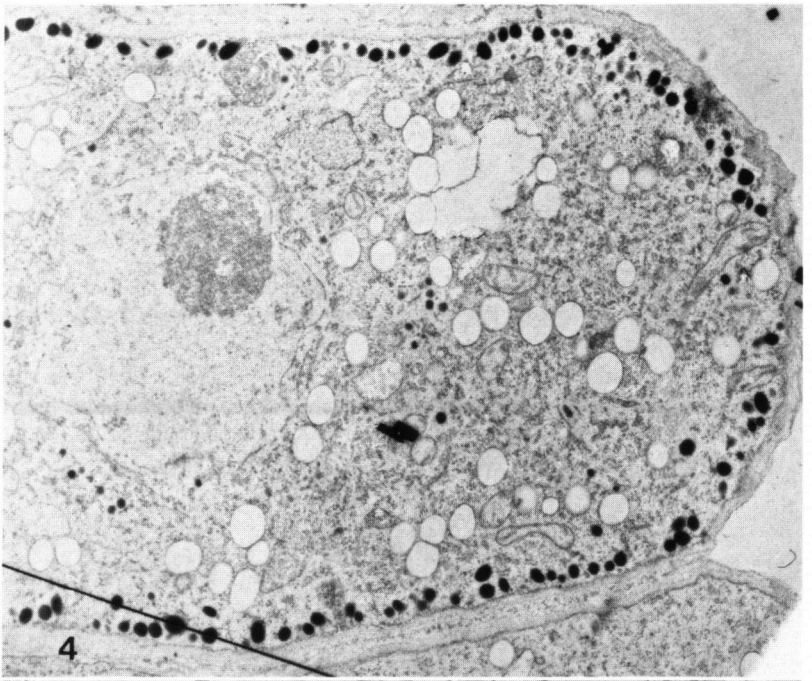
This is the classical siderophilous granulation found in *Lyophyllum*, *Calocybe*, and *Asterophora*. Mature basidia contain very numerous, heavily stained, mostly round granules 0.2–0.6 μm in size, rarely reaching 1.5 μm. The granules are more or less uniformly distributed in the basidium, but mostly leaving a zone of lower density just above the middle of the cell. Because of the high number, size, and good stainability they are easily seen and identified with the light microscope.

This type of granulation occurs outside the Lyophylleae in three closely related species of *Rhodocybe*, actually classified in the Rhodophyllaceae. This lead Clémençon (*1968c*) to transfer two of them into *Lyophyllum* (Tricholomataceae), a proposition that has not been accepted by Singer (*1975*). Since siderophilous granules commonly occur in Rhodophyllaceae, I see no objection in placing the species in question in the genus *Rhodocybe*. These are *Rhodocybe suburens* and *R. leucopaxilloides*. The third species, my collection *77/73* could not be identified yet and represents perhaps a new species close to *Rhodocybe alutacea* Singer.

There is some variation in the final size and form of the siderophilous granules. In most species they stay moderately small and round or even spherical, but in *Lyophyllum* section *Difformia* they grow bigger and become elongate-difform in age. It is quite possible that this behaviour is taxonomically significant.

### (B) THE OLIGO TYPE

It is well known that in basidia of *Lyophyllum ulmarium* there may be none or only very few siderophilous granules, a situation that is frequent in an unrelated species, *L. connatum*. If well developed granules are present, they are not very numerous and are situated in the lower part of the basidium. The granules are of the same size and form as in the macro type, but they develop later and rather unwillingly.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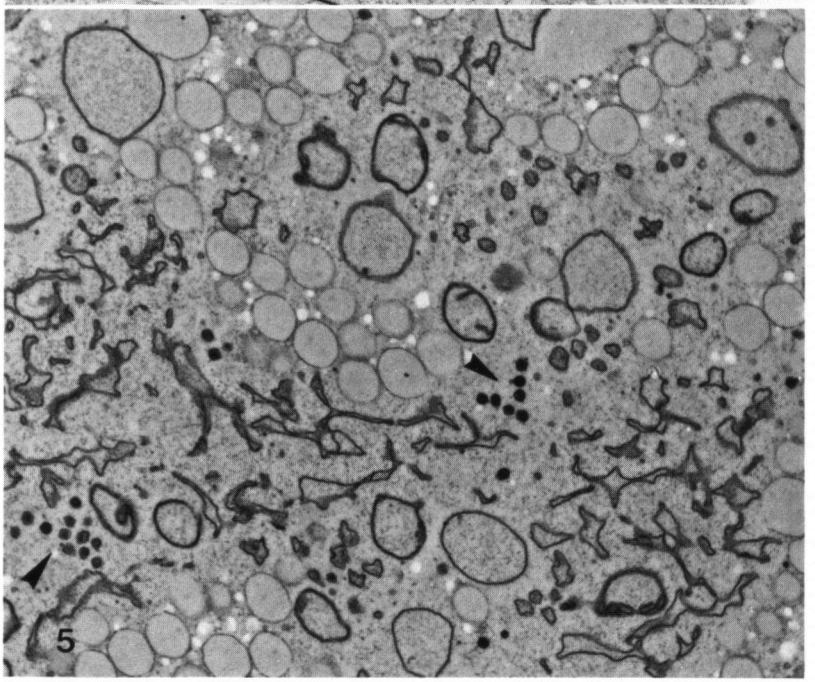
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There is considerable hesitation about the generic distinction of *Lyophyllum ulmarium* and *Hypsizygus tessulatus*, the latter mainly differing in the absence of siderophilous granules. I am not at all convinced that '*Pleurotus ulmarius*' is a *Lyophyllum*; rather would I admit that there is a *Hypsizygus* type of siderophilous granulation. The other species with the oligo type, *L. connatum*, is not a typical member of *Lyophyllum*, and perhaps should be removed, too.

For the time being I réfrain from proposing taxonomical changes waiting for culture work to give better answers.

### (C) The gigas type

In 1975 Clémençon reported siderophilous granules in the basidia and cystidia of American *Tylopilus felleus*. At first the few granules are small and easily overlooked, but in the cystidia and in some basidia they grow very large and become striking features of the cell. Frequently the very big granules are hollow.

A very similar though not hollow granulation occurs in the basidia of quite unrelated a fungus, *Lindtneria trachyspora* (Aphyllophorales). Here they have been described as cyanophilous granules (e.g. Eriksson & Ryvarden, *1976*). They are more or less uniformly distributed in the basidium and measure up to 2–3 μm. It is not unfrequent that the granules become somewhat difform, especially in *Tylopilus felleus*, and then obscure entire parts of the cell.

### (D) The micro type

It is a known fact that many species of *Rhodophyllus* (*Entoloma*) show distinct but small siderophilous granules in some of their very mature basidia (e.g. Moser, *1978*). It was therefore not very surprising to find nice siderophilous granules in almost every mature basidium of that genus so far studied. In comparison with the macro type the granules of the micro type are rather small, the typical size ranging from 0.05 to 0.18 μm. But it is not infrequent to see isolated granules reaching a diameter of 0.3 or even 0.4 μm, thus overlapping somewhat in size with the macro type. Therefore they are sometimes easily seen with the light microscope, but very frequently they are overlooked or go undetectable.

The distribution of the micro type siderophilous granules in the basidium may be uniform in the cell, including the sterigmata and even the young spore, but frequently they take a predominantly peripheral position, as in many species of the section (or subgenus) *Entoloma* of *Rhodophyllus*.

Siderophilous granules of the uniformly distributed micro type have also been detected in *Rhodocybe nitellina*.

Figs. 4, 5. Distribution of micro type siderophilous granulation in mature basidia. — 4. Distribution mainly peripheral. In the center there are small groups of small granules. The peripheral granules are just visible in the light microscope. The two rectangular bodies just below the center are cristals of uranyl acetate used to stain the section. *Rhodophyllus* (*Entoloma*) *bicolor*, × 12,500. — 5. Micro type more or less uniformly distributed in the basidium. *Russula adusta*, × 25,000.

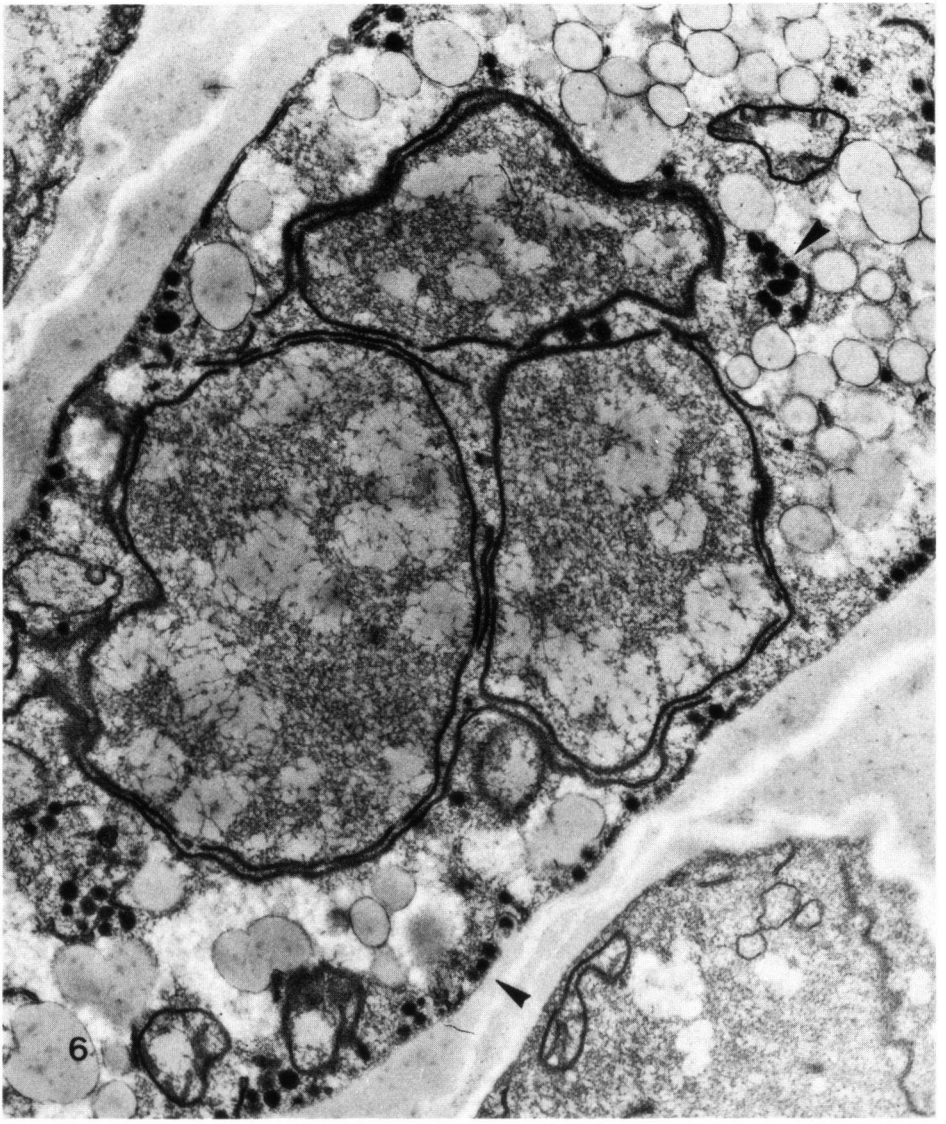
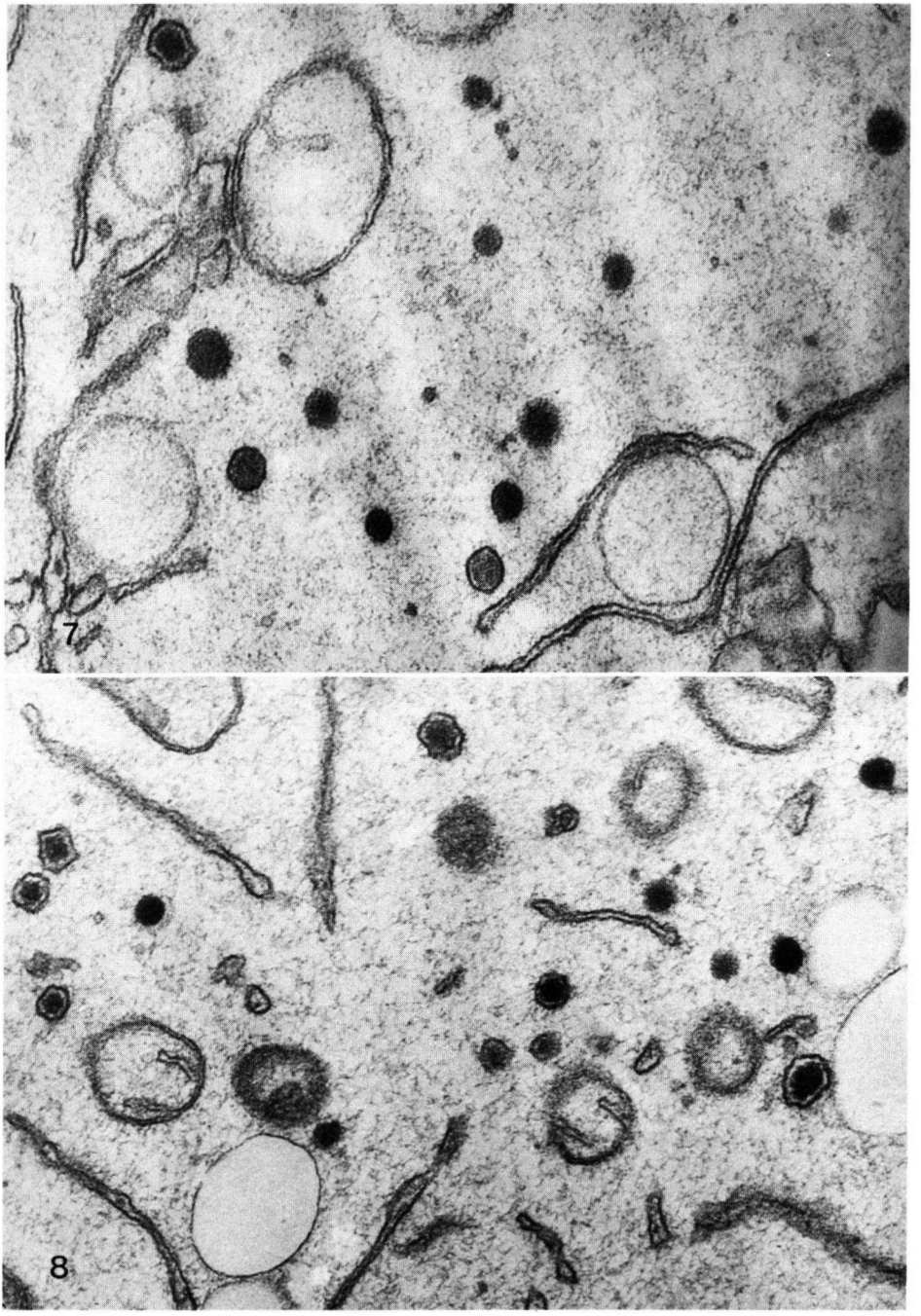



Fig. 6. Micro type siderophilous granulation with peripheral and central distribution in the basidium of *Melanoleuca alboflavida*, ×25,000. Arrows indicate some granules. The three big membrane bound bodies in center are postmeiotic nuclei.

Figs. 7, 8. Micro type granulation with uniform distribution, ×50,000. — 7. *Rhodophyllus incanus*. — 8. *Russula sardonia*.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It came as a big surprise that every species of *Russula* and *Lactarius* so far studied has plenty of uniformly distributed siderophilous granules of the micro type in mature basidia. It seems that in some species the ability to bind metal ions is somewhat attenuated, giving less intense staining in the light microscope and less electron opacity in the electron microscope. Thus some of the *Russula* type siderophilous granules may intergrade with common ERV, and then become totally undetectable with the light microscope. In most species, however, the ERV are quite typical and strongly stained micro siderophilous granules.

It may be mentioned here that in *Melanoleuca alboflavida* from Michigan, U.S.A. the mature basidia contain numerous siderophilous granules of the micro type, arranged peripherally. Unfortunately I do not have data on other species, but my research continues in this direction.

(E) THE CRYPTO TYPE.

When the micro type of siderophilous granules becomes sparse, so that there remain only a few isolated, single granules in a basidium, then they are very hard to discover. Common ERV usually are present in these basidia, differing strikingly from the isolated, dark and small siderophilous granules. So far, this type has been detected in two quite unrelated agarics, *Rhodocybe parilis* and *Agrocybe praecox*, both from Michigan, U.S.A. The main difference between the micro type and the crypto type is the paucity and rare occurrence of the latter in the cell.

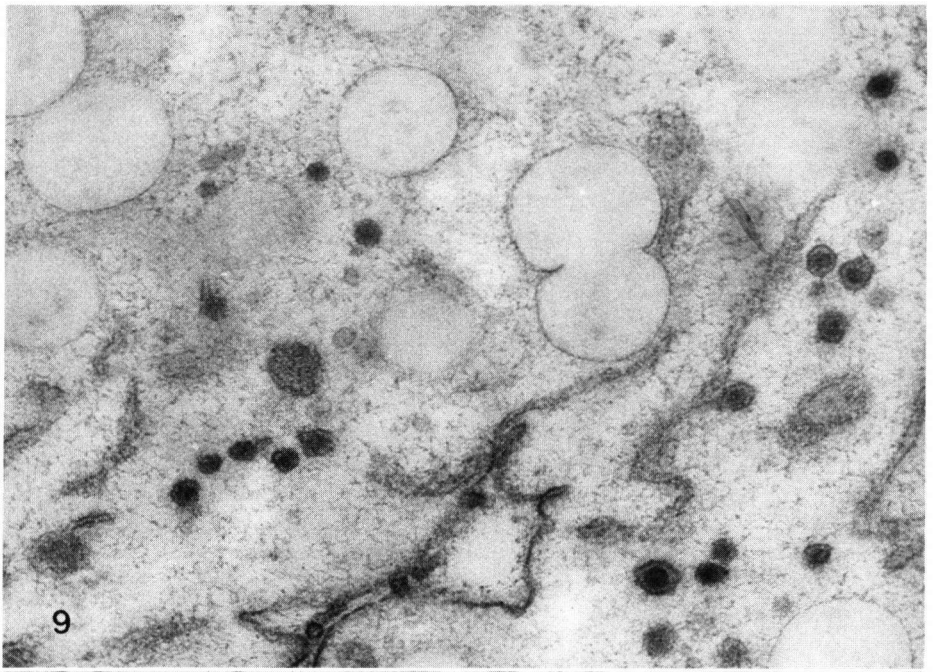
Actually, since this type is easily overlooked, it may be more frequent than our experience suggests.

## General considerations

It is a fact that vesicles derived from the endoplasmic reticulum and filled with fibrillar masses are formed in every meiotic and postmeiotic holobasidium so far studied. In most species these ERV have no special affinity for metal ions and therefore do not exhibit enhanced electron density after treatment with uranyl acetate and lead citrate, nor do they stain with acetocarmine or hematoxylin. In some species, however, they do accumulate large amounts of metal ions and then they stand out with high contrast both in the electron microscope, and in the light microscope. These siderophilous granules range from very small to very large (approx. 0.05–5 μm) and their frequency in a baidium goes from very low (a few granules per basidium) to very high (the basidium seems 'filled' with granules).

If only very few granules are present they may go totally undetected with the light microscope if they stay very small (crypto type), but they may become very conspicuous if they grow very large (gigas type). It is therefore not surprising to observe both types in the same carpophore, as in *Tylopilus felleus*. Here the crypto type is propably merely an early phase

Figs. 9, 10. Micro type siderophilous granulation with uniform distribution, × 50,000. — 9. *Russula fragilis*. — 10. *Russula adusta*.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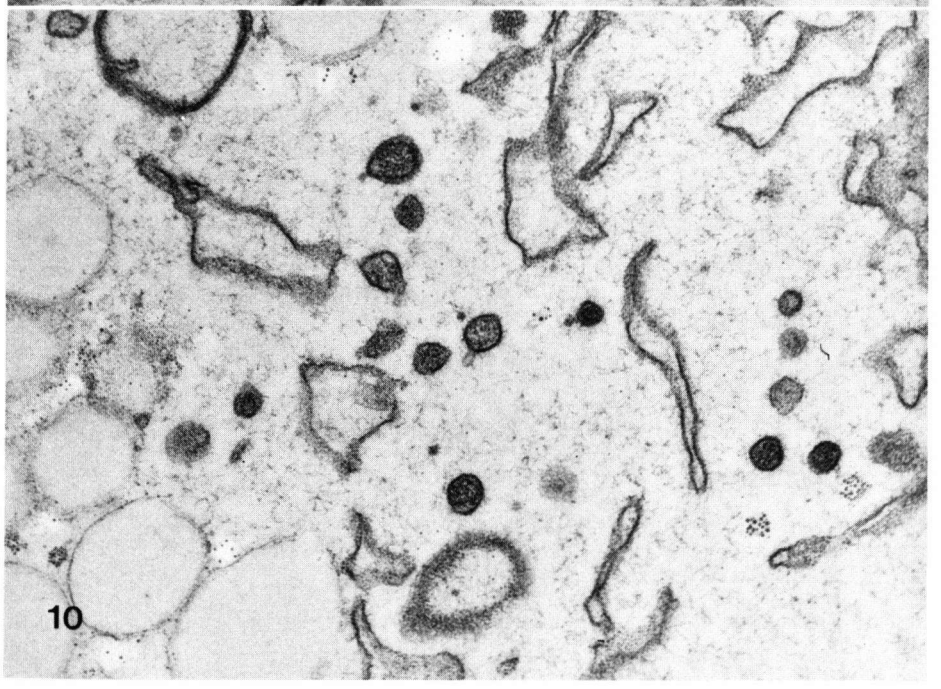
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of the gigas type. In other fungi, e.g. *Agrocybe praecox*, the granules never grow big enough to be seen, and the crypto type stays that way all the time.

For taxonomical mycology the easily observable macro type certainly is of significance. I think that the micro type is of equally high importance for *Rhodophyllus* (*Entoloma*) and for the Russulaceae.

For the moment being, I do not believe that the crypto type, the gigas type and the oligo type have comparable taxonomic weight, although here more observations could give valuable help for taxonomists on the specific level. It is certainly worth while to continue this work.

## Collecting data of the fungi mentioned

*Lindtneria trachyspora* (Bourd. & Galz.) Pilát, *J. Kubička*, Sept. 2, 1976, Switzerland, Bulle FR, Bois de Bouleyres (LAU, Fungarium Clémençon).

*Tylopilus fellus* (Bull. ex Fr.) Karst., *A. H. Smith & H. Clémençon*, U.S.A., Michigan, Aug. 1, 1968 (LAU, 68081 N).

*Lyophyllum leucophaeatum* (Karst.) Karst., *H. Clémençon*, September 1962, Switzerland, Belp BE, Groβe Au. (LAU, Fungarium Clémençon).

*Lyophyllum favrei* Haller & Haller, *H. Clémençon*, September 1962, Switzerland, Belp BE, Groβe Au (LAU, Fungarium Clémençon).

*Lyophyllum fumosum* (Pers. ex Fr.) Kühn. & Romagn., *H. Clémençon*, Aug. 21, 1973, Biel BE, Bözingenberg (LAU, 73/42).

*Lyophyllum anthracophilum* (Lasch) M. Lge & Silvertsen, *H. Clémençon*, Nov. 25, 1967, U.S.A., Missouri (LAU 671125 A).

*Calocybe gambosa* (Fr.) Donk, *H. Clémençon*, May 6, 1973, Switzerland, Bremblens VD (LAU, 73/11).

*Calocybe ionides* (Bull. ex Fr.) Kühner, *H. Clémençon*, September 1962, Switzerland, Belp BE, Groβe Au. (LAU, Fungarium Clémençon).

*Asterophora lycoperdoides* (Bull.) Ditm. ex S. F. Gray, *H. Clémençon*, July 7, 1968, U.S.A., Michigan (LAU, Fungarium Clémençon).

*Rhodophyllus prunuloides* (Fr.) Quél. *H. Clémençon*, Switzerland, Lausanne, Bot. Gard., May 18, 1971 (LAU, 710518).

*Rhodophyllus hirtipes* (Schum. ex Fr.) Quél., *H. Clémençon*, May 9, 1973, Switzerland, Le Mont sur Lausanne, Les Liaises (LAU 73/13).

*Rhodophyllus incanus* (Fr.) Quél., *H. Clémençon*, July 12, 1967, U.S.A., Michigan (LAU, Fungarium Clémençon).

*Rhodophyllus turci* (Bres.) Romagn., *H. Waridel*, April 24, 1975, Switzerland, Lausanne (LAU, 75/2).

*Rhodophyllus viridulus* Herink,. *H. Clémençon*, June 24, 1977, Switzerland, Le Mont sur Lausanne, Les Liaises (LAU 77/43).

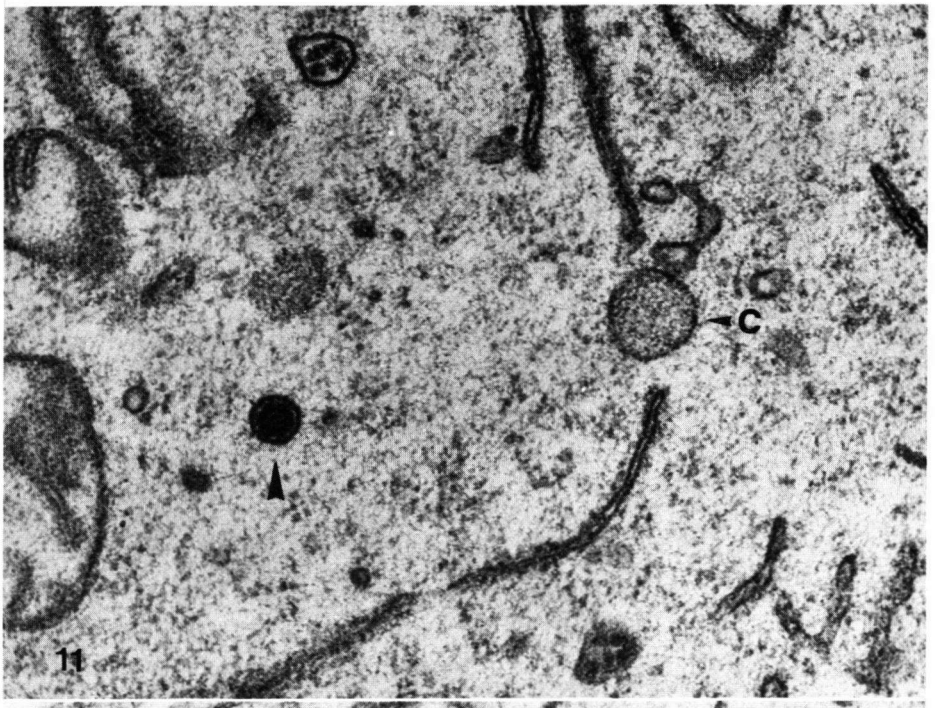
*Rhodophyllus bicolor* (Murr.), *H. Clémençon*, July 4, 1967, U.S.A., Michigan (LAU, 670704 A).

*Rhodocybe suburens* (Clémenç.) Sing. & Clémenç., *H. Clémençon*, July 26, 1967, U.S.A., Michigan (LAU, 670726 O).

*Rhodocybe leucopaxilloides* (Smith & Bigelow) Sing., *H. Clémençon*, July 24, 1967, U.S.A., Michigan (LAU, 670724 C).

*Rhodocybe* spec. Clémenç. 77/73, *F. Marti*, Aug. 18, 1977, France, Klingenthal-Rothbach (LAU).

Figs. 11, 12. Crypto type siderophilous granulation, × 50,000. — 11. *Agrocybe praecox*, c indicates a common ERV. — 12. *Rhodocybe parilis*. — Arrow indicates the isolated siderophilous granule.

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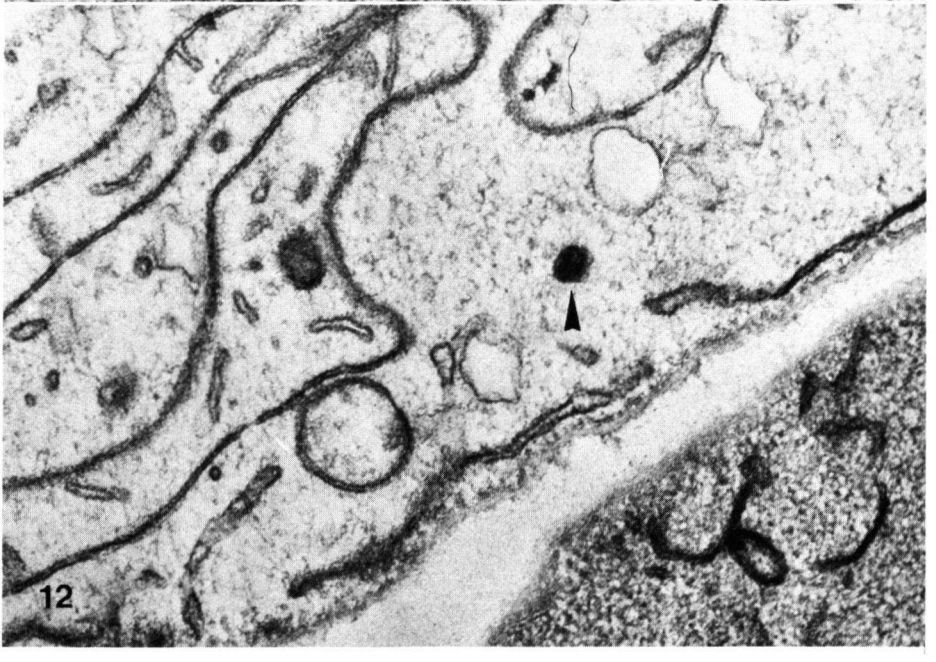

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*Rhodocybe parilis* (Fr.) Sing., *H. Clémençon*, July 19, 1967, U.S.A., Michigan (LAU, 670719 B).
*Russula fragilis* (Pers. ex Fr.) Fr., *H. Clémençon*, July 21, 1967, U.S.A., Michigan (LAU, 670721 A).
*Russula adusta* Fr., *H. Clémençon*, July 21, 1967, U.S.A., Michigan (LAU, 670721 B).
*Russula sardonia* Fr. em. Romagn., *H. Clémençon*, June 30, 1967. U.S.A., Michigan (LAU, 670630 B).
*Lactarius maculatus* Peck, *H. Clémençon*, July 30, 1967, U.S.A., Michigan (LAU, 670730 A).
*Lactarius griseus* Peck, *H. Clémençon*, Aug. 14, 1967, U.S.A., Michigan (LAU, 670814 D).
*Melanoleuca alboflavida* (Peck) Murr., *H. Clémençon*, June 25, 1967, U.S.A., Michigan (LAU, 670625 C).
*Agrocybe praecox* (Bolt. ex Fr.) Sing., *H. Clémençon*, April 19, 1967, U.S.A., Missouri (LAU, 670419 A).

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