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DEVELOPMENTAL ANATOMY OF COPRINUS

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The aim of this study has been to compare the ontogenetic structures of as many species of *Coprinus* as possible in order to obtain a better insight into their mutual relationships. The sequence of development of the parts in the first phases of primordium development has been traced with greater precision here; several degrees of rupthymenial hymenophore development have been distinguished; the veil and pileipellis structures and the corresponding terminology have been critically discussed. Finally, an attempt has been made to establish phylogenetic relationships between about 27 species, but to acheive a higher degree of accuracy in this field, ontogenetic information concerning still more species is required.

INTRODUCTION

The genus *Coprinus* is one of the most intensively studied genera of the higher Basidiomycetes. As early as the time of Brefeld (*1877*) and de Bary, the anatomy of the fruit bodies was well known. Brefeld also laid the basis for a series of investigations about the influence of external factors, such as light, on the fructification and morphogenesis of *Coprinus*. Very well known are the observations of Buller (*1909, 1924, 1931*) concerning the finer anatomy and sporulation of a series of species of *Coprinus*. Since the discovery of incompatibility by Bensaude (*1918*) with *Coprinus fimetarius*, species of this genus have played a very important role in the genetic research of the Basidiomycetes. Moreover, new research about speciation has usually been done on species of *Coprinus*: well known are the crossings of species of the Setulosi group by M. Lange (*1952*), by which it was possible to delimit these species much better. Also, the experiments of Kemp (*1977*) concerning sympatric speciation (homing of oidea, etc.) were done with species of *Coprinus*.

The fact that many species of *Coprinus* can be easily cultivated and also that they fructify in cultures is probably the main reason for the preference shown by research workers for this genus. It was also Brefeld (*1877*) who gave a good account of the development of the fruit bodies of several species, illustrating it with several splendid lithographs. Since then, a rather large number of species has been studied in this respect (Reijnders, *1963*: 190–197).

When we had the opportunity several years ago to collect the primordia of some not previously examined species (mainly in cucumber hothouses, through the kind intervention of my friend, J. Daams), we decided to continue the research in order to be able to compare as many species as possible from all sections and sub-sections. In this way, we have now acquired a general knowledge, as far as the development is concerned, of 27 species which belong to this heteromorphous genus.

Classification within the genus is now based mainly on the properties of the veil and the pileipellis, and they have lead, for example, Kühner & Romagnesi (*1953*) and Singer (*1975*) to discern very nearly the same sections and sub-sections, although there is a slight difference in the order of these groups and in the names. We will investigate the degree to which the characteristics of the development agree with this classification and the degree to which they can improve or supplement it. Fries (e.g. *1874*) made use of the presence or absence of a veil and some of its characteristics to a great degree in his classification. However, it was J. E. Lange (*1938*) who emphasized the importance of the structure of the pileipellis. In the 'Flore analytique', the system that is now commonly used appeared.

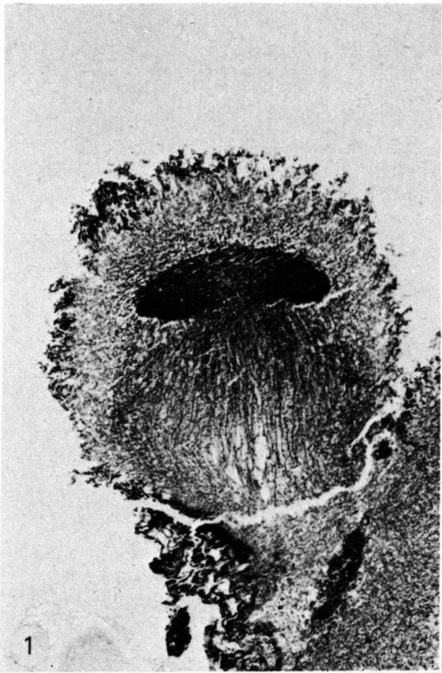
The question of why this big, polymorphous genus has not been split up can be asked. Attempts to do this have probably been made, but there are two main objections: First, the whole group is bound together by one characteristic which is unique in the system of the Agaricales: the deliquescence of the gills (pileus), although this characteristic does not appear in all cases. And second, there are many intermediate forms. It is clear that the structure of the veil and the pileipellis should receive a great deal of attention in this study. In general, we see that the dermium is strongly developed when the veil is reduced, and vice versa. But in reality, the situation is more complicated. There does not yet exist unanimity in the terminology concerning the pileipellis, and this can cause confusion. It will not, however, be difficult to arrive at an adequate nomenclature.

Apart from the development of the velum and the pileipellis, there are two other ontogenetic structures of extreme importance during the primordial condition of Agaricales. The sequence of development of the most important parts of the carpophore (stem, cap, hymenophore) is not the same; we discussed this not-yet-fully-conceived topic of ontogeny of agaric fruit bodies several times (Reijnders *1963*: 235–245; *1979*a). Moreover, it is precisely the case with *Coprinus* that the development of the hymenophore is remarkable, for here a different structure was discovered as compared with other Agricales (Levine, *1914*). This problem became controversial after Atkinson (*1916*) thought that he had to dispute the results of Levine (Reijnders *1948*: 266–267; *1963*: 245). In connection with the deviating initial structure of the gills, attention should be paid to their open edge during a great part of the primordial condition. This edge is turned toward the stem. The problem is now whether the young gills are attached to the tissue below (lipsanenchyma). This comparative research on *Coprinus* can therefore serve to facilitate some taxonomic conclusions and can also produce some precision concerning some ill-defined ontogenetic structures.

We would prefer to postpone a treatment of these questions until the discussion below. We can then draw our conclusions on the basis of what was already known.

As for materials and methods used, there are no novelties. The fixative was Bouin's liquid. The stains were Mayer's haemalum or saffranin – gentian violet.

Figs. 1–5. *Coprinus phaeosporus*. — 1. Isocarpous young stage with wide protenchymatic zone around the base of cap and stem between which the lipsanenchyma is perceptible. The universal veil consists of radiating dichophysoid hyphae × 80. — 2. A tangential section of an intermediate developmental stage with open edge of the primary gills × 80. — 3. The universal veil and the protenchyma under it at an intermediate stage × 200. — 4. The pileus-trama and the veil in the lateral part of a primordium at a more advanced stage × 200. — 5. Tangential section of a young primordium showing three groups of palisade-hyphae × 320.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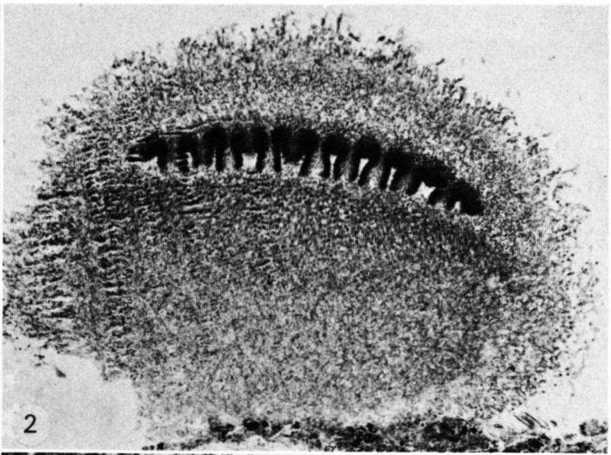
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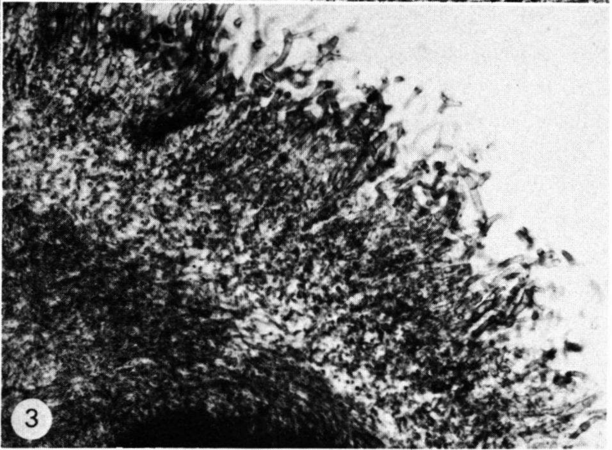
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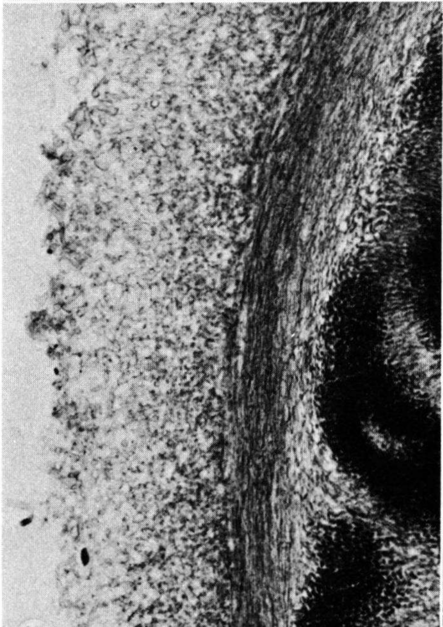
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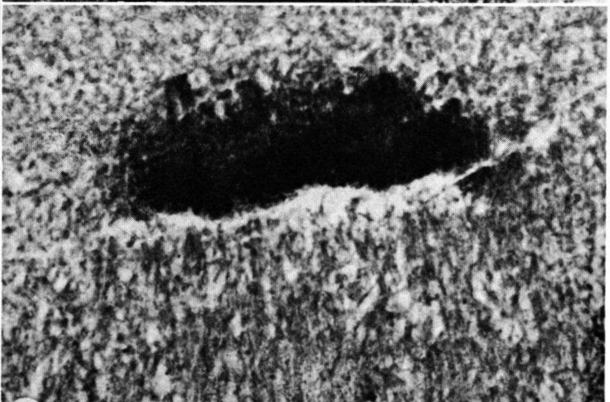
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DESCRIPTIVE PART

COPRINUS PHAEOSPORUS AND COPRINUS BRASSICAE

Dealing with these closely related species together may have some advantages. The ontogenetic structures are slightly different in some respects, but on the whole, they are congruent and deviate somewhat from those of other Coprini, as does the group to which they bellong: the section *Impexi* Romagn. apud Kühn. & Romagn. (= subsection *Alachuani* Sing.)

(1) The youngest stage studied (Fig. 1, height 567 μm, width 529 μm) of *C. phaeosporus* has an isocarpous structure. The longitudinal hyphae of the stem, the chromophilous cap, and the still darker stained, downward-growing hyphae of the hymenophore and pileusmargin show up clearly. Remarkable is a broad zone of protenchyma enveloping these parts; at the outside of this, the universal veil arises through the appearance of radiating hyphae which widen somewhat and have thicker walls toward the periphery, while the small branches at the extremity are already present, giving this veil what Singer (*1975*: 63, 493) calls a dichophysoid structure. The lipsanenchyma between stem-surface and pileus margin is made up of longitudinal, protenchymatic hyphae.

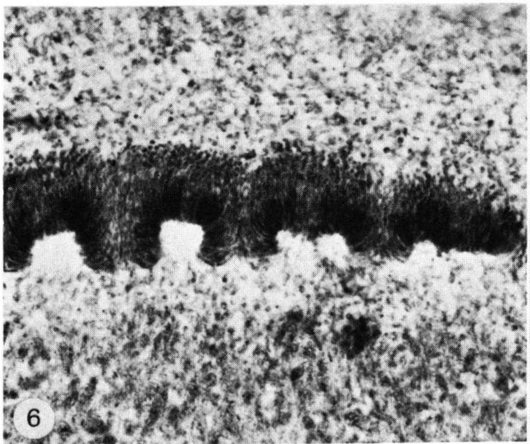
It would be inaccurate to consider the whole protenchymatic peripheral layer as belonging to the universal veil. Certainly, it represents in part a matrix-layer of the veil, the radiating hyphae of the latter originating from this tissue. Though the demarcation between cap and surrounding tissue seems to be quite conspicuous, there is no real segregation of the hyphae at the centre of the cap-surface where the hyphae remain interwoven, and this continues to be the case in later stages. It is quite possible that during the extension of the cap, this tissue is partly absorbed into the rapidly growing hyphae of the cap-trama. We have met with this not very well-defined position of cap and veil in several other species (e.g. *Coprinus*), and Atkinson (*1914*) observed this for *Lepiota clypeolaria*.

Coprinus brassicae seems to be more strictly pileocarpous. The youngest primordium we can represent (Fig. 8, height 378 μm, width 592 μm) shows a deeply stained area in the centre, surrounded by a wide zone of protenchyma. Here the veil is far less differentiated than in the preceding species, as it is in mature specimens. Beneath the chromophilous dome, there is a zone where the tissue also has a dark colour, and this may be the rudiment of the stem (cf., e.g. very young stages of *Amanita*, etc.). But the longitudinal direction of the hyphae in this zone is hardly noticeable, and no inflation has as yet taken place.

It is possible that in still younger primordia, the pileus also arises first in *C. phaeoporus*, but in

Figs. 6–7. *Coprinus phaeosporus*. — 6. Tangential section of an intermediate stage showing the young gills × 320. — 7. Tangential section of a somewhat more advanced stage showing the open gill-edges and hyphae which pass into the lipsanenchyma × 200.

Figs. 8–11. *Coprinus brassicae*. — 8. Median section of a young stage showing the pileocarpous origin × 125. — 9. Intermediate stage with wide protenchymatic zone around the primordium (universal veil) × 63. — 10. Pileus-margin and lipsanenchyma at an advanced stage × 125. — 11. Upper portion of the pileus-trama with hyphae merging into the universal veil and peripheral zone of the latter with somewhat altered hyphae × 125.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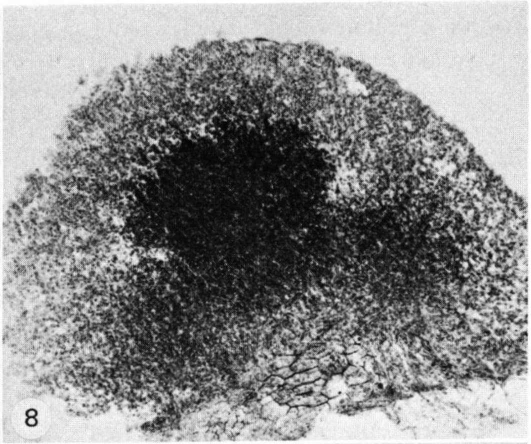
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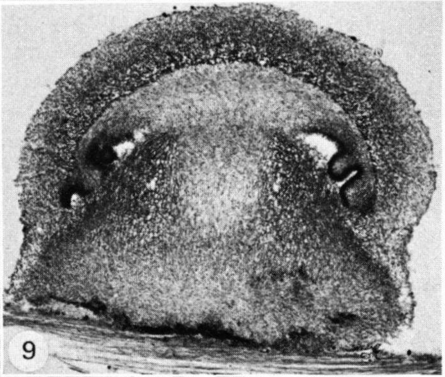
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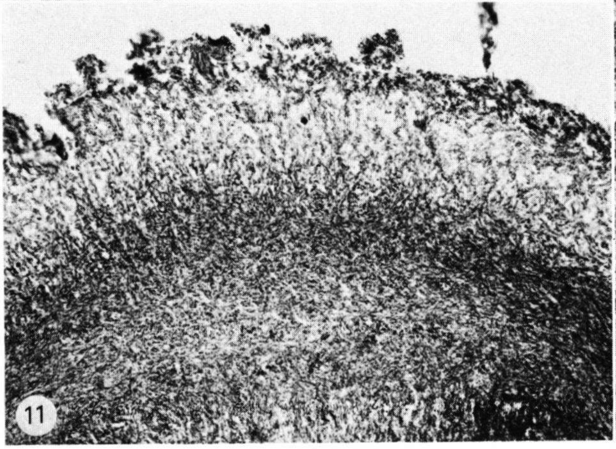
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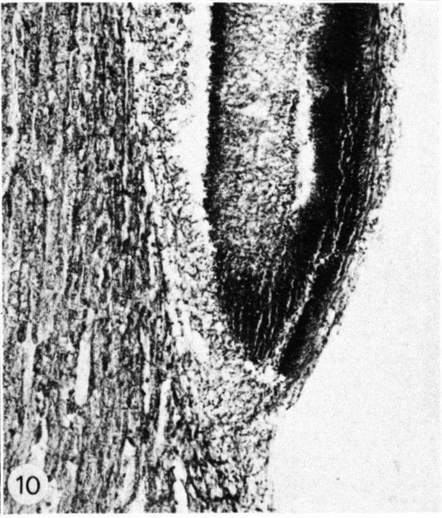
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the section of the minute primordium of Fig. 1, the stipe is already so well-marked that an isocarpous or even a pileo-stipitocarpous mode may be present as well.

(2) In both cases, the shape of older primordia is striking and somewhat characteristic, the primordia being very wide and less high. To demonstrate this, we inserted Fig. 9, which represents an approximately median section of a stage of *C. brassicae* with a height of 807 μm and a width of 1 mm, and Fig. 2, a tangential section of *C. phaeosporus* (height 630 μm, width 857 μm; cf., here the open edge of the lamellae and the continuous palisade-layer over the lamellulae).

A section of a very advanced stage, representing the pileus-margin and what is for Coprini a rather abundant lipsanenchyma along the elongated stem is given in Fig. 10 (width of the glandiform cap 1.4 mm) for *C. brassicae.*

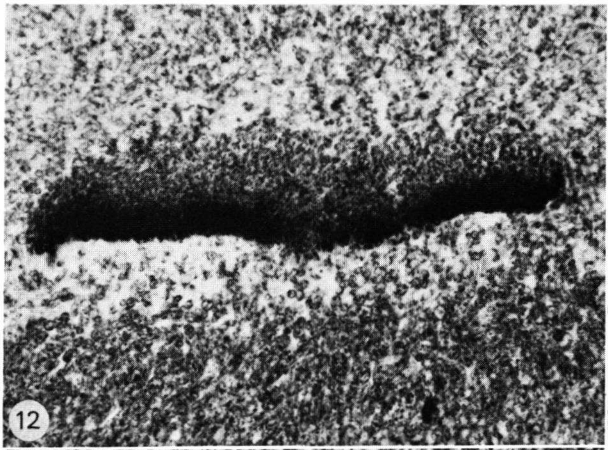
During the course of the entire development, the layer of interwoven protenchyma between the pileus-trama and the veil (new branches of the latter continue to appear between the older ones) merged into the pileus-trama, at least in the centre of the cap. This phenomenon is obvious in Fig. 3, a photomicrograph of a somewhat older stage of *C. phaeosporus* (width 1 mm) and in Fig. 11 for *C. brassicae* (width of cap about 1.5 mm). This connection is of course less evident at the side of the cap in older specimens, where the hyphae of the pileus-trama are parallel and directed downward for the purpose of cap-growth. A pileïpellis is missing in these two species as it is in *C. macrocephalus* and *C. macrorhizus*, but in contrast to these species, there is no meristemoïd at the contour of the pileus to produce the radiating hyphae of the veil (the latter arises from the layer of protenchyma at the outside of the cap. Fig. 4, width of the unexpanded cap of *C. phaeosporus* about 1.8 mm). The nature of the universal veil in *C. phaeosporus* has been described above. In more advanced stages there are no important transformations in this respect. The hyphae of the veil are initially radiating, slightly widening toward the tip, metachromatic with rather thick walls, and provided with short, spine-like branches (dichophysoïd structure). In older primordia, these peripheral hyphae are more interwoven, resulting in a rather coherent cover.

The veil in *C. brassicae* has a much more simple structure. The peripheral hyphae are still interwoven, and the protenchyma underneath is slightly altered and somewhat more inflated and metachromatic.

(3) Here again we find a similar development of the hymenophore in these two species. Initially, the layer of palisade-hyphae may be interrupted here and there (Fig. 5, width of the primordium 491 μm, *C. phaeosporus*; Fig. 12, width 882 μm, *C. brassicae*), and no regular arched structure can be observed or arches alternating with sterile bands. Very soon after this initial stage, however, the rupthymenial structure becomes apparent through the activity of the downward-growing hyphae of the gill-trama in horizontal stripes radiating outward from the centre toward the periphery (Fig. 6, width 724 μm, *C. phaeosporus*). From the beginning, the edge

Figs. 12–13. *Coprinus brassicae.* — 12. Tangential section of a young stage showing the uninterupted layer of palisade-hyphae of the hymenophore × 320. — 13. Tangential section of an intermediate stage showing the open gill-edges × 200.

Figs. 14–16. *Coprinus patouillardii.* — 14. Nearly median section of young primordium × 200. — 15. Median section of an intermediate stage with universal veil consisting of an inner layer of protenchyma and serried sperocysts × 125. — 16. Upper portion of the cap of a more advanced stage. Nearly the whole weil consists of spherocysts × 80.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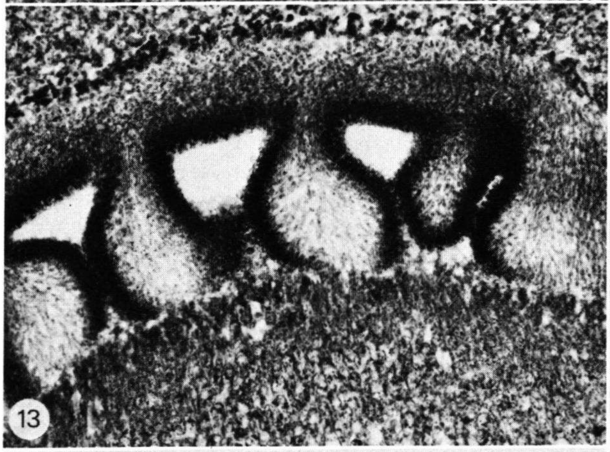
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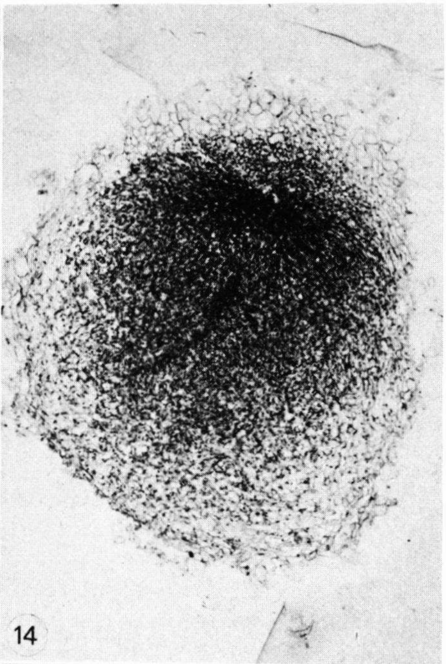
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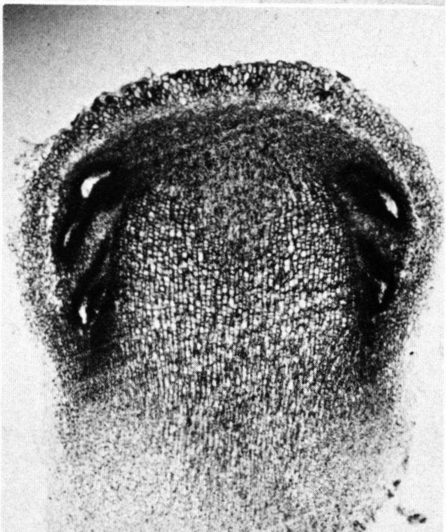
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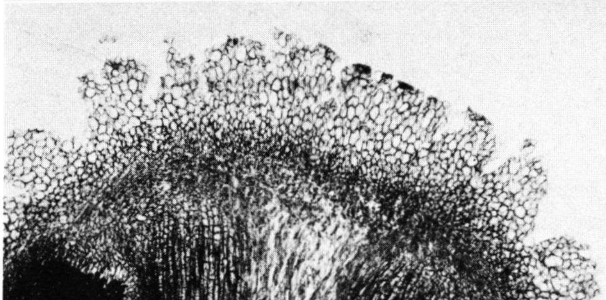
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of the gill is open and there is, especially in these two species, a larger number of hyphae passing from the gill-trama into the underlying lipsanenchyma (Fig. 7, width of the primordium at the level of the hymenophore 945 μm, *C. phaeosporus*. Note the loose texture of the divergent gill-trama). The hyphae at the demarcation between lamellae and lipsanenchyma are not in any way crowded together; on the contrary, the hyphae appear to be drawn out in that region. (Cf., Fig. 13, *Coprinus brassicae*, and 'Development of the hymenophore').

COPRINUS PATOUILLARDII

(1) The youngest stage (Fig. 14, largest diameter 277 μm) shows the structure typical of the initial phase in *Coprinus*. In the centre, the thin protenchymatic hyphae, which extend mainly in a longitudinal direction, and over this part a darker staining zone with miniscule cells, can be observed where this orientation of the hyphae is less evident. Immediately over this darker spot is the universal veil, consisting of cells with very thin walls, of which the diameter increases toward the periphery. The cells of the basal plectenchyma are already strongly inflated, and their walls are equally thin. It is clear that the dark-staining part of the section represents the rudiment of the cap, and soon thereafter the hymenophore appears in the usual manner (cf., e.g. *C. macrophizus*: Fig. 33).

(2) A median stage is represented by Fig. 15. As there are no deviations here from the ordinary mode of development in *Coprinus*, we shall pay attention to the universal veil only, and to its relationship to the pileus-trama. The entire universal veil has a cellular structure. At the outside of the stem and the pileus-margin, the ascending hyphae are divided into short cells, acquiring the character of the veil to an increasing degree toward the exterior. Over the cap, there are rows of oblong cells (or spherocysts, somehow reminiscent of the radiating hyphae in *C. microrhizus*, etc.; width of the veil *c.* 64 μm). Immediately over the cap, the hyphae are divided into short cells. There is no sharp boundary between veil and pileus trama, and this is also the case in older stages. Figure 16 represents the top of a young carpophore (the pileus is not yet spread out). We observe the cells of the veil and beneath the repent hyphae of the thin pileus-trama (Fig. 17).

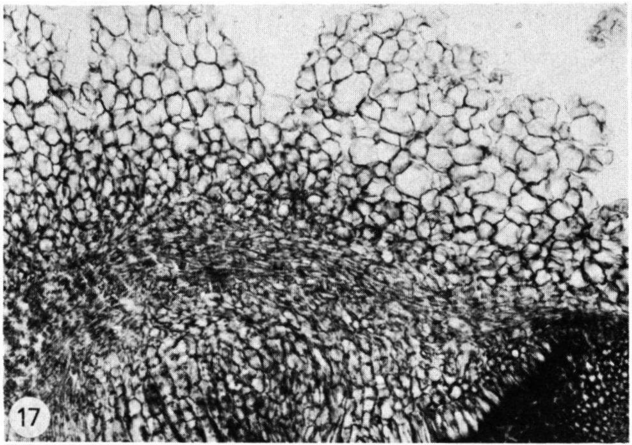
(3) The development of the hymenophore is rupthymenial. At first, there are isolated arched groups of palisade hyphae. The lipsanenchyma is scanty and not subject to further development (Fig. 18).

COPRINUS POLIOMALLUS

This species and the preceding one belong to the same section, called by Singer (*1975*) *Cycloidei* Fr. and by Kühner & Romagnesi, *Vestiti* (Lange) Kühn. & Romagn. It is to be

Figs. 17–18. *Coprinus patouillardii.* — 17. Detail of the section shown in Fig. 16 demonstrating the narrow pileus-trama of repent hyphae between the spherocysts of the veil and the pseudoparenchyma of the stipe × 200. — 18. Tangential section of a young stage showing the tendency towards the rupthymenial mode of hymenophore formation × 320.

Figs. 19–22. *Coprinus poliomallus.* — 19. Very young stage: the pileo-stipitocarpous primordium and the veil × 160. — 20. Young isocarpous primordium × 160. — 21. Intermediate stage × 100. — 22. Detail of the the preceding section showing the veil-meristemoid and the rows of spherocysts formed by it × 320.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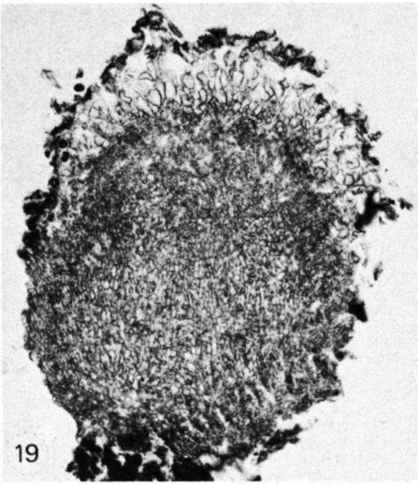
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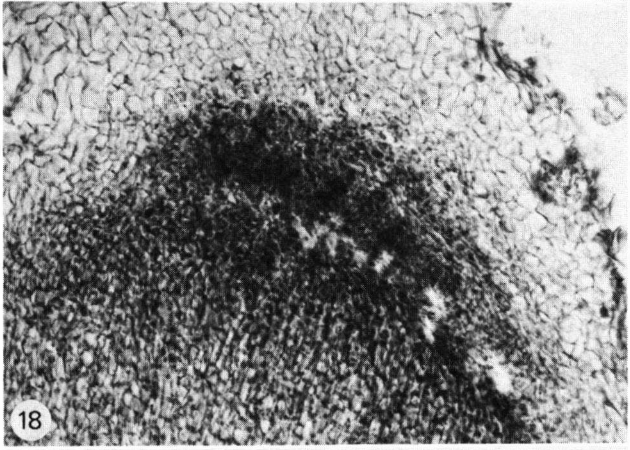
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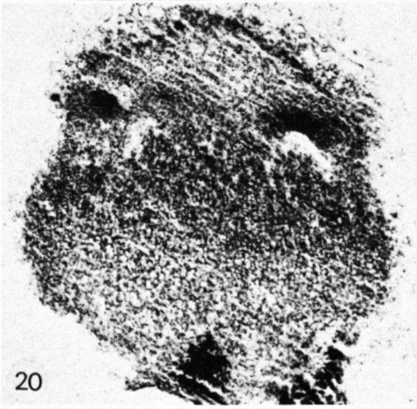
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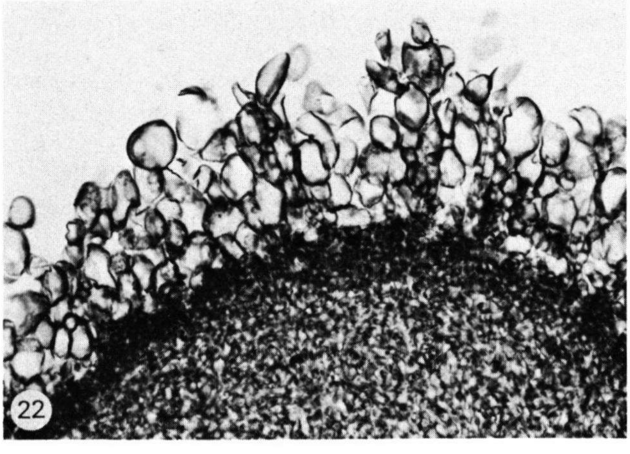
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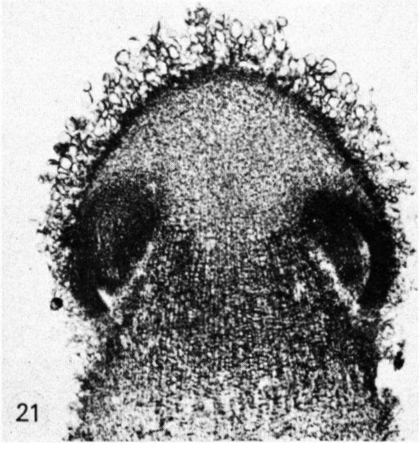
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expected that their development will not differ a great deal, the most striking difference being the fact that the veil in older stages is not completely concrete with the pileus, but that a rather inconspicuous dermium is formed instead. Furthermore, the veil seems to be specialised in this species.

(1) Three successive young stages reveal the same disposition of the tissues as in the preceding species. At first, there is a central core of longitudinal hyphae, but somewhat beneath the upper end of the primordium there is a region where the hyphae are somewhat more intricated and consist of short cells (Fig. 19). The universal veil is present in the youngest stages and is made up of chains of rounded cells, radiating outward and widening. Soon the palisade-hyphae of the hymenophore, growing downward, appear, and the three fundamental parts of the carpophore are present (Fig. 20 at the level of the hymenophore 265 μm). At this point, no more important changes of this disposition occur, as is shown in Fig. 21 (diameter 536 μm).

(2) For a considerable time during development, the pileus-trama and the veil pass into each other without perceptible demarcation. At the base of the veil there is a kind of meristemoid where cell division is frequent. The rows of spherocysts or oblong cells are here more individualised than in the preceding species, and the cells themselves are also more specialised, their walls being rather thick and often brown in colour (Fig. 22, diameter 536 μm). Finally, in a still more advanced stage, the veil is separated from the pileus-trama by a single layer of isodiametric cells, and the outer layer of the pileus-trama becomes pseudoparenchymatic, so the chains of spherocysts are pinched off and become loose flocks on the pileus-surface (Fig. 23).

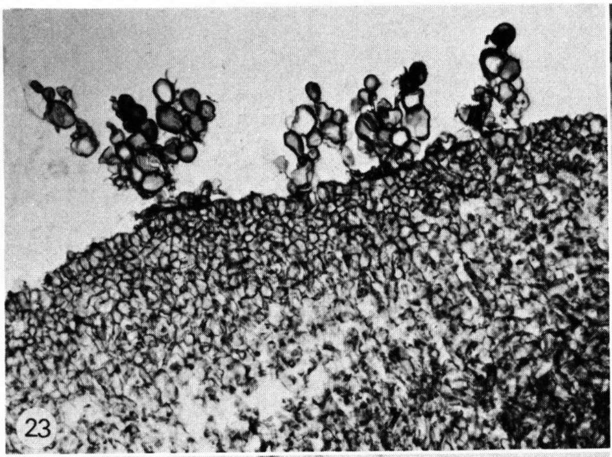
(3) The development of the hymenophore is of the rupthymenial type. There is evidence that the arches of the palisade-hyphae are isolated from the beginning. Through the activity of the downward-growing hyphae of the gill-trama folds, there are soon salients developing in a radial direction, but there remain some original protenchymatic hyphae passing from the lipsanenchyma into these folds (Fig. 24 and Fig. 25). These hyphae are nearly always divided into short cells. It is quite probable that in this species hyphae from the gill trama also grow afterwards into the lipsanenchyma. So the connections between gills and lipsanenchyma last for some time (Fig. 26).

COPRINUS FLOCCULOSUS

(1) The youngest primordium of *Coprinus flocculosis* (Fig. 27, diameter of the widest part 410 μm) consists of a centre of longitudinal hyphae surrounded by large cells of the universal veil (diameter up to 13 μm). On the left-hand side, the veil has been torn off. The veil is particularly

Figs. 23–26. *Coprinus poliomallus.* — 23. Rests of the veil and epithelium with individualized outer layer at a more advanced stage × 160. — 24. Young gill with open edge and hymemial palisades in a rather young primordium × 320. — 25. Detail of the preceding section showing the nature of the connection of the gill-trama and the lipsanenchyma, the latter being composed of short cells × 800. — 26. The open gill-edge of the primary lamellae and the edge of the secundary lamellae surrounded by hymenial elements, the lipsanenchyma over the stem surface at a more advanced stage × 320.

Figs. 27–28. *Coprinus flocculosus.* — 27. Youngest stage with longitudinal rather uninflated hyphae in the centre, and a meristemoid in the upper portion surrounded by the veil × 125. — 28. Detail of the preceding section, showing the nature of the veil × 320.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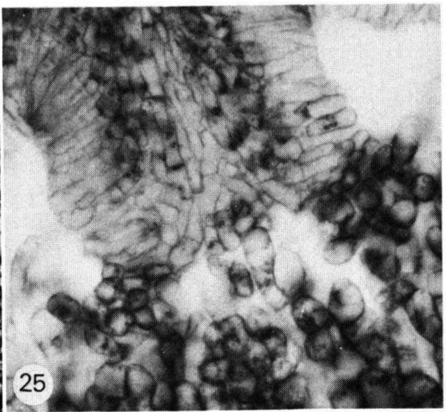
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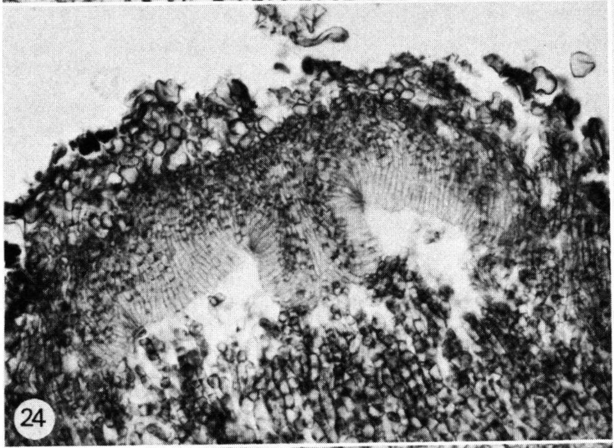
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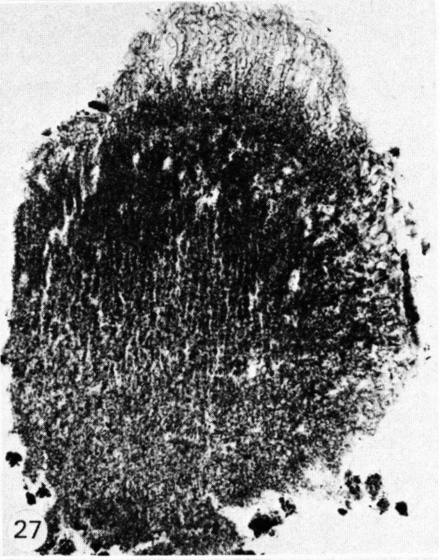
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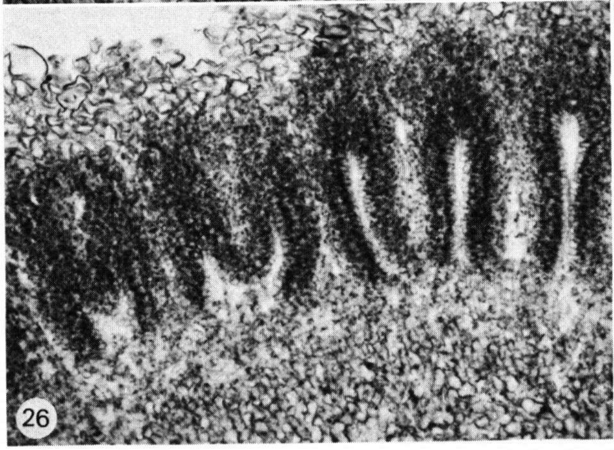
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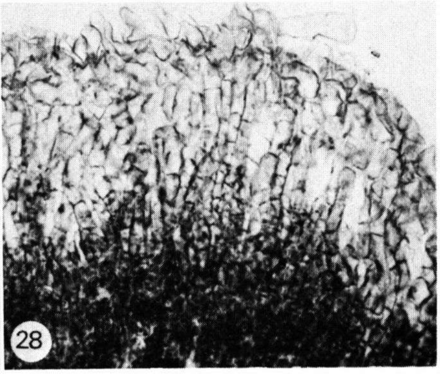
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well-developed at the tip (width *c.* 130 µm). Here we find gradually widening hyphae, divided into short cells (but no spherocysts) with a diameter up to *c.* 13 µm (Fig. 28, detail of the same section under higher magnification). Between the stem part and the ascending hyphae of the veil, a zone of narrow protenchymatic hyphae made up of isodiametric small cells is to be found. One can observe a longitudinal direction in these hyphae too, but the mass of small cells rather represents a meristemoid which gives rise to the pileus-trama and the undermost layer of the veil, which consists of short cells. Beneath the parallel hyphae of the stem is a basal plectenchyma with already inflated cells (diameter up to 6.5 µm).

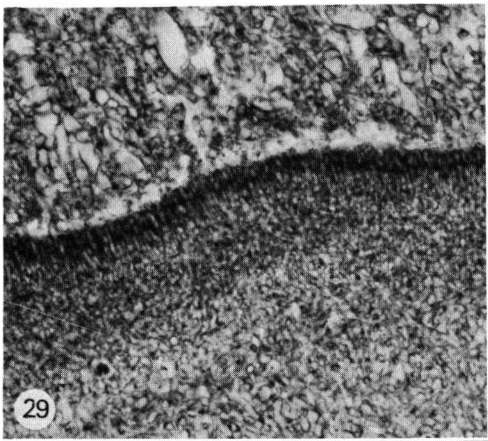
(2) The next stages are not described in detail here as they show a normal coprinoid development. Soon after the initial structure observable in stage 1, the palisade-hyphae of the hymenophore manifest themselves, giving rise to an isocarpous primordium, in which all parts develop proportionally. The extension of the veil is notable in older stages: width over the tip of the cap is 479 µm in a primordium with a diameter of 3.5 mm; width over the stem is only 126 µm. Though immediately over the pileus-surface the hyphae are still radiating, they are more interwoven toward the periphery (diameter of the largest cells about 16 µm).

We will describe here only the development of the pileipellis, which in this case is a true palisadodermium. When the primordium has reached a diameter of about 0.9–1 mm, we see between the narrow lower end of the hyphae which pass into the veil, peg-like cells—at first only a few but thereafter rapidly increasing in number. In a young carpophore of 3.5 mm width, the palisade-layer is well-established (Fig. 29), with transversal cross-walls in the cells which give rise to the hymenium-like dermium. As these cells multiply and enlarge (length up to 20 µm, diameter *c.* 3 µm), the hyphae of the universal veil are pinched off, and the veil is separated from the pileustrama and can be found on mature specimens as loose flocks.

(3) The development of the hymenophore is decidedly rupthymenuous, as Fig. 30 shows. The primordium to which the tangential section of this photomicrograph belongs has a diameter (at the level of the hymenophore) of 718 µm. The radiating rows of palisade-hyphae, cut transversally, alternating with sterile bands with hyphae passing into the lipsanenchyma which descends from the pileus-trama, are conspicuous. This structure is present from the beginning throughout the entire layer of palisade-hyphae. Nevertheless, there may be a peripheral portion where the bands of protenchymatic hyphae are less striking; this is the youngest part of the palisade-layer, at the exterior of which new elements are deposited during the primordial growth (Fig. 31, diameter 441 µm). As in most other species of *Coprinus*, the edge of the lamellae is open in older primordia, and there is no indication of the lamellae pressing against the stem, and some hyphae run across interspaces.

Figs. 29–31. *Coprinus flocculosus.* — 29. The palisadodermium at an intermediate stage × 225. — 30. Rupthymenial orgin of the hymenophore × 200. — 31. Peripheral palisade-hyphae in a somewhat later stage × 415.

Figs. 32–34. *Coprinus macrorhizus* — 32. Youngest stage. As in *C. flocculosus* × 160. — 33. A somewhat older stage. The initially at most pileostipiticarpous development has changed into an isocarpous one × 200. — 34. Intermediate stage with profusely developed veil and meristemoid under it × 100.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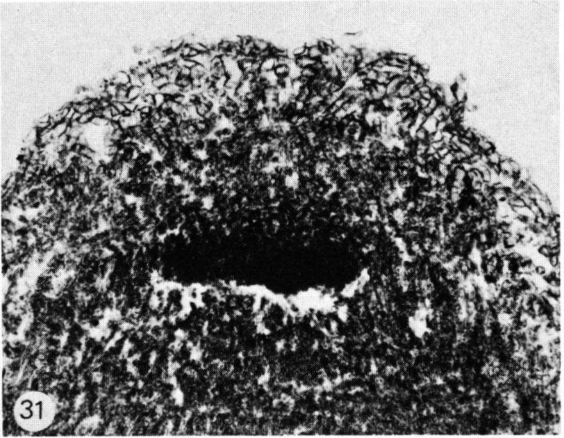
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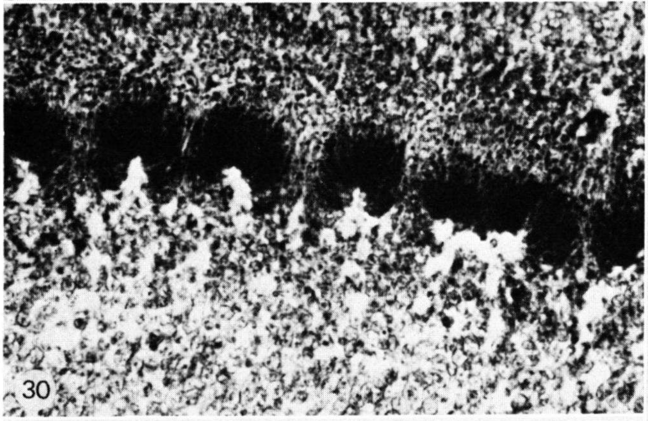
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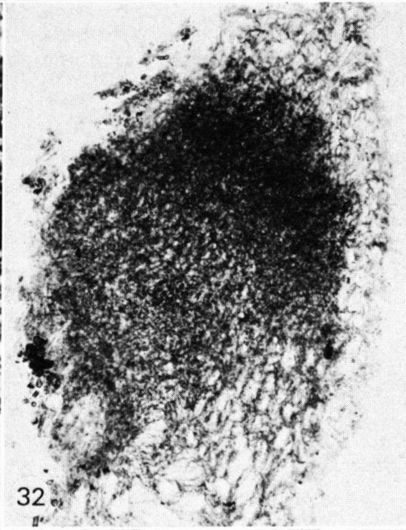
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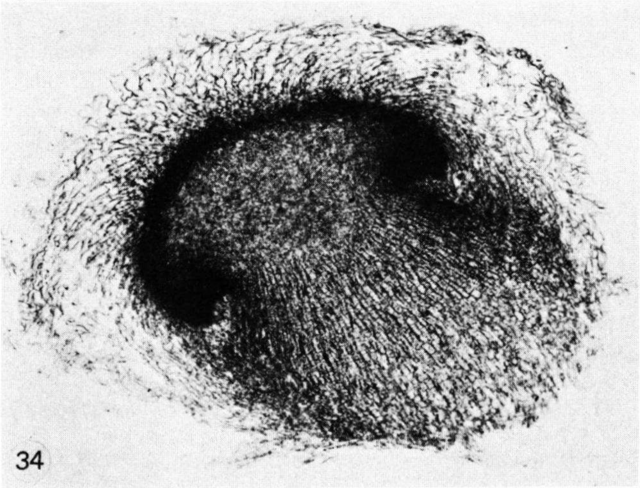
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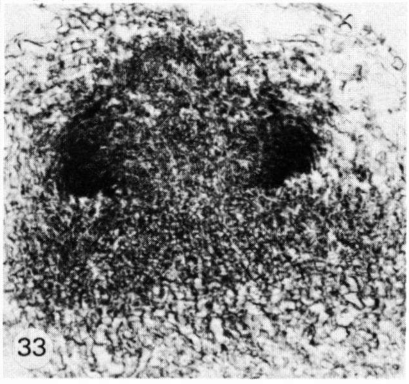
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COPRINUS MACRORHIZUS AND COPRINUS MACROCEPHALUS

It is appropriate to treat the closely related *Coprinus macrorhizus (Pers. ex Fr.)* Rea and *C. macrocephalus* Berk.—of which the latter is far less well known—together. The differences between our specimens, collected in hothouses at Kortenhoef, corresponded to those mentioned by Orton in his key (*1957*: 270), with the exception of the fact that the spores of our *C. macrocephalus* were narrower, the longest ones having a somewhat cylindrical shape and therefore probably belonged to another race. The other characteristics of the two species were in agreement with those described and depicted by J. Lange (*1939*: 110). Measurements of our spores were as follows: *C. macrorhizus* 9.5–11.2(–12.8) × 6.5–7(–8) μm. Orton's were 10–11.5 × 6–7 μm. For *C. macrocephalus*, we found: (9.5–)11–14.5 × 6.5–7.5 μm. Orton's were 11–14 (–17) × 7–9 μm.

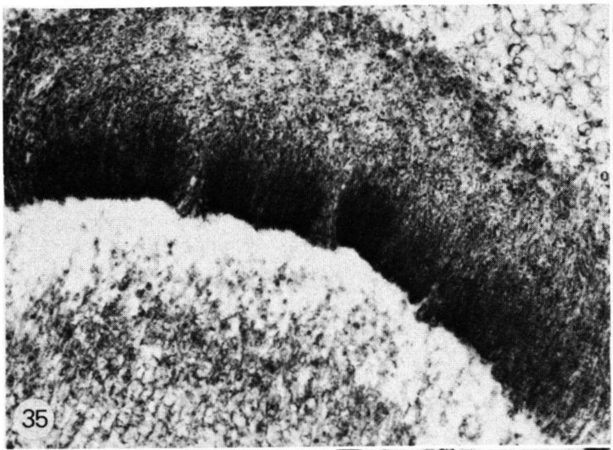
There is a high degree of conformity between the ontogenetic structures of the two species. A similar form has been described earlier, which we called *C. fimetarius* (Reijnders, *1952*: 73, cited in my book under the erroneous name *C. radiatus*).

(1) The youngest stages of these species have the same construction (length of *C. macrorhizus* 504 μm, width 288 μm; length of *C. macrocephalus* 182 μm, width, including veil, 315 μm; Figs. 32, 36). The center consists of longitudinal, somewhat inflated hyphae (diameter up to 5 μm), and over it is a zone of dark staining hyphae which are divided into short cells, the longitudinal direction of which is less evident. Towards the tip, longitudinal hyphae can again be observed, widening gradually into inflated cells (up to a diameter of about 10 μm), with a reduced protoplasmic content. These are the hyphae of the universal veil, which is concrete with the inner portion of the primordium. The veil also covers the sides of the primordium where it is narrower and consists of ascending hyphae with large cells which are formed at an early stage. The radiating hyphae of the veil at the upper part of the primordium are particularly conspicuous in *C. macrocephalus*. In both cases, beneath the dark staining centre, there is a well-developed basal plectenchyma with inflated cells (up to about 8 μm).

(2) Soon after this stage, the inner aspect of the primordium is changed considerably by the appearance of an annular bundle of chromatic hyphae in the upper part, growing outward and downward. This represents the origin of the pileus margin and the hymenophore. No annular gill-cavity precedes this formation. Beneath it, we find the hyphae of the lipsanenchyma in sections of *C. macrorhizus* (Fig. 33). In *C. macrocephalus* the hyphae of this annular bundle project through the longitudinal hyphae of the lower part of the primordium and a lipsanenchyma is still lacking. The sections in *C. macrorhizus* have a height and a diameter of 315 μm; the height is 454 μm and the diameter 441 μm in *C. macrocephalus*. In the sections of the latter species, the hyphae of this bundle are convergent in the beginning (see, e.g. Reijnders, *1971*: 306, pl. 1 fig. 2b). With the

Fig. 35. *Coprinus macrorhizus*. Tendency towards a rupthymenial origin of the hymenophore. The layer of palisade-hyphae is locally interrupted × 320.

Figs. 36–39. *Coprinus macrocephalus*. — 36. Youngest stage with remarkable development of the universal veil × 200. — 37. Section, showing a lateral portion of the cap grown together with the veil meristemoid × 200. — 38. The universal veil forming a coif over the pileus in a more advanced stage × 80. — 39. Tangential section of a rather young stage demonstrating the tendency of the palisade hyphae to become arranged in groups × 200.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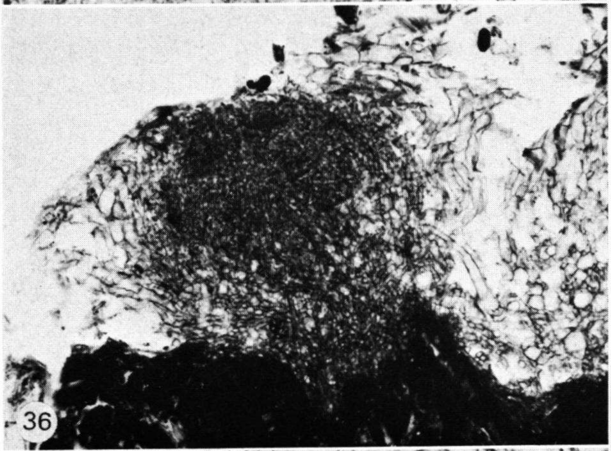
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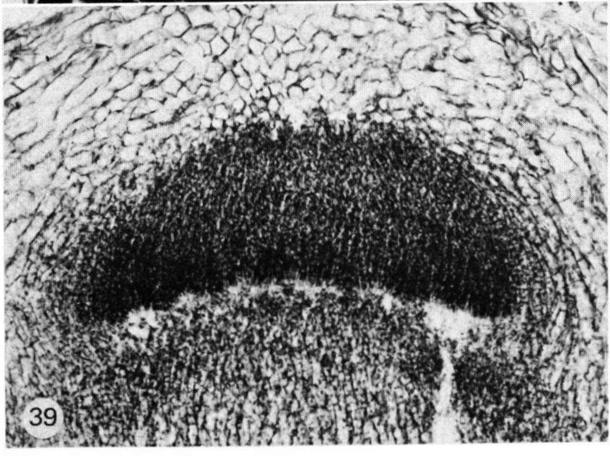
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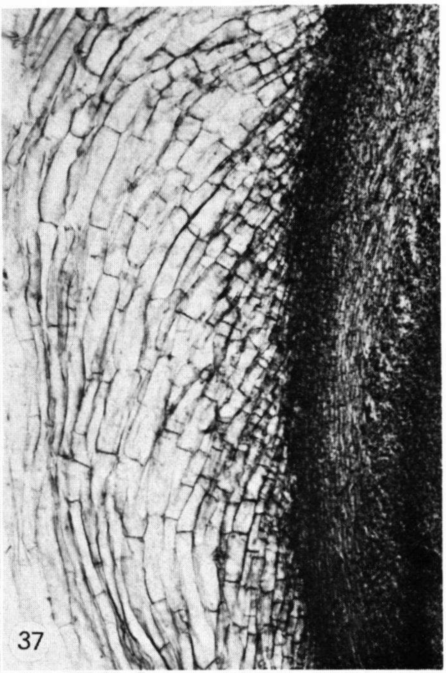
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appearance of this structure, the primordium shows three clear rudiments: that of the stem, that of the pileus (the tissue of the pileus-trama is still very dense but the hyphae are more or less interwoven) and that of the hymenophore. The stem is still low, its longitudinal hyphae are somewhat inflated, and the demarcation between stem and pileus-trama is abrupt.

(3) In the course of development, a few more small changes take place in the proportions of these parts. The veil increases, principally over the cap, where the radiating hyphae are abundant. Their oblong cells remain coherent and widen towards the periphery. The veil is always concrete with the cap. Between the trama of the cap and the differentiated cells of the veil is a zone with a strong cell-division—a tissue we called meristemoid (Reijnders, *1977*). Only one older stage of *C. macrorhizus* was depicted (Fig. 34, diameter 756 μm), which shows the disposition of the growing tissues clearly. The lipsanenchyma is scanty and consists merely of hyphae which are directed obliquely upward: these are the outermost hyphae of the young primordium which are not incorporated into the stem.

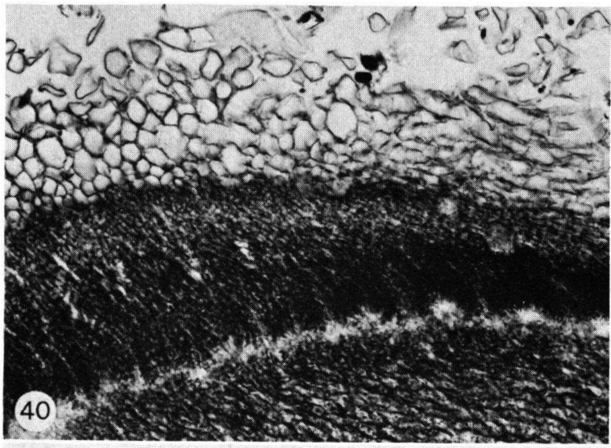
The veil over the cap is extremely well-developed in *C. macrocephalus;* there it forms a coif (Fig. 37). It also extends over the stipe; at the outside of the pileus margin are longitudinal hyphae, a remnant of the original longitudinal hyphae. The lateral trama of the cap consists of parallel protenchymatic hyphae, and the veil and its meristemoid between them have been somewhat more highly magnified in Fig. 38.

(4) We can study the continuity or interruption of the layer of the palisade-hyphae, which always precedes the formation of the hymenophore in tangential sections. Figure 35 represents this layer in *C. macrorhizus* (diameter of the section at the level of the hymenophore: 441 μm). In this section, the layer is interrupted here and there, but not regularly. A similar section, but somewhat further from the axis, shows an almost continuous layer of palisade-hyphae (diameter of the section 504 μm). It is therefore impossible to speak of a rupthymenial origin of the hymenophore in this species. With *C. macrocephalus* on the other hand, we find a more pronounced rupthymenial origin. Figure 39 represents this layer in a section, which has a diameter of 567 μm; Fig. 40 shows a somewhat older stage (diameter 756 μm). One observes clearly the arched arrangement of the palisade-hyphae.

Another interesting point in the development of the hymenophore is the fact that the edge of the gill salients in the primordia are mainly open toward the stem. This is also the case in these two species. Much has been written about this subject (the literature has been compiled in Reijnders, *1948*: 266–277; *1963:* 248). Ascending hyphae, running from the stem or lipsanenchyma into the gill-trama are generally present (Fig. 41). These hyphae, divided into short cells, are wider than the hyphae of the trama but sometimes merge into each other. In the beginning, the palisade-hyphae push their way through the longitudinal hyphae of the primordium. Also in cases where the layer of palisade-hyphae is continuous, some hyphae of the orginal protenchyma remain between them. When an annular gill-cavity has formed, these hyphae initially traverse the

Figs. 40–41. *Coprinus macrocephalus.* — 40. Tangential section of a similar stage as in Fig. 39 showing the same phenomenon × 200. — 41. Tangential section of a more advanced stage showing the open gill-edges and the ascending hyphae which continue into the trama of the lamellae where they narrow down × 400.

Fig. 42–44. *Coprinus curtus.* — 42. Very young stage showing an isocarpous origin and the first spherocysts of the veil × 400. — 43. Somewhat later stage showing the structure of the veil × 250. — 44. Intermediate stage × 125.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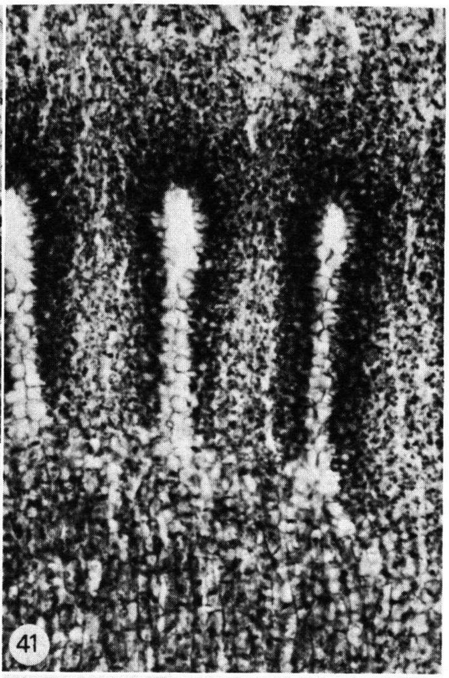
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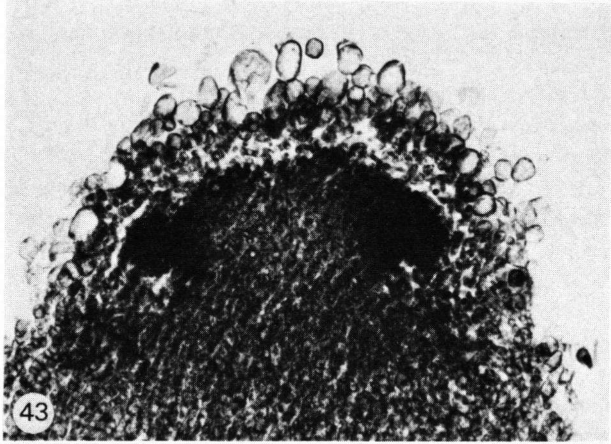
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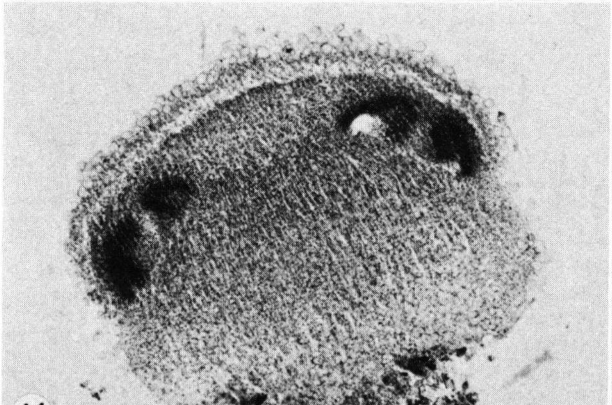
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cavity; later, they are torn apart (see, e.g. Reijnders, *1952*, pl. 7 fig. 5). Here there is no cavity; the hyphae of the lipsanenchyma are wider than those of the gill-trama, but it is quite possible that these cells of one hypha differ under the influence of local factors. With these two species no hyphae growing from the gill-trama into the lipsanenchyma could be observed to reinforce this—a phenomenon which is to be found in *Coprinus* as well. (This will be dealt with later.) The open edge of the lamellae has been imputed to a pressure excerted by the young gills against the stem (Atkinson, *1916*), but this cannot be the case. In all the series of sections which are being and have been analysed by the author, no indication of pressure in this region has been found (e.g. compressed hyphae). On the contrary, we stated that the tension arising during the development produces a stretching in this part of the primordia.

COPRINUS CURTUS

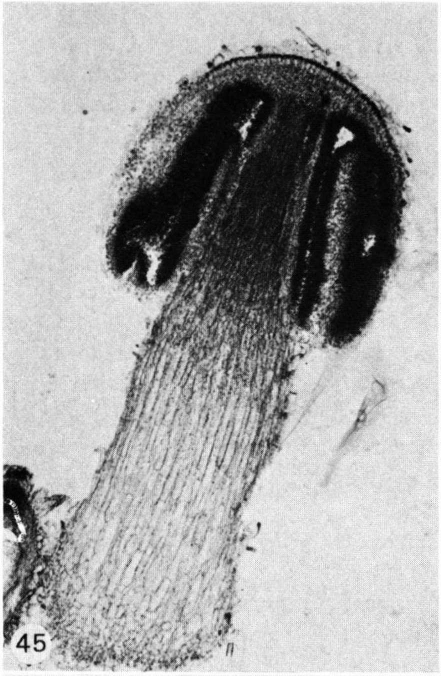
This species has already been subjected to thorough investigation by Buller (*1931*). The anatomy of the mature carpophores is, therefore, well-known. However, our results concerning the ripening of the spores are not in complete agreement with Buller's findings. This question will be treated following the description of the development.

(1) The youngest stage, a microtome-section of which has been presented in Fig. 42 (length 160 µm, diameter 114 µm), already shows the rudiments, however small, of all important parts: the veil, the stem, the pileus-trama and the palisade-hyphae of the hymenophore. Comparison with other species of *Coprinus* leads to the conclusion that this isocarpous construction has been preceded by the presence of the longitudinal hyphae only, giving rise in the lower portion to the stem and in the upper part under the veil to the pileus-trama. But in a very early developmental stage, the palisade-hyphae appear and spherocysts become apparent in the veil.

(2) Three photomicrographs of median sections have been included here. The primordia are respectively: very young (Fig. 43, diameter 256 µm), somewhat more developed (Fig. 44, diameter 460 µm), and representing a young toadstool (Fig. 45, height 820 µm, width of the cap 180 µm). As the development is quite normal, no further description is necessary. Before stretching of the stem, the primordium remains low for a long time (Fig. 44). It appears that the veil is made up of two layers: an inner portion consisting of interwoven hyphae and an outer envelope of spherocysts, which are also present at the sides of the stem. No chains of spherocysts exist, however. These cells appear at the boundary between these two layers, and some cells in the hyphae enlarge considerably to become spherocysts. The hairs typical of this species are still lacking in the stage shown in Fig. 44. It may be that this section shows the very beginning of the

Figs. 45–47. *Coprinus curtus.* — 45. Median section of an advanced stage, with a conspicuous palisadodermium over the centre of the cap, spherocysts of the veil, and hairs on the cap and, more scattered, along the stem × 100. — 46. Detail of the upper portion of the cap; showing the palisadodermium with dark staining protoplasm in the upper end of the cells, spherocysts of the veil, and club-shaped dark staining hairs × 250. — 47. Tangential section of a young stage showing isolated arches of palisadehypae × 400.

Figs. 48–49. *Coprinus hexagonosporus.* — 48. Youngest stage, with centre of subparallel hyphae, upper part of very small isodiametric cells, and universal veil reduced the some vesiculose elements × 250. — 49. Somewhat later isocarpous stage. The universal veil is not coherent and consists for the greater part of vesiculose elements with a long narrow neck; the lipsanenchyma is evident × 160.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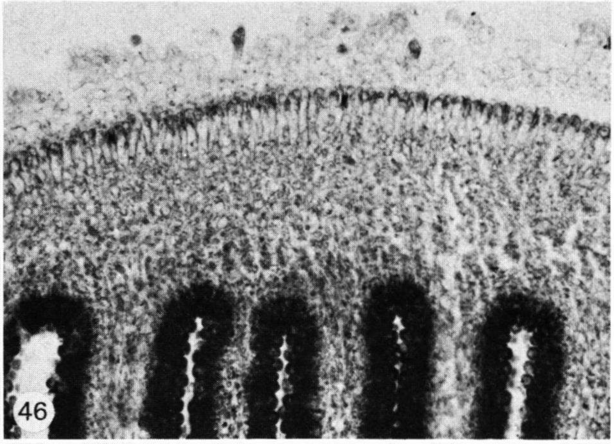
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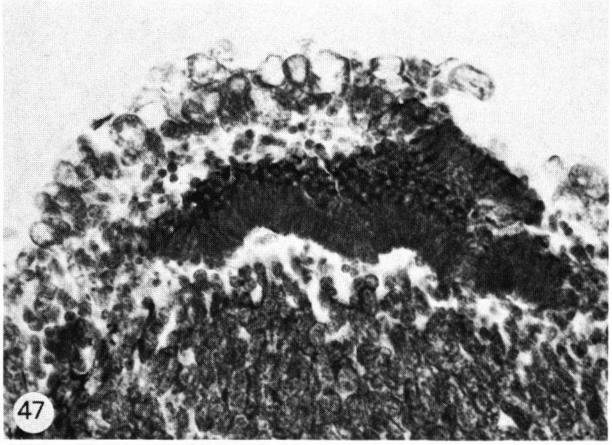
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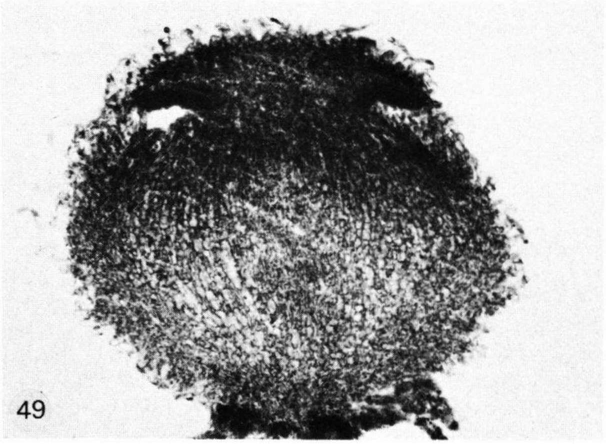
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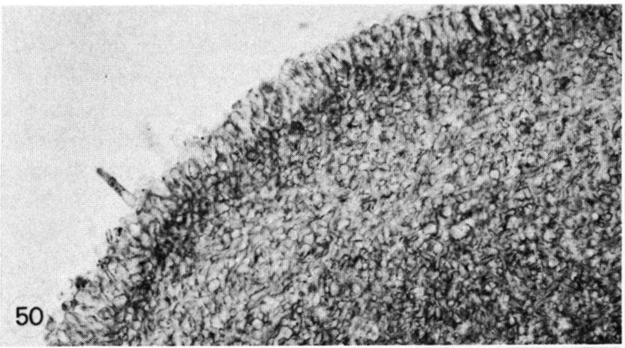
palisadodermium over the centre of the cap surface; however, the palisades are barely distinguishable. The construction of the outer layers has changed notably in the third of the median sections (Fig. 45), and we note the important development of the palisade of the pileipellis (cf., also Fig. 46, pileipellis and veil of a still more advanced young toadstool, height 2.8 mm, width of the cap 907 μm). These palisade-cells have a dark-coloured tip, which is caused by a protoplasmic concentration. The inner layer of the veil has disappeared completely, all the cells of which probably having changed into spherocysts. Some of these cells at the outside have a thicker, brown-coloured cell-wall, but these modified veil-cells (used in systematics for the distinction of the species) are not always present. The hairs characteristic of the species, deeply stained by protoplasmic contents, clavate and *c.* 5–8 μm at the ends (at this stage) appear over the entire cap, but also at the side of the stem. The late appearance of these hairs and the fact that young, dark-staining elements are present between the palisade-cells of the dermium, lead to the assumption that they belong to the pileipellis, rather than to the veil. Nevertheless, their presence at the side of the stem, where they arise from the cortex, seems to contradict this. We still note that the development of this palisadodermium, which becomes afterwards a hymenidermium (the cells become cubical), begins at the centre of the pileus and progresses toward the exterior (Fig. 45).

(3) The development of the hymenophore is rupthymenial (Fig. 47, diameter of the primordium 235 μm). The layer of palisade-hyphae growing downward and representing the first differentiation of hymenophore development, is not continuous, and, as can be seen in this tangential section, there are sterile bands between the arches of the palisades.

(4) In very young specimens, sporulation begins. We measured the spores which were present in microtome-sections of very young carpophores. The first spores formed in *Coprinus curtus* appear to be different from spores produced in more mature carpophores. They are less pigmented, have a rather thin wall, and the germ-pore is less evident or lacking. Sometimes they are somewhat deformed and do not have the regular eliptical shape which is usually apparent; at any rate, they are smaller. Spores from exsiccata of specimens growing on the same dung which yielded our primordia measured 11–13.5 × (6.5–)7–8 μm; the first spores in question 6.7–9 × 5.5–6.5 μm (in a longitudinal section of a glandiform, unexpanded cap with a height of 457 μm and a width of 208 μm) or 8–9.5 × 6.5–7 μm (when the cap was 956 μm high and 819 μm wide). Nevertheless, it is highly unlikely that we are dealing here with young, unripe spores, as spores were measured which were generally well-pigmented and had been released from the basidia which had already aged and thrown off their spores. Unpigmented, unripe spores were as much as ever present in the preparations (perhaps only a few of these were measured). So we must conclude that the spores which are formed first are smaller, and that their sizes increased gradually until spores of normal dimensions were released. This is in agreement with the observations of Clémençon (*1979*).

Figs. 50–52. *Coprinus hexagonosporus*. — 50. Palisadodermium and pilocystidium of an advanced stage. Note the cellular structure of the cap-trama × 200. — 51. Hymenidermium with young hairs in an advanced primordium × 250. — 52. Rupthymenial hymenophore in a young stage × 200.

Figs. 53–55. *Coprinus angulatus*. — 53. Young stage showing a somewhat isocarpous texture × 200. — 54. Somewhat older stage with remnants of the veil (lipsanenchyma) under the pileus-margin and with a palisadodermium × 100. — 55. More advanced primordium with hymenidermium and pilocystidia × 160.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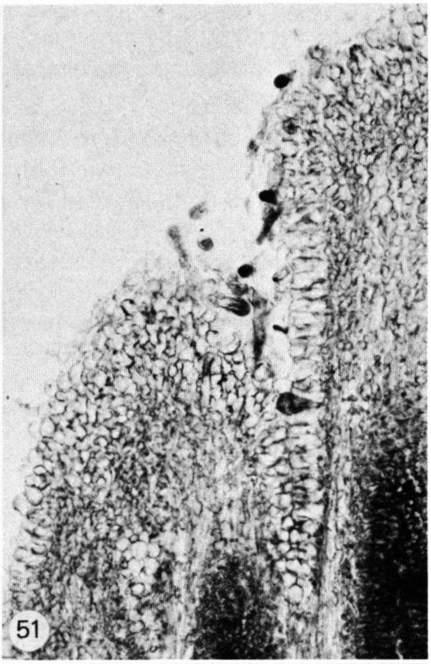
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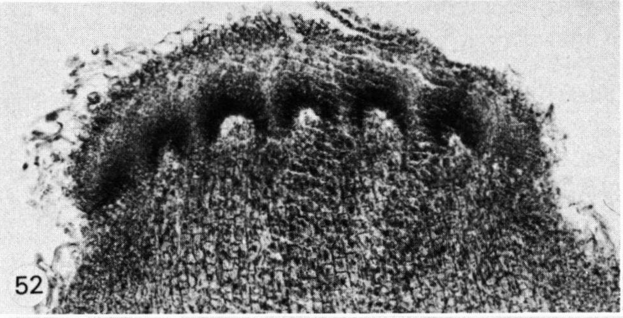
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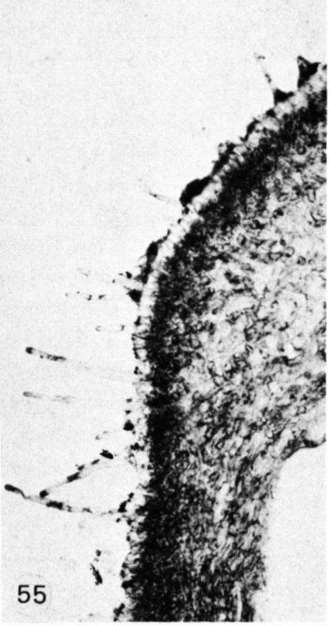
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Another feature which may be significant is the simultaneous ripening of the spores over the whole gill from the youngest stages. We observed this peculiarity in several sections, for example, of the primordium, the cap of which had a height of only 457 µm (see above). To corroborate this observation, we examined very young specimens of the related *Coprinus hepthemerus*, obtained from a culture from the Centraalbureau voor Schimmelcultures, Baarn, and the same phenomenon was observed. That the spores formed in early stages are smaller—mature specimens: (11–)12–15.5 × 6.5–8(–8.8); very young (8–)9.5–12 × 6.5–7(–8)—was also clear in this case, as well as the simultaneous ripening of the spores over the whole young gill. This is in contradiction to Buller's assertion that in *Coprinus curtus* the ripening of the spores begins in a zone near the margin of the pileus and progresses centripetally, as in so many other species of *Coprinus*. Neither in *Coprinus curtus* nor in *C. hepthemerus* can this be true. We must also call into question the deliquescence of these small membraneous species of the genus. Non-deliquescence has been reported only for *Coprinus disseminatus*, but the relationships of the latter have been disputed. Very detailed observations have still to be made concerning these generalized phenomena in this genus, particularly concerning the direction of spore-ripening along the gills.

COPRINUS HEXAGONOSPORUS

This species is also related to *C. curtus*.

(1) In the youngest stage (Fig. 48, largest dimension 315 µm, width 208 µm), we can see that some differentiation has already taken place. A basal plectenchyma with somewhat inflated hyphae (diameter up to 8 µm) occupies the lowest part and the centre. From a point at about one-third of the height from the bottom, hyphae extend toward the tip and toward the left side (they are therefore not only parallel to the axis), and they are somewhat interwoven, although they are generally parallel.

In the upper part of the section, the hyphae are again divided into small isodiametric cells, and the intertwining of the hyphae is here somewhat more striking. There is no well-defined universal veil, although some larger, round cells at the periphery (diameter up to 7 µm) certainly belong to this structure, which appears to be much reduced in this species. It is somewhat more conspicuous in the next stage (Fig. 49, height 334 µm, width at the level of the hymenophore 277 µm). It comprises several elements: the rounded or club-shaped cells mentioned above, which are also present at the stem-surface; some undefined extremities of protenchymatic hyphae; and bottle-shaped, lageniform hairs (length 25–37 µm, diameter of inflated portion 6.5 µm, diameter at neck 3–4 µm), which are in no way incorporated into the tissue of the cap, but rather lie on it. There is as yet no rudiment of the pileipellis. At the left side we see a scanty lipsanenchyma, and at the right the gill-chamber is filled with this tissue or with the hyphae passing between the arches (rupthymenial hymenophore). The narrow zone of parallel stem-hyphae is evident; below it is the basal plectenchyma.

(2) When the buds have reached a diameter (at the level of the hymenophore) of about 400 µm, the palisade-cells of the pileipellis appear and push forward between the inflated lower ends of

the velar hairs (diameter *c.* 13 μm, length 60 μm). Most of these inflated elements are the lower ends of hairs with or without a visible neck (often the neck will be cut off) and not, properly speaking, spherocysts. They are empty at this point and have thin walls, and they are almost colourless. But between the palisade-cells of the pileipellis, new hairs appear, initially as deeply-staining club-shaped elements. Most of these hairs remain blunt, somewhat clavate (length *c.* 60 μm, diameter at tip up to 10 μm; Fig. 51, height of cap 1.5 mm), but the neck of some is narrow, tapering somewhat (Fig. 50, diameter of the cap 1.15 mm). In these stages the hymenidermium is made up of wide, almost cubical cells.

(3) It is beyond question that the rupthymenial mode of hymenophore construction is to be found here. Arches of palisade hyphae, interrupted by bands where they were lacking, were always observed (Fig. 52). As with other species of *Coprinus*, the edges of the gills are widely opened, there is no indication that they press against the stem. Hyphae passing from the gill-trama into the scanty lipsanenchyma are frequently present.

COPRINUS ANGULATUS (= *C. boudieri*)

(1) The youngest stage represented (Fig. 53, height 265 μm, width 202 μm) already demonstrates some differentiation. The material used in the investigation of this species deteriorated to some degree (there were traces of putrefaction, not of drying). So for cytological research, it was of little use, but it did permit some observations on the histological texture of the primordium. Though a coherent universal veil seems to be lacking, there are, particularly at the side of the stem, masses of short cells, originating partly from longitudinal hyphae, with many cross-walls, and partly from short, club-shaped branches, directed outward and also divided into short cells. This peripheral tissue also reaches over the point where the palisade-hyphae of the hymenophore will arise more internally. There are already long hairs, which are in this stage mainly restricted to the pileus (length up to 80 μm, diameter at the lower end 9 μm, at the neck about 6 μm, rounded at the tip). The areas of pileus-trama, stem and hymenophore are well-demarcated.

(2) From a stage with a diameter of 472 μm and a length of 548 μm, the palisadodermium becomes evident (Fig. 54). Over the cap, there are only the long hairs which have arisen partly between the palisade-cells. Some of these hairs may also occur at the side of the stem, but we find here chiefly the club-shaped, outward-directed elements. In a still older stage (Fig. 55, diameter of the cap 2.1 mm), there are large hairs over the cap (about 60–100 μm long, lower end 10 μm wide, neck about 5 μm), but now they are present over the stem as well (e.g. 102 μm long, 20 μm wide, neck 6 μm). As is the case with the preceding species, it remains problematic whether these hairs belong to the universal veil or to the pileipellis. Their presence along the stem (in older stages) and their early appearance (before the palisododermium) would appear to suggest the first supposition, but when the hymenidermium (the cells of which become more or less cubical) is well-established, they continue to appear.

(3) We observed the rupthymenial mode of gill-formation, but because of the poor condition of the material, this conclusion is somewhat questionable.

DISCUSSION

THE SEQUENCE OF DEVELOPMENT

The sequence of the development of the parts is one of the most remarkable features of carpophore development in Agaricales. On the basis of an analysis of several hundreds of species, in 1963 we made up a scheme of various sequences in the earliest phases of development. Nevertheless, the number of species investigated appeared to be insufficient to determine exactly the systematic value of these phenomena. At first, we thought that differences in sequence were significant only for larger groups, as we found concentrated forms with reversed sequences of development only in well-defined, highly evolved sections of the system. But exceptions were encountered, as Singer (*1975*: 28–29) states in his 'Agaricales in Modern Taxonomy'. Since then, we have considered the subject several times (Reijnders, *1974a*: 363; *1975*: 308, 309; *1979a*: 345–346), and it is especially in the context of this investigation into many species of *Coprinus* that we can focus on this problem with greater precision.

It is obvious that all species of this genus have a concentrated development: in general, and under normal conditions, the primordia remain short and wide for a long time. Nevertheless, external factors seem to have a great influence upon the shape of the young primordium. A lack of light causes the primordia to become extended (etiolated), yet it may be admitted that such environmental factors affect the sequence of development far less, as we have stated, for example, in *C. stercorarius* (Reijnders, *1948*).

Examining the very young primordia of *Coprinus* (the dimensions of which amounted to only some tenths of a mm.), we frequently encountered somewhat confusing structures, which might have resulted in some erroneous interpretations. In this initial phase, the hyphae most often run in a longitudinal direction, so the primordia must be considered stipitocarpous, and this mode does not occur in concentrated types. In reality, the longitudinal hyphae in the earliest stages of *Coprinus* indicate something quite different from what they do in the primordia of the unquestionable stipitocarpous types: in the latter slender primordia, they curve outward at the tip to form the pileus which is continuous with the stipe. When we carefully observe the longitudinal hyphae in the initial phases of *Coprinus*, we always find in the upper portion more or less interwoven hyphae—or, when this is not the case, a meristemoid, i.e. a zone where the longitudinal hyphae are divided into isodiametric cells which increase rapidly.

Furthermore, the time factor interferes with these processes. We are obliged to draw our conclusions from series of fixed images, so it is difficult to estimate the speed of these processes. The primordia of *Clitocybe*, *Tricholoma*, *Hygrophorus*, etc., being composed of a slender stalk, remain stipitocarpous for a long time; in those of *Coprinus*, the phase with longitudinal hyphae is soon succeeded by the isocarpous state, which seems to be the real starting point for the development of the parts. So the longitudinal hyphae in the first stages of *Coprinus* (and this will probably also be the case in some other genera of Agaricales) are merely uninflated protenchymatic hyphae which are not interwoven, and here they cannot be considered indicative of beginning stem development. We have, therefore, classed these species with the pileostipitocarpous ones; when we do not have the youngest stages at our disposal for study, we arrive at the somewhat questionable conclusion that these species are isocarpous.

We found only one exception: the pileocarpy of *Coprinus brassicae*. In the description of the species and in the phylogenetic considerations we paid some attention to this phenomenon which up to now seems isolated. We should also take into consideration the possibility that pileocarpy can be close to isocarpy, while in other cases pileocarpy means a quite different sequence of development. Here the time factor is again significant; it is the appearance of stem-, cap-, and hymenophore-rudiments which tips the scale (cf., Singer *1975*: 29, on *Psathyrella pyrotricha*).

DEVELOPMENT OF THE HYMENOPHORE

According to the investigations of Atkinson and his school, the origin of the hymenophore seems to be a rather consistent process in Agaricales. Atkinson (*1914b*) recognizes only one exception: the gill formation in *Amanita*, which we called the schizohymenial mode. However, since Levine (*1914*) described another deviation in *Coprinus micaceus* and afterwards (*1922*) in *Agaricus* and some other Coprini, this question has become a controversial one and has been strongly contested by Atkinson (*1916*). Kühner (*1928*), who at first adopted Levine's view, described a somewhat intermediate mode in *Coprinus disseminatus*; Chow (*1934*) agreed with Atkinson for quite a number of species, although in our opinion his observations cannot be exact in all cases, and his descriptions and representations are not convincing. We dealt with the history of these opinions already in an previous article (Reijnders, *1948*: 266–277). It is not necessary to recapitulate the implications of this question (cf., Reijnders, *1963*: 247–248), but, after many comparisons, we think we better understand the complicated nature of these elusive structures now.

The existence of a conspicuous mode is undeniable. It can be demonstrated in series of tangential sections from the axis toward the periphery, where, in all these sections, the palisade hyphae (the first hymenial elements) show up as groups, alternating with lighter staining bands of sterile tissue. In reality, these are horizontal, radiating stripes, alternating with sterile tissue. We met this most typical form of rupthymenial development earlier in *Coprinus ephemerus* (Reijnders, *1948:* pl. 12 fig. 64) and in *C. stellatus* (at that time erroneously called *C. miser;* Reijnders, *1948*: pl. 13 fig. 69, 71). The groups of palisade hyphae soon become arched (cf., Fig. 30, *Coprinus flocculosus;* Fig. 52, *C. hexagonosporus*). A less obvious expression of this mode exists when the sterile interspaces are narrow and we see somewhat arched groups of palisade hyphae which almost touch each other (Fig. 39, 40, *C. macrocephalus*).

Another modification which seems to occur rather frequently is caused by the very early action of the gill-trama. For a short time, there is a continuous layer of palisade hyphae, but almost simultaneously, growth in the trama of the arising gill folds begins, pushing the palisade hyphae sideways. This development begins near the centre and is directed outwards (Fig. 6, *C. phaeosporus*). Kühner (*1928*) described this process in *C. disseminatus*; it is evident that in this case, a peripheral continuous layer of palisade hyphae will often be found, while the sections nearer the centre show the rupthymenial structure.

Finally, cases can be found where the layer of the palisade hyphae is interrupted only here and there, in an irregular manner (Fig. 35, *C. macrorhizus*). These unexpected interruptions seem not to be very rare; we met with such structures earlier, e.g. in *C. radians* (Reijnders, *1952*: pl. 22 fig. 4). All these modifications occur, and we are not well informed as to the variation of these phenomena in a single species: these facts may at least partly explain the controversial opinions on this question.

Apart from the fact that the first palisades develop between the protenchymatic hyphae which are continuous with the tissue underneath (the gill cavity is therefore often traversed by such fringed elements (e.g. Reijnders, *1952*: pl. 7 fig. 5, *Tricholoma focale*), connecting elements in the spaces between the rupthymenial hymenophore are quite obvious. So we find from the beginning some hyphae which run from the lipsanenchyma over and into the young gill-trama. As we stated before, the hyphae in the initial phases of *Coprinus* often run in a longitudinal direction, such hyphae can be found which pass into the gill-trama (Fig. 41, *C. macrocephalus*). In most species of *Coprinus*, if not in all, the edge of the gills (primary lamellae) remains open for a long time during development. Some authors (Atkinson, *1916*: 123, 124) believe that this opening of the gill edge is due to pressure exerted against the stem. This cannot be the case, however. We analyzed hundreds of sections with such structures, and only in a few exceptions could we find a disposition that could be explained by pressure (Fig. 13 *C. brassicae*). Usually, the tissues of the gill trama and the lipsanenchyma seem to be stretched out locally by the tension caused by the growth processes of the primordium (Fig. 7, *C. phaeosporus*; Fig. 24, *C. poliomallus*; Fig. 41, *C. macrocephalus*). We encountered the same facts in other species with a rupthymenial development (Reijnders, *1952*: pl. 16 fig. 3–5, *Bolbitius vitellinus*). We believe that the wide, open gill edge in *Coprinus* represents an efficient adaptation: it may explain the parallel faces of the lamellae in *Coprinus*. However, the secondary gills or lamellulae have an edge surrounded by hymenial elements and, in primordia, they are wedge-shaped, as in other Agaricales. Moreover, the open gill edge is necessary for the further joining of stipe and lamellae.

Atkinson (*1916*), as well as Kühner (*1928*) and Chow (*1934*), observed the fact that soon after their origin, the lamellae become attached to the stipe. This involves a situation in which hyphae grow out from the gill-trama into the lipsanenchyma. An extension of the gill-trama with no bordering elements of the hymenium was encountered in several cases (Reijnders, *1963*: pl. 17 fig. 6, *Tricholomopsis rutilans; 1971*: pl. 2 fig. 6a–c, *Agrocybe aegerita*); it appears to be quite common in *Coprinus*.

As a rule, the lipsanenchyma itself does not increase significantly (e.g. by intercalary growth) in this genus. This might explain why these species, discounting a few exceptions, have no ring. But the sometimes scanty lipsanenchyma is a bit reinforced by hyphae coming from the gill-trama. This process serves to fix the lamellae to the stem. When this has happened and the primordium is surrounded by a universal veil, it is sufficiently protected, for example against drought (evaporation). We mentioned the extension of the lipsanenchyma and the connections between lamellae and stipe for each species separately in Table I. We photographed such connections in Fig. 7 (*C. phaeosporus*), Fig. 13 (*C. brassicae*), and Fig. 26 (*C. poliomallus*). The passing hyphae have been photographed once more under higher magnification in a younger stage of the latter species (Figs. 24, 25). This photomicrograph might demonstrate a striking case of cells of the same hyphae, which have differentiated into other forms where they have been influenced by various developmental factors: the cells of the lipsanenchyma are larger and nearly isodiametric (cf. Reijnders, *1963*: 277–278).

VEIL AND PILEÏPELLIS

The great variation in the veils in *Coprinus* gives us an excellent opportunity to deal once more with the 'cortical layers' in general, particularly in view of the difference between universal veil and pileipellis. Recent descriptions of pelles have demonstrated clearly that this important distinction has not been observed everywhere, probably because a determination of the universal veil is somewhat hazardous in mature specimens. So we will first treat the modifications of the veils in *Coprinus*, and consider afterwards the differences between veil and pileipellis and questions of terminology.

Many *Coprinus* species have a well-developed universal veil, while in others this veil is lacking. A lipsanenchyma is present, except in *Coprinus plicatilis*, which has no veil at all. As a consequence, most *Coprinus* species are bivelangiocarpous; if one does not take into consideration the same peculiar hairs which are present on the cap as well as on the stipe, the *Setulosi* are paravelangiocarpous, and *Coprinus plicatilis* is gymnangiocarpous. As we stated before, the lipsanenchyma is in most cases not very voluminous, but the gills are attached to it. Our comparisons of hundreds of species has led us to the conclusion that the protenchymatic veil, consisting of generative hyphae, is the most primitive and the extending lipsanenchyma often remains for a longer time in this condition (Reijnders *1963*: 224–232, 351–352). We found the most undifferentiated veil in this genus in *C. brassicae*. We shall comment only on some questions concerning the modifications of the universal veil which are useful for the survey: the details for each species are dealt with separately in Table I.

First, one encounters the problem of the protenchymatic tissue, which partly constitutes the universal veil, but in the beginning, it is not separated from the pileus-trama. A wide zone of protenchymatic tissue between the developing cap and the veil has been described in many species. After the downward growth of the hyphae of the cap-trama has become evident, there is a clear demarcation between these tissues in the lateral portion of the pileus, but in the centre they remain continuous for a long time. Nevertheless, a distal zone of this protenchyma belongs to the veil and takes part in its further differentiation; as it also envelops the pileus-margin or the point where the hymenophore will appear, it must be considered a universal veil. It also happens that such a zone does not exist; in very early stages, the pileus surface is present immediately under radiating hyphae and at the contour of the pileus a meristemoid develops.

The universal veil is subject to some characteristic transformations in *Coprinus*, including the formation of chains of long, wide cells, a dichophysoïd structure, spherocysts, and isolated hairs. The spherocysts arise scattered throughout the protenchymatic layer of the veil, but they can also be arranged into chains, formed by a meristemoid. We notice that only in the *stercorarius* group and some other species spherocysts are lacking over the stem: this is one case of a peculiar differentiation of the veil over the pileus (see below).

We are inclined to consider isolated hairs, which are present over the cap as well as along the stipe, to be a transformation of the veil as well. Sometimes, these are visible in the veil itself without being connected to the underlying tissue (Reijnders, *1952*: Pl. 19 fig. 5, Pl. 20 fig. 2, *Psathyrella multipedata*; Fig. 49, *C. hexagonosporus*, etc.). In most cases, they arise between the cells of the pileipellis, and then it becomes interesting to determine whether or not these hairs of later origin have quite the same shape as those of the universal veil. Indeed, such hairs, with their specialized appearance, cannot represent the first stages of universal veil development. We met with such features when the young primordium was encircled by a few protenchymatic threads, having an innate veil (e.g. Reijnders, *1948*: Pl. 8 fig. 35–37, *Alnicola melinoides;* Reijnders, *1963*: Pl. 34 fig. 3–6, *Hypholoma elongatipes* Peck, as *Nematoloma polytrichi*) and when it was surrounded by outward-growing generative hyphae, we encountered an emanated veil (Reijnders, *1948*: Pl. 18 fig. 101, 102, *Gomphideus roseus*; Pl. 22 fig. 129–133, *Strobilomyces floccopus*). These are examples of very primitive veils, as the evolution of the veil has not started with the formation of isolated, particular hairs. Nevertheless, an interpretation of their nature becomes somewhat confusing when we find the same hairs arising abundantly afterwards amid the palisades of the pileipellis. Many authors have called them pilocystidia.

Above, we mentioned the fact that spherocysts are sometimes present only in the veil over the cap: this is one of the instances in which influences from the developing pileus interfere with structures in the veil. As this phenomenon seems to be rather rare in Agaricales, it is easily passed over. It may, however, once more corraborate the conclusion that the development of structures in agaric fruit bodies is locally determined (Reijnders *1963:* 277–278; Reijnders *1975*: 309, *Chamaemyces fracidus;* Reijnders, *1979b*, *Limacella glioderma*). However, even if some structures exist which seem to make the distinction between the universal veil and pileipellis somewhat indefinite (our schemes are seldom adequate to account for all modifications in nature), we should always be careful not to confuse these notions: the veil and the pileipellis are essentially different structures. We analyzed these questions in our book in 1963: the universal veil shows up in the youngest stages and is characteristic for the primordium as a whole; the pileipellis originates later on and is restricted to the pileus. The first mentioned organ sometimes increases considerably, serving the purpose of protecting the developing carpophore, the pileipellis forms a roof only over the mature cap, and its function is in accordance with this fact. Nevertheless, in mature carpophores, the remnants of the universal veil cannot be easily distinguished from the pileipellis. This fact might be of paramount importance.

Two recent publications (Bresinsky & Schwarzer, *1969;* Watling & Largent, *1976*), Singer's (*1975:* 60–69) detailed account, and the problems of nomenclature which are urgent here lead us to make some observations. Although the authors of these publications emphasize the importance of this distinction, only in the second are misinterpretations absent. The opinion of Bresinsky & Schwarzer that the veil has to be considered 'funktionell und ontogenetisch als Schicht des Hutes' is erroneous (see Reijnders, *1963*: 13, 14, 224). The universal veil has been treated as equivalent to pileipellis structures, e.g. in fig. 5 (*Cortinarius*), fig. 6 (*Rozites*), figs. 7, 8 (*Hypholoma*), fig. 22 (*Naucoria*), fig. 23 (*Phaeolepiota*), figs. 24, 29 (*Lepiota*), etc. The covering of *Amanita* and *Phaeolepiota* is not an epithelium but a universal veil (Singer, *1975*: 62). The dichophysoid structure in the *Coprinus* sect. *Alachuani*, is relative to the universal veil, not to the pileipellis, etc.

As we stated above, it is not always easy to determine whether we are dealing with the universal veil or with the pileipellis in mature carpophores. But we suppose that some authors could strive for greater accuracy here. When scattered, repent, narrow hyphae are encountered over the cap, one should be very careful about their interpretation, and the same applies to all kinds of spherocysts, loose or coherent (for the cells of the hymenidermium are mostly cubic). Finally, a great number of genera has been examined ontogenetically, exact descriptions of these developments have been published since Atkinson's times, and monographers studying genera or anatomists examining pileipellis structures should use these data. Furthermore, one can profit by including very young specimens (not necessarily primordia) in the examination; the nature of the universal veil may often be determined by freehand sections of the outer portion of the pileus margin, also in fresh material.

The diverse modifications and the corresponding terminology of pileipellis formation have been thoroughly analyzed in Watling and Largent's paper, and we agree with their conclusions in every respect. As the homology of the pileipellis layers can as yet be established only in genera with closely related species, it is reasonable to distinguish structural and topographical modifications and to create two series of names for them. As for the terms which denote morphological differences, we adopted Lohwag's terminology in our book in 1963, as many authors have done (e.g. Singer). As it may be superfluous to repeat all the definitions, the names in question are: 'trichodermium', 'palisadodermium' ('hymenodermium'), 'epithelium', 'cutis' and 'cortex'. We note that only the difference between palisadodermium and hymenodermium is somewhat arbitrary: we called that layer with greatly inflated cells which become cubic in the end the hymenodermium. We use the term 'epithelium' in the same sense as Singer: a pluristratous complex of isodiametric cells (definitely excluding the spherocysts of the veil). We prefer the term 'cortex' to Singer's 'dense layer'; a cortex is merely a condensation of the unaltered underlying hyphae. The cortex represents the transition to the complete absence of the pileipellis, so we must often refer to the periclinal hyphae of the outer zone of the pileus-trama (under the veil) as a cortex. There may be no differentiation and we find such hyphae often at the periphery of the stem as well. As the terms 'dermium' and 'cutis' have been used to designate morphological differences, we agree with Watling and Largent that it is highly confusing to use them in compound words in order to describe topographical layers. It is far better to apply to the latter category the terms proposed by Bas (*1969*).

PHYLOGENETIC CONSIDERATIONS

Although phylogenetic hypotheses are seldom satisfactory in mycology, one can scarcely avoid, when examining a series of morphological structures, attempting to imagine how one could have developed out of another. When doing this, however, one has to be well aware of the fact that present forms can seldom be derived from other, still extant, ones; it is better to consider which of the attributes can be considered primitive and which specialised, and then to try to find a modern species that shows many of the primitive characteristics. In this manner, one can more or less imagine the course of evolution.

As far as the structures which we have dealt with are concerned, in the interest of attempting to decide which are primitive and which have developed further, we make the following assumptions.—

(1) We consider the generative hyphae, which combine to form the protenchyma, more original than all the cells which developed therefrom. Inflated cells can in any case be considered characteristic of evolution, and this is especially true of certain types, such as palisades, spherocysts, pseudo-paraphyses, etc. There are certain groups of carpophores (e.g., *Mycena*,

TABLE I

Developmental anatomy of *Coprinus*

species	succession	origin of the hymenophore	universal veil, lipsanenchyma	pileipellis
Coprinus phaeosporus Karst.	the youngest stage available (567 × 529 µm) is isocarpous; young prim. wide and short	somewhat rupthymenial (by the activity of the young lam. tr. the groups of palisade-hyphae are pushed apart); gill-edge open; connections gill-tr. and lipsanenchyma evident	u. v. consisting of a wide protenchymatic zone and a layer of radiating threads becoming dichophysoid at the periphery. Lips. rather abundant, somewhat reinforced by h. coming from lam. tr.	none; the h. of the pileus-tr. merge into the prot. zone of the u. v.
Coprinus brassicae Peck	pileocarpous; young prim. wide and short	levhymenial; gill-edge open from the outset; connections evident	u. v. a wide zone of protenchyma, only slightly altered at the periphery in older stages; lips. rather abundant, reinforced by h. from lam. tr.	none; as in the preceding species
Coprinus niveus (Pers. ex Fr.) Fr. R., *1948*	pileo-stipitocarpous, soon isocarpous; young prim. slender	levhymenial; gill-edge also in older stages for the greater part surrounded by cells of the hymenium	a large zone of protenchyma remains outside the cap; in this tissue spherocysts arise by inflation of cells; short rows are present only in older stages; lips. rather abundant	none; also in older stages the tissue of the pil. tr. merges into the outer prot. zone; spherocysts over the cap only
Coprinus stercorarius Bull. ex St-Amans) Fr. Brefeld, *1877*; Levine, *1922*; R., *1948*	pileo-stipitocarpous, soon isocarpous	levhymenial; gill-edge open in later stages, at first surrounded by hymenial cells	protenchymatic zone over the cap less extensive, with radiating threads, but in young stages spherocysts not in rows; lips. rather abundant	in older stages the upper surface of the cap is delimited by short cells, a sort of epithelium, but outward they pass into the isodiametric elements of the veil; spherocysts over the cap only

Coprinus narcoticus (Batsch ex Fr.) Fr. R., *1963*	unknown	unknown	a wide zone of protenchyma over the cap, at the outside radiating threads with short rows of spherocysts	in the etiolated specimen which has been examined the tissue of the pil. tr. is continuous with the protenchyma of the u.v.; spherocysts over the cap only
Coprinus patouillardii Quél.	pileo-stipitocarpous	at first the layer of palisade hyphae is continuous but soon afterwards there are arches, probably by the activity of the young lam. tr. — a tendency towards a rupthymenial mode	over a narrow protenchymatic zone surrounding the cap there is a close layer of isodiametric cells; the veil along the stem (also short cells) is somewhat different; lips. scanty	finally the whole veil is divided into cells which inwardly are not well delimited from the peripheral layers of the pil. tr. which forms an epithelium; spherocysts over the cap only
Coprinus bulbillosus Pat. Chow, *1934* (as *C. hendersonii*)	stipitocarpous or pileo-stipitocarpous, probably soon isocarpous	levhymenial; in a later stage: 'les arêtes des feuillets se soudent au pied'	the description of Chow is not clear; probably there are isodiametric elements at the outside of the u. v. and a prot. layer within, the latter grown together with the pil. tr.; lips. abundant	'Le chapeau ne présente pas un revêtement différencié à sa surface'
Coprinus cortinatus Lange R., *1952* (as *C. roris*)	not well known	levhymenial; edge of gills also in later stages not open and surrounded by cells of the hymenium	a wide zone of prot. around the cap and an outer layer with spherocysts or oblong cells, which are absent along the stipe; lips. rather abundant	an epithelium, at last not well demarcated from the veil
Coprinus cubensis Berk. & Curt. Johnson, *1941*	'Stipe, pilear, and hymenial primordia have almost simultaneous origin.'	levhymenial; (structures as described by Levine occur in abnormal buttons?); gill-edge afterwards open: 'loosely attached to the stipe'	the layer of prot. over the cap appears to be thin or absent according to the photographs; lips. not mentioned	'a single layer of highly differentiated cells is formed just below the upper blematogen'

TABLE I (cont.)

species	succession	origin of the hymenophore	universal veil, lipsanenchyma	pileipellis
Coprinus poliomallus Romagn.	pileo-stipitocarpous, soon isocarpous	a tendency to the rupthymenial mode; by the activity of the young lam. tr. the palisades which arise at the same time are pushed apart (arches); gill-edges open; connections from the beginning	there is practically no layer of prot. underneath the radiating h. forming the spherocysts, also occurring along the stipe; lips. consisting of longitudinal h.	in later stages there is an epithelium, the upper layer well differentiated and made up of cubic cells (somewhat hymeniform)
Coprinus flocculosis DC. ex Fr.	pileo-stipitocarpous, soon isocarpous; a portion with small isodiametric cells over the longitudinal h. of the stem rudiment	rupthymenial; palisade-h. in isolated groups from the beginning; edge of lam. open from the beginning	there is a wide zone of protenchymatic h. over the cap and an outer portion of radiating h., which widen gradually (without spherocysts), the prot. later divided into short cells and less evident along the stipe; lips. scanty	a palisadodermium also in later stages (width 3.5 mm). the palisades arise already when the prim. has a width of *c.* 900 μm
Coprinus radians Desm. R., *1952*	pileo-stipitocarpous, soon isocarpous	rupthymenial	prot. zone over the cap narrow, disappearing in more advanced stages; the outer part of the veil consisting of radiating rows of spherocysts, also present along the stem; lips. scanty	a palisadodermium (also in 3 mm wide prim.) developing centrifugally at the pileus margin and centripetally over the centre
Coprinus micaceus (Bull. ex Fr.) Fr. Levine, *1914*; Atkinson, *1916*	pileo-stipitocarpous, soon isocarpous	rupthymenial (denied by Chow, *1934*); gill-edge open; connections in later stages admitted by Atkinson	u. v. and lips. as in the preceding species	a palisadodermium already present in young stages (width ±400 μm

Coprinus macrorhizus (Pers. ex Fr.) Rea R., *1952* (as *C. fimetarius*)	*pileo-stipitocarpous, soon* isocarpous; in a very young prim. the h. in the centre longitudinal; the upper portion is a meristemoid	*there are no regular arched* groups of palisades, but they alternate with strips of ordinary prot.; tendency towards the rupthymenial mode; gill-edge open; connections present	*u. v. consisting of radiating* h. and present in a very young stage (less conspicuous over the cap-centre); somewhat later a veil-meristemoid is formed at the surface of the cap; lips. made up of ascending h.	*none; at maturity the cells* of the outer part of the cap tr. gradually enlarge towards the adjacent cells of the veil
Coprinus macrocephalus Berk.	pileo-stipitocarpous, soon isocarpous	somewhat rupthymenial; isolated arches of palisades really present; edge of gills open; hyphae of the lips. merge into the lam. tr.	as in the preceding species; u. v. in later stages luxuriant over the cap, reduced along the stem; lips. consisting of ascending h. of small cells	none
Coprinus lagopus (Fr.) Fr. Chow, *1934*	'Le prim. du chapeau commence à se différencier tardivement par rapport au développement du pied.'	levhymenial; gills in a later stage connected with the stipe	u. v. presumably as in the preceding species	none
Coprinus comatus (Müller ex Fr.) S. F. Gray Atk., *1916*	probably pileo-stipito-carpous, soon isocarpous	levhymenial; after having grown across a wide gill-cavity the edges of the gills press against the stem (?), open and some elements interweave with the lips.	'An outer zone of radiating threads ('blematogen') remains 'concrete' with the pileus; lips. present	none
Coprinus atramentarius (Bull. ex Fr.) Fr. Atk., *1916*	youngest stages not studied 'Fundament of pileus present in the earliest stages'	with slight tendency towards rupthymenial structures; later on the gills become attached to the plectenchyma surroundig the stem	an outer zone of radiating h. remains concrete with the pileus; lips. as in the preceding species, probably abundant	none
Coprinus auricomus Pat. R., *1974*	the palisade hyphae of the hymenophore develop somewhat lately; by this phenomenon the species seems to be pileocarpous, but it is almost isocarpous	levhymenial; gill-edges soon open towards the stem; gill-trama connected with the lips.	the u. v. of repent h. has its greatest extension outside the cap margin; it consistst of long and thin yellow hairs, mainly along the stipe	a serried palisadodermium develops from a stage of about 300 μm wide (at the cap margin), it contains large yellow hairs with the walls thicker than those of the hairs in the veil

TABLE I (cont.)

species	succession	origin of the hymenophore	universal veil, lipsanenchyma	pileipellis
Coprinus curtus Kalch. s.s. J. Lange	the youngest stage available (height 160 µm, width 112 µm) is isocarpous	rupthymenial; only the most peripheral part of the layer of palisade hyphae can be continuous; gill edge wide open in later stages; as to the ripening of the spores see Descriptive Part	with an inner initially rather thick, protenchymatic layer and short rows of spherocysts at the outside; soon the radial arrangement of these rows is lost; lips. scanty	a palisadodermium already present in young prim.; the protoplasmic content is to be found in the upper part of the palisades, which afterwards become about cubical; most hairs arise between the cells of the dermium, a few also along the stem
Coprinus disseminatus (Pers. ex Fr.) S. F. Gray Kühner, *1929*	pileostipitocarpous, soon isocarpous	according to Kühner, in the initial stage levhymenial, but by the activity of the lam. tr. the palisades are pushed sidewards and form isolated arches; the gill trama becomes attached to the lips. and is open at later stages	as in the preceding species; protenchymatic part perhaps less wide; lips. scanty	a palisadodermium which becomes a hymenidermium, with large hairs arising between its cells
Coprinus hexagonosporus Joss.	in the youngest stage available the upper part of the prim. is occupied by a meristemoid; this may be a pileostipitocarpous structure, but soon the prim. is isocarpous	rupthymenial (from the beginning — a classical case)	the u. v. is not coherent even not in young prim.; with some scattered h., a few spherocysts, and vesiculose elements usually showing a long neck; these presumably empty elements are less frequent along the stem; lips. scanty	since a young stage a palisadodermium which becomes afterwards a hymenidermium; with characteristic dark staining clavate hairs arising between the cells of the dermium; the vesiculose cells of the veil mostly on the outside
Coprinus angulatus Peck (= *C. boudieri* Quél.)	pileostipitocarpous soon isocarpous (youngest stage 277 × 214 µm)	probably rupthymenial	the u. v. is not coherent and consists of scattered h. and long lageniform hairs	a palisadodermium arising in a young stage (diam. 440 µm), with cells becoming

			which are present in the youngest stages; lips. present	much inflated later on (hymenidermium); many hairs present between the cells of the dermium
Coprinus ephemerus (Bull. ex Fr.) Fr. R., *1948*	at least isocarpous	rupthymenial; gill-edge in later stages wide open; some slight connections	the young u. v. does not cover the whole prim.; it consists of longitudinal h. which run along the stipe and the pileus margin, and of long vesiculose hairs with a tapering neck all around the prim.; lips. consisting of ascending h.	a palisadodermium with long hairs, similar to those of the veil, between the cells
Coprinus bisporus J. Lange Kühner, *1926*	unknown	rupthymenial	not described in detail	a palisadodermium present when young, with hairs between its cells
? *Coprinus stellatus* Bull. R., *1948*, *1963* erroneously called *C. miser*, because of the absence of pilocystidia in sections of many advanced primordia; cf. R., *1948*: 293–295)	the youngest stage available is isocarpous	rupthymenial; gill-edge open from the beginning	as in *C. ephemerus*; lips. rather scanty	a palisadodermium present from the youngest stages, with the cells soon inflated (hymenodermium), with numerous acuminate small hairs (length 30–40 μm) in young prim., almost disappearing with age
Coprinus plicatilis (Curt. ex Fr.) Fr. R., *1952*	at least isocarpous, possibly hymenocarpous (youngest stage 350 μm wide)	rupthymenial; gill-edge open from the beginning but no connections because lips. is absent	u. v. and lips. absent; gymnangiocarpous	a palisadodermium present from the youngest stages; the palisade-cells becoming large and strongly inflated

ABBREVIATIONS USED. –

Atk. = G. F. Atkinson
h. = hyphae
lam. tr. = trama of the gills
lips. = lipsanenchyma
pil. tr. = pileus trama
prim. = primordium
prot. = protenchyma
R. = A. F. M. Reijnders
u. v. = universal veil

Conocybe, *Coprinus*), in which the primordia are already distinguished by a dominant pseudoparenchymatic texture, and this may be considered an indication of specialisation.

(2) A universal veil with spherocysts is even more specialised than a veil consisting of long series of inflated cells.

(3) When the same kind of hairs is found over the cap and along the stem, this is considered a reduced and altered universal veil, and a characteristic of specialisation.

(4) A universal veil which no longer has a plectenchyma, but which is formed by a meristemoid, is more specialised.

(5) The simplest enclosure of the adjacent tissue of a carpophore is a condensation of this tissue, as can often be seen at the surface of the stem. This is called a cortex. A single cortex is also often found on the upper side of the cap in advanced primordia, despite the fact that in mature carpophores, it may have altered.

An epithelium, which consists of a few layers of somewhat isodiametric cells, is more evolved than a cortex, although the difference is not always easily seen in the case of a dense cortex in sections. There is also an epithelium with a clearly distinguishable outer layer, as in *Coprinus poliomallus*.

A palisadodermium is without question an even more specialised enclosure of the cap, the difference between a palisadodermium and a hymenidermium being minimal: we could consider the latter to be a palisadodermium with strongly inflated cells.

(6) As far as the sequence of the emergence of the first structures is concerned, it is not quite clear whether the pileocarpous structure is more evolved than the isocarpous one; the latter is certainly more highly developed than the pileo-stipitocarpous mode. For an exact definition of these terms, see the section on succession.

(7) Compared to the greater part of the Agaricales, the rupthymenial development of the hymenophore is a peculiarity which is seen only once in a while with very highly concentrated primordia. Usually, a continuous layer of palisade-hyphae is formed (levhymenial mode). There are, however, all kinds of transitional forms between these structures, and they are often present with *Coprinus* (see 'Origin of the hymenophore').

We will now try to apply these points of view to the facts of development of *Coprinus* species which are presently known and which have been presented in the table. We can start from the assumption that a species in *Corpinus* is primitive when it has a veil that remains protenchymatic, that is pileo-stipitocarpous (but soon to be isocarpous), levhymenial, and that does not have a differentiated pileipellis. Of those studied in this article, we will find no species that correspond exactly. Those species, however, which, according to Romagnesi, belong to the *Impexi*, and, according to Singer, to the *Alachuani*, come closest.

One could object that the wide veil which consists mainly of unchanged generative tissue, has a special texture in *C. phaeosporus* because of the radiating dichophysoid hyphae, and, further, that this species is more or less rupthymenial, whereas *C. brassicae* is the only species studied till now which shows a clear pileocarpous primordium. These facts cannot be denied, and they do detract somewhat from the plausibility of the hypothesis that the *Impexi* should be the most primitive Coprini. We have to realise, however, that the pileocarpous and isocarpous types appear to be close together (in some cases the former might even be more primitive than the latter) and that the rupthymenial development of *C. phaeosporus* is not the most explicit form of this structure.

In this connection, it is regrettable that the development of so few species of this group is known, but it is not always easy to find material. According to the descriptions in the 'Flore analytique', the veil can vary a great deal in this group.

Other groups that might come close to a primitive form are those of *Coprinus niveus* and *C. stercorarius* of the section Singer called *Cycloidei* Fr., and Kühner & Romagnesi termed *Vestiti* J. Lange. *Coprinus narcoticus* is probably linked to these species. These species are characterised by a thick layer of spherocysts over the cap, the function of which is not very well known, but which appears in different non-related genera (*Cystolepiota, Phaeolepiota, Phaeomarasmius,* etc.) among the Agaricales.

It is peculiar that these spherocysts in the species mentioned, form only over the cap and not along the stem, although they originate in the universal veil (see 'Veil and pileipellis'). If we consider a universal veil to be a tissue which surrounds part of the primordium or the complete primordium, but which, in any case, stretches along the pileus-margin, then this consists here of a wide band of protenchyma, in which the spherocysts develop in a rather irregular way. These spherocysts are pushed outside, but no chains develop.

Coprinus niveus and *C. stercorarius* do not have a differentiated pileipellis; the original protenchyma of the pileus-trama is confluent with the inner layer of the veil. These species are levhymenial.

In *C. patouillardii,* we find in the universal veil a thinner layer of protenchyma over the cap and, outside that, short chains of spherocysts, that, however, do not appear along the stem. In later stages, the upper part of the pileus consists of an epithelium, which is difficult to distinguish from the short cells belonging to the veil which has almost completely split into cells (Fig. 16, 17). This species shows rupthymenial structures, but we also found peripheral tangential sections with continuous layers of palisades (see 'Origin of the hymenophore'). *Coprinus cortinatus* is linked to *C. patouillardii.* In this case as well, no spherocysts form along the stem, and a thinner layer of protenchyma in the veil and, finally, an epithelium over the pileus appear. However, the species is probably levhymenial, which could be connected to the fact that we also found a closed edge of the lamellae in later stages (an exception!).

What appeared only in later stages in the last two species—i.e., that between pileus and veil a zone of cells appears that belongs partly to both tissues, we find with *C. poliomallus* from the beginning. Almost no plectenchyma appears in the veil here, the first spherocysts being formed in a very early stage (to a lesser extent also along the stem) and a meristemoid develops in which the cell divisions occur mainly in a radial direction, so that the spherocysts often range in chains. In later stages, there is an epithelium over the cap with an outer layer of one-cell thickness, and this layer can be distinguished from the other cells. This species also shows a more or less rupthymenial hymenophore (see Table I).

The species from the section, *Micacei* Fr., can be compared with *C. poliomallus. Coprinus radians* already has almost no plectenchyma over what will later become the demarcation line between pileus and veil. This zone is very narrow, and it forms radiating hyphae which divide into short cells out of which series of spherocysts originate. This tissue is possibly less a meristemoid than is the case with *C. poliomallus.* According to Atkinson's description (*1916*),

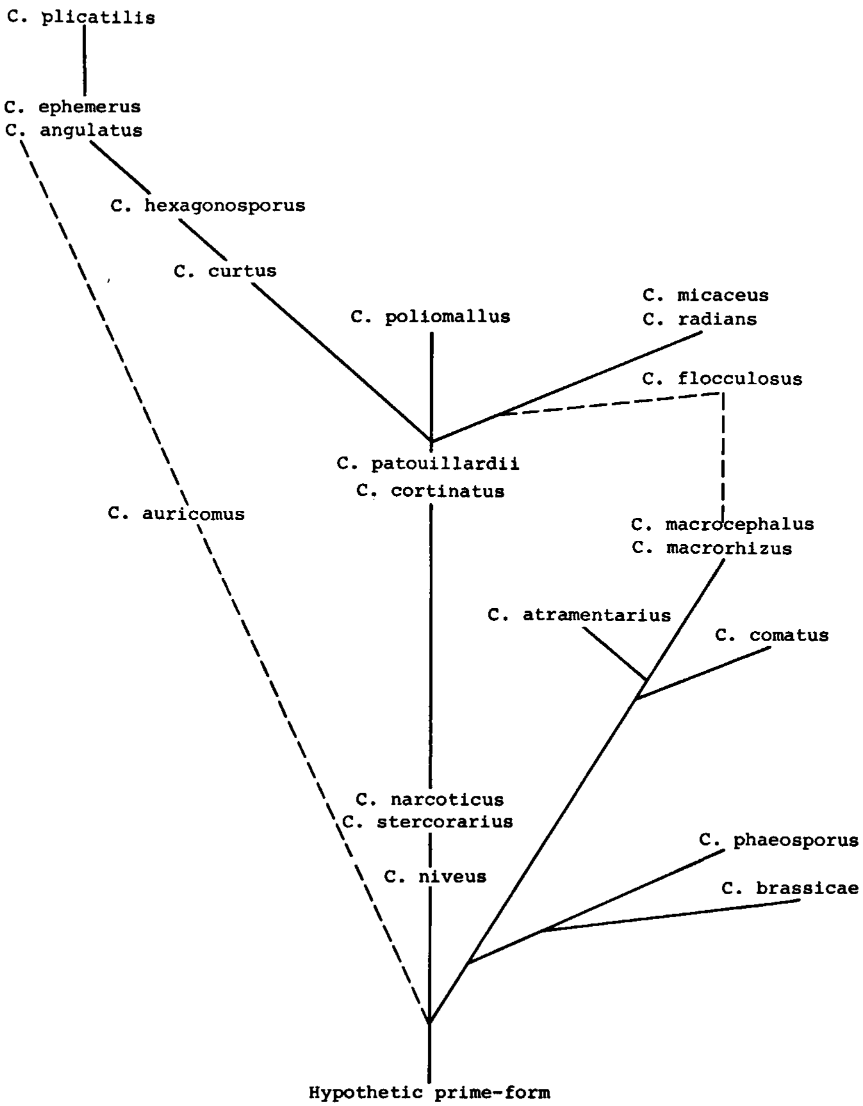

TABLE II

Possible interrelationships of *Coprinus*-species there is no plectenchyma present in the veil of *Coprinus micaceus*. Singer divided the *Micacei* into *Domestici* Sing., with filaments between spherocysts, and *Exannulati* Lange, where this is not the case. Both types have clearly rupthymenial structures, and the pileipellis is made up of a palisadodermium, which distinguishes the *Micacei* from the *Vestiti*. In the *Lanatuli* Fr. that have been studied up to the present, a meristemoid also plays a large part in creating the veil. In this way, these species can be compared with *C. poliomallus* as well. Virtually the entire trama of the very young pileus has been divided into cells in *C. macrorhizus* and *C. macrocephalus*. From the centre of this trama, radiating hyphae arise, which have already also divided into short cells. Later, the pileus-trama and the universal veil will separate because of the formation of a meristemoid at the juncture. This continues to form externally radiating filaments, the cells of which increase in breadth toward the surface. Very thin protenchymatic hyphae, growing downward, appear over the rim of the cap. No pileipellis is present. The strongly developed velum probably takes over the function of the pileipellis. The universal veil is highly specialised in these species, as far as its growth and development are concerned. There is a clear tendency toward the formation of a rupthymenial hymenophore in these species, although this structure is not present in its most conspicuous form. It has been claimed that *C. flocculosus* is an intermediate form of *Lanatuli* and *Micacei*. This is due to the structure of the veil, which will eventually consist of round and long elements (Kühner & Romagnesi, *1953*: 383). In earlier stages, there is quite a lot of protenchyma present in the veil as an inner layer; however, this soon divides into small isodiamatric cells. The veil is notably less radiating than in *Lanatuli*—at least in later stages. There are no real spherocysts present, although thin-walled, round elements do occur in the veil. The species has a well-developed palisadodermium and is clearly rupthymenial. It shows little similarity with *Lanatuli*, although it could be grouped with *Micacei* if one assumes that the spherocysts in this group have developed later. But if one assumes that in the course of evolution, spherocysts occurred even before the development of a palisadodermium (*C. poliomallus*), then the position of *C. flocculosus* remains somewhat enigmatic.

Singer (*1975*) divides the *Hemerobii* into three subsections: *Setulosi* Lange, *Auricomi* Sing., and *Glabri* Lange. Kühner & Romagnesi (1953) mention only *Setulosi* Lange and *Hemerobii* Fr. The first are divided into three groups, of which two, apart from peculiar hairs on the cap, have spherocysts as well.

There are three species of these *Setulosi* with spherocysts of which the development is known. *Coprinus curtus* has a universal veil from the beginning, with a protenchymatic inner layer and short series of spherocysts. Soon, however, the hyphae of this layer divide into round cells which become spherocysts, which are also present along the stipe. The number of spherocysts is much smaller than in, for example, the *Micacei*.

With these partly thick-walled and specialised spherocysts, the peculiar hairs which grow only more numerous in later stages, the strongly inflated palisadodermium, and a strongly pseudoparenchymatic texture, *C. curtis* is certainly a highly evolved form.

Probably *C. disseminatus* does not deviate from this very much. Judging by Kühner's figures (*1928*: pl. 1 fig. 1-8), protenchyma is present in the veil here as well, and on the outside of the veil and along the stipe, spherocysts occur. So these species, except for the dermium and the hairs, can very well be linked with certain *Vestiti*, although *C. patouillardii* has no spherocysts along the stipe.

The veil of *C. hexagonosporus* is different from the beginning. Besides some few spherocysts, we also find inflated elements with long, tapering necks here. The pilocystidia that occur later are different from these veil hairs. Because of the special nature of the veil, we can endorse the conclusion of M. Lange (*1952*: 110) in which he states: 'This species is probably the best link between the *Nudi* and *Farinosi*.'

We found these three species to be rupthymenial, although Kühner also draws a section with a continuous layer beside an interrupted one. Apart from that, *C. disseminatus* is of the type that becomes rupthymenial very quickly through the activity of the gill-trama.

The layer of spherocysts in the veil in these species is probably in a state of reduction, since *C. hexagonosporus* points to that conclusion. In this way, we could derive the *Nudi* among the *Setulosi* from this group with spherocysts. From a number of distinguishing characteristics, we can conclude that the *Setulosi* without spherocysts are a very specialised form.

We consider specialised hairs (pilocystidia, caulocystidia) that occur over the cap and along the stem as an altered universal veil and not as an initial phase (see 'Veil and pileipellis'). These species have all been provided with a palisado (hymeno)-dermium, and in this group one finds the typical rupthymenial formation of hymenophore with groups of palisade-hyphae occurring even before there is any growth in the trama of the lamellae. Here the remarkable thing is that deliquescence is often less strong or does not occur at all (*C. disseminatus*). According to M. Lange (*1952*: 108–130), deliquescence occurs in different degrees and is dependent on environmental factors. We are inclined to take the decreased deliquescence as a reduction: these small species with an often expanding cap do not need deliquescence. Although *C. heptemerus* deliquesces according to Lange, we had a culture of this species from Baarn, the well-developing fruit bodies of which did not deliquesce.

A somewhat amazing experience was discovering, in young primordia of *C. curtis*, that the initial ripening of spores occurred over the complete lamella. We have already mentioned the particulars in the description of this species. We could establish the same fact with very young specimens of *C. heptemerus* (cultivated). For these species, this disposes of the significant distinguishing peculiarity of inequihymeniferous fruit bodies, which is considered a characteristic of the genus. We also found that the initially formed spores were smaller than the ones which developed later (Clémençon, *1979*). Closer scrutiny of these smaller species could demonstrate that certain opinions concerning the genus *Coprinus* are generalizations.

Coprinus auricomus (Reijnders, *1974b*) occupies a very special position, which is not easily defined. Here the universal veil contains protenchyma, beside the known yellow-brown hairs which cover the greater part of the young primordia. It is notable that the bristle hairs which develop later in the pileipellis are larger and have a thicker wall than the original hairs in the veil. The species has a welldeveloped palisadodermium, but is levhymenial, and, in that respect, primitive.

It could be supposed that further reduction of the veil could have resulted in the origination of the *Nudi* (*Setulosi*) form types like *C. auricomus*. But we would rather derive this group from *Setulosi* with spherocysts via, for example, a type like *C. hexagonosporus*. It is quite possible that the numerically rich group of *Setulosi* without spherocysts is polyphyletic. Only a very exact knowledge of the development of a great number of species of this group could solve this problem.

The *Glabri* Lange or *Hemerobii* Fr. (sensu Fl. anal.) should also be considered. *Coprinus plicatilis* is the best-known representative of this small group. If we take the reduction hypothesis further, then this species (which has no veil at all) would stand for the final stage. There is neither universal veil nor lipsanenchyma. We called such forms gymnangiocarpous if the hymenophore has an endogenous origin. The palisadodermium occurs in an early stage and assumes huge proportions. The mature carpophore consists mainly of isodiametrical cells. The hymenophore is rupthymenial, although perhaps not in its most significant form. We unfortunately did not have a section of a primordium which was young enough to determine this exactly.

In Table II we have assembled the more or less probable relationships existing between sections and species of *Coprinus* discussed above; we are, however, very well aware of the fact that this table can only be approximate and incomplete because of the insufficient number of species studied, especially in the *Impexi* and in the *Setulosi*, and because of the difficulty in proving phylogenetic relationships.

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Résumé

Ce travail vise à approfondir notre connaissance des relations mutuelles d'espèces du genre *Coprinus* a l'aide de recherches ontogénétiques. La comparaison d'environ 27 espèces a mis à même l'auteur de préciser quelques critères du développement surtout en ce qui concerne la succession des structures initiales des parties principales du champignon, et l'origine rupthyméniale de l'hyménophore. La thèse que tous les Coprins sont du type inéquihyménifère est une généralisation inadmissible. Les variations dans les voiles et du piléipellis, si importantes pour la classification des espèces dans ce genre, ont été considérées intégralement; la terminologie générale des structures corticales, qui paraît être très confuse de nos jours, a été discutée en détail. Pour finir, l'auteur a essayé de dresser un schème des relations phylogénétiques dans le genre mais il faudrait la connaissance du développement d'un plus grand nombre d'espèces pour acquérir des résultats plus exacts dans ce domaine.

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