CRITICAL SURVEY OF THE CHARACTERS OF THE *AGARICALES* AS THE BASIS OF THEIR TAXONOMY

The basic characters used by taxonomists in the field of Higher *Basidiomycetes* have twice been augmented and revised in the history of systematics. We shall not review the characters on which the Persoonian and Friesian systems of classification were based, nor shall we repeat the fundamental discoveries in the field of anatomy and the revaluation of certain macroscopical characters that have taken place in the second half of the past century, inspired by the general search for the « Natural Classification » of the fungi, and by the activities of such influential mycologists as de Bary. All these facts are now duly understood and can be found in any good text book on mycology. The present treatment starts where a new development has taken the lead without as yet offering a comprehensive resumé of facts and results. The author (1936) has discussed this last period (starting in the early twenties of this century) as the third period in the development of systematics in the *Agaricales,* characterized by an immense accumulation of additional descriptive facts and new theories to explain them. The resulting modification in the appraisal of certain known characters and the addition of new characters now considered important is the subject of the following chapters.

I. THE VEIL

The veil in the widest sense, i. e. the involucrum in Persoon's terminology has been considered as being of the utmost importance in taxonomy as far as Fries and his school were concerned, and an improportionate overemphasis was put on it in such classifications

as those proposed by Karsten, Schröter, and especially Earle. This was partly understandable since the veil was considered as a first step toward the higher forms, or rather a criterion of higher forms as such. It is only fair to mention here that some authors still continue to think so — however with certain modifications, based on the difference in interpretation of veils.

Several basically different organs have been confused in the term « veil »

1. *The volva.* This is a general enveloping layer in the « egg stage » of the carpophores and subjects the primordium to a certain centripetal pressure. It is never thin and arachnoid. A volva has been observed in the *Agaricaceae* (*Clarkeinda*), the *Amanitaceae* (*Termitomyces*, *Amanita*, *Volvariella*), the *Coprinaceae* (*Coprinus* spp., *Macrometrula*), and in a more reduced, indistinct or fugacious form in other related genera. In all these forms, the volva remains more or less distinct in the adult carpophores as a cup or concentric scales at the base of the stipe, and/or as coarse warts or volva-patches on the surface of the pileus. Parts of double annuli viz. the outer-lower portion of the annulus of *Catathelasma* and some species of *Agaricus*, probably also *Rozites,* may logically be considered as a special form of volva where, on the lower part of the stipe, the volva is appressed or reduced to an innate covering. (Atkinson's blematogene). The annuliform, i. e. ring-like portion of the volva may be referred to as an « annular volva » or the «volval portion of the annulus ». The counterpart of the agaric volva in the *Gastromycetes* is the volva or so-called peridium of the *Phallineae,* the genera *Montagnea*, *Gyrophragmium*, *Battaraea*, some species of *Tulostoma*, and *Torrendia.*

2. *The pellicular veil and the cortina.* These are remnants of a layer or all the layers of the cortical tissue of the primordium, and are later ruptured by the expansion of the pileus whereby they are extended and thinned. If the cortical layer is gelatinized and the stipe absent, this kind of veil is called pellicular veil by Lohwag ; if it is dry and arachnoid, and the carpophores are stipitate, it is, since Friesian times, called cortina. Both pellicular veil and cortina are essentially the products of hemiangiocarpous development of the hymenophore in the young carpophores, and should not be applied unless it is reasonably certain that the organ referred to belongs to a species with hemiangiocarpous development. The thinness of this veil is an important feature because the layer taking part in its formation is a thin layer from the start, and is reduced by the tension

from the margin when the pileus expands. It is conceivable that, phylogenetically, the pellicular veil as well as the cortina may be derived from the volva.

3. *The marginal veil.* This type of veil formation is due to the incurving margin whereby the covering layer of the apex of the stipe is brought in intimate contact with the tissue of the margin of the pileus. Later, when the pileus is expanded, the separation may not take place exactly at the plane of the original contact but parts of the marginal tissue take part in the formation of an organ that consists largely of an outgrowth of both marginal and stipe-hyphae, and is stretched by the reopening or the margin. If the final separation takes place near the surface of the stipe, the marginal veil will then hang down from, or adhere to the margin of the pileus; if, however, the separation takes place farther outside, an annulus will be formed that remains on the apex of the stipe or slides down it. In the first case, it is called velar appendiculation of the margin, in the second, marginal annulus on the stipe. If separation takes place on both stipe and margin of the pileus, the annulus mobilis results. The marginal veil can best be studied on such species as *Boletinus cavipes* or *B. appendiculatus*, in *Macrolepiota procera*, *Chlorophyllum molybdites*, etc. In the two species of *Boletinus* the development of the hymenophore in the young carpophores is pseudoangiocarpous (see p. 30); in *Macrolepiota* and *Chlorophyllum*, it is hemiangiocarpous (see p. 30).

4. *The annulus superus.* This organ has, as receptacle, first been distinguished in the *Phallales* (*Gastromycetes*) where it has no velar character. However, Lohwag (1926) showed convincingly the homologies of the organs of *Phallus* and *Clathrus*, *Dictyophora*, and other Phalloids with such agarics as *Amanita*. It turns out that we have reason to consider the so-called annulus superus, or apical veil (Lohwags « Manschette ») as corresponding to the receptacle of the *Phalloideae*. It is not an annulus of the kind that was discussed under the term of « marginal annulus » above nor is it part of the volva, but rather is it formed under pressure of a volva against the pileus and herewith against the hymenophore growing into the covering tissue of the young apex of the stipe. Such outgrowths of palisade-structures against dense tissue tend to be pseudoparenchymatic and if tramal elements are involved, partly pseudoparenchymatic according to Lohwag, and others. The « annulus » of the Amanitas is, indeed, mainly composed of isodiametric and inflated elements. Limiting ourselves to the agarics, we, consequently, distinguish the apical

veil or annulus superus from other annular formations by its origin which is not marginal nor volval but hymenophoral. It may be suspected that some smaller or larger portions of certain annuli in the agarics (e. g. *Stropharia coronilla*) belong to this category but since the apical veil can, a priori, exist only in a volvate species where the margin is not strongly enough incurved or convex to separate most of the hymenophore from the stipe, the chances are remote. The typical example of an annulus superus is *Amanita caesarea* and its relatives.

The above categories do not seem to be immediately applicable to all velar formations. It is not fully proved that all the annular formations of the boletes are actually marginal annuli as long as their development has not been studied. Many velar formations are still puzzling, even if some ontogenetic hypothesis is temporarily admitted. We do not know exactly what to think about the annulus of *Chamaeota* and many other genera. In other cases, as was to be foreseen, the annulus is complex and its formation is partly that of one category of veils, and partly that of another.

However that may be, it is considered to be a wise course to continue using the general terms annulus and veil in the original sense where a closer interpretation would be mere guesswork.

The presence or absence of volva, pellicular veil, cortina, marginal veil, or apical veil (annulus superus) is not in itself, i. e. unless accompanied by correlated characters, a decisive character for taxonomic purposes. Very natural groups such as *Suillus* among the *Boletaceae* and *Russula* and *Lactarius* among the *Russulaceae* contain species with well-developed marginal veil and some without any veil, and the veiled forms are often more closely related to evelate forms than to other veiled forms. This is especially easily demonstrable in *Suillus*, sect. *Granulati*, and in *Russula annulata* (that has an evelate form). It is well known that *Amanita*, without any appreciable hiatus grades from forms with well-developed volva into such with friable volva which is often obliterated; and forms otherwise closely allied to each other, in the *Agaricaceae*, either have or lack a volva. The annulus superus is rather inconstant in certain species of *Pseudoamanita* (subgenus of *Amanita*) and *Vaginaria* (subgenus of *Amanita*), e. g. *Amanita gemmata* and *A. fulva*. In the genus *Gymnopilus*, closely allied species are distinguished almost exclusively by the presence or the absence of the cortina.

On the other hand, some significance should be conceded to the

veil especially on the species level in spite of the fact that in some particular cases (the amanitas named above, *Russula annulata*, *Agrocybe praecox*, and even *Suillus brevipes* and *S. Grevillei*) this character is not useful for the distinction of species. It can also be used in order to dispel ancient superstitions such as the alleged close affinity of the *Agaricaceae* and the *Amanitaceae* because of the presence of the annular veil wich has been established as of essentially different origin.

In a few cases, the anatomy of the veil has some significance, e. g. in the intrageneric taxonomy of *Gomphidius,* and possibly, in future investigations on the Amanitas.

II. THE SPORE PRINT

The only macroscopical character available that concerns the basidiospores in the *Agaricales* is the color of the spore print. This was first emphasized in a classification by Fries. However, Fries minimized (using words like «sordidae» for the description of the spore color), overlooked, or merely ignored certain complications that make it impossible to use his classification, even for an artificial system, without introducing important modifications.

1. *The green spored group.* This group of agarics, considered as a taxonomic unit in some artificial classifications, belongs in various families and genera in the *Agaricales*, viz. the *Agaricaceae*, *Amanitaceae,* probably the *Tricholomataceae,* and the *Boletaceae.* The green spored group has no place in the Friesian classification because Fries misinterpreted either the spore color (in *Phylloporus*), or the species (all the tropical green spored agarics); it has no place in the modern classification because it contains elements from four different spheres of affinities.

2. *The pink spored group.* In spite of the combination by Fries of the pink spored agarics in one group and the pink spored boletes in another, the former is not a homogeneous taxonomic group. The two largest constituents are *Rhodophyllus*, and the *Pluteeae.* The former group belongs in the large family *Rhodophyllaceae,* and the latter in the family *Amanitaceae,* none of them related to the other. Fries, and the key-writers following him, especially Saccardo, paid no attention to the fact that there are many other agarics with pink spores, and in order to get to the right genus, in their schemes, it is necessary,

as is so often the case, to assume the spores to be white rather than pink. This holds true for such genera as *Rhodocybe* (*Rhodophyllaceae*), some species of *Clitopilus* (id.), *Phyllotopsis, Schizophyllum,* several common *Collybiae,* one or two species of *Amanita* and the *Agaricaceae* (*Leucocoprineae*).

3. *The yellow spored group.* There are numerous species in several genera of «white spored agarics» and «white spored boletes» (the latter term is superfluous since no such thing exists) that have yellowish-cream-colored to ochraceous or citrinous spores but have passed as white-spored because of errors of observation. Part of the error of the observation was due to the fact that in obtaining the spore print, the pilei were formerly (and still are according to the recommendations of recent books) put over black or blue paper, the latter in the erroneous assumption that this color did not occur in basidiomycete spores. In order to discover pale colors which are easily misinterpreted as white on a dark background, it is necessary to use paper as pure white as is used and recommended by Crawshay (1930). This is also true for the paler tints of pink.

4. *The black spored («melanosporous») group.* This group intergrades with the brown and purple spored groups at certain levels, as has been recognized by Britzelmayr and other earlier writers. The Friesian *Melanosporae* fall now entirely into the *Coprinaceae* with the single exception of the genus *Gomphidius,* which belongs in the *Gomphidiaceae,* near the boletes. Even *Lacrimaria,* once wrongly incorporated into the purple spored group by Fries, is now considered as belonging to the *Coprinaceae.*

5. *The brown spored («ochrosporous») group* is not a homogeneous group as was anticipated by Fries. Some genera, in their present, narrowed sense, come close to, and form a parallel series with the *Stropharia-Naematoloma-Psilocybe-Deconica-Melanotus* group, a series so closely related that it is often difficult to separate the corresponding genera and sections of both series. Another series parallels the *Coprinaceae* in a much less strict manner, and has since been separated from the other *Ochrosporae* as a family by itself, the *Bolbitiaceae.* It differs from the *Cortinariaceae.* In the *Cortinariaceae* we have again two parallel series, already partly recognized by Fries; one contains the genera with argillaceous-fuscous spore print such as *Inocybe* and *Hebeloma,* the other the genera with vividly rusty colored spore print such as *Cortinarius* and *Gymnopilus.*

The above examples show that the spore print colors are not as

such, and in themselves, indicative of an affinity between groups according to general classes of colors (white, pink, purple, black, brown, etc.). They can be used on the family level only if modified by other correlated characters, and only on a lower level can they be used as the leading characters of taxonomic groups. This shows that Fries' discovery of the spore print colors as a taxonomic character of first grade importance was certainly a fortunate and valuable contribution to the systematics of the *Agaricales,* however it should be used with reason, without generalizations, and never in a spirit of dogmatic schematism.

The colors observed in fresh spore prints are apt to change in the herbarium due to further dehydration, and in some cases they loose the olive hue, so characteristic for the spore print for several genera of the *Boletaceae,* in other cases they bleach to almost white, after having been a distinct vinaceous pink in some species of *Tylopilus* (*Boletaceae*) while in *Russula, Melanoleuca, Cantharellula,* and other « white spored » agarics, the pale colored fresh spore print eventually darkens to decidedly cream color or ochraceous, especially if prepared with some fixative. In *Gomphidius* the deep fuscous or olive-black spore print becomes deep rusty brown in a few years of preservation. Since many tedious observations by the author (1945-1946) have shown that the taxonomically important differences are found in the fresh non-dehydrated spore prints, it is necessary to identify the color immediately with the help of a good color chart [2]. The pale

[2] Many mycologists, unfortunately, do not use charts at all but rely on color terms that do not mean the same thing to other people, especially when translated into foreign languages. Some still use Oberthuer, or Klincksieck, but the majority uses Ridgway, *Color Standards and Color Nomenclature,* Washington, D. C. 1912. This book is now difficult to obtain, and besides has the disadvantage of being subject to drastic color changes in the plates after some time of exposure to light. Therefore, many scientists use a newer chart, Maerz, A. and M. Rea Paul, *Dictionary of Color,* New York, 1930. The plates are said to be light resistant, and besides the number of colors shown is larger than in Ridgway, especially in some colors frequently found in *Agaricales.* The richest and most vivid colors of spore prints, such as those of *Gymnopilus,* are nevertheless often hard to match in any color chart, and until a special chart for these tinges is published, the mycologist will do well to get the nearest approximately corresponding number, adding « deeper », or whatever the difference may be. The spore prints between pure white and deep ochraceous, such as found in the *Russulaceae, Melanoleuca, Drosella,* etc. should be compared with Crawshay's plate (Crawshay, *The Spore Ornamentation of the Russulas.* London 1930).

tints should be rigorously observed on paper of the whiteness of that used in Crawshay's (*l. c.*) plate; the discussion of even whiter ground colors (salts, etc.) is rather theoretical than practical.

III. THE MYCELIUM

A. Cultural characters

The mycelium has not been used thus far for taxonomic purposes on a large scale. It is obvious, however, that differences of color, zonation, consistency and manner of growth in standard cultures, as employed for *Porias* by Baxter should also be of diagnostic value in the *Agaricales*. We know that some species of *Agaricales* have luminescent mycelia. There is now available a rather long list of agarics with luminescent mycelia, a character, with certainty demonstrable only in laboratory cultures. Some species have mycelia with a characteristic odor. This character can be used for the determination of ectotrophic mycorrhiza. The mycorrhiza of *Russula punctata* and *R. Dadmunii* has a characteristic odor of iodoform which can be obtained in test tube culture.

Some mycelia form sclerotia (see under B), rhizomorphs (see under B), oidia (or what is called so in the literature on life cycles and sexuality of the *Basidiomycetes*), conidia, chlamydospores (or chlamydosporoid oidia), oleiferous hyphae (see chapter IX), and even mycelial basidia, and mycelial cystidia. The latter have been named allocysts by Kühner, a term that should be accepted in view of the original definition of the word cystidia. These allocysts often resemble the cystidia or cheilocystidia of the hymenophore of the same species, or of allied species, but in other cases, they do not remind one of any analogous bodies in the carpophores. All these characters will undoubtedly be used for taxonomic purposes as soon as more data become available. The main difficulty here arises from the variance of conditions necessary to grow mycelia of *Agaricales*, and even so, the mycelia are often short-lived and obviously not in normal growing condition. Under these circumstances, a standard method that makes cultures possible and comparable for taxonomic purposes cannot yet be indicated. Most non-mycorrhizal fungi can be grown on malt agar and on Lütz' synthetic medium, also in liquid media of analogous composition. A widely applicable medium has been indicated by

Kühner, and it has been tested, along with many other media, by the author. It is a modified Lütz medium [3], which appears to be suitable for almost all non-mycorrhizal species and many mycorrhizal species of the agarics and boletes. The cultures can be started from spores, from the internal tissue of the pileus or stipe, or from the hymenophore (hymenium plus subhymenium). The separation of the pieces to be inoculated should be made under binocular in order to avoid infected places; the interior of young and fresh carpophores is safest in regard to possible contamination. Bacterial contamination is most difficult to avoid in many cases, and separation of the fungus mycelium from the bacteria is not always possible. The culture methods indicated above cannot be applied to certain species of *Amanita* and certain boletes and *Gomphidius,* certain *Cortinarii* and *Russulaceae.* Their culture requires special techniques, e. gr. sterilisation by filtration through a bacterial filter (Seitz or Berkefeld), addition of growth substances, root extracts, etc. In a few cases, all attempts at culturing have thus far been unsuccesful.

A pure culture is also necessary for studies on the sexuality of the *Agaricales* (see chapter XVI). In this connection it is often necessary to start from a single germinating spore, and later confront the resulting primary mycelia. As for the technique involved, the reader is referred to Vandendries's papers (see literature); some interesting technical information can also be found in Kühner's *Recherches morphologiques et caryologiques...* (1946).

[3] The formula used by the author :

Water	1000	gr.
Difco agar	25	»
Vitrums Maltextrakt (Stockholm)	10	»
Ammonium nitrate	1	»
Ammonium phosphate	1	»
Magnesium sulfate	0.1	»
Ferric sulfate	0.1	»
Manganese sulfate	0.05	»

As has been pointed out by A. B. Hatch & C. T. Hatch (*Journ. Arnold Arb.* **14** : 325. 1933), the American brands of malt extract are not suitable. The brand obtained from Apoteksvarucentral, Stockholm, Sweden, proved to be superior to American brands in all cases.

B. Characters observed in nature

On the base of the stipe or the point of attachment of the pileus to the substratum, a tomentose or strigose or silky-arachnoid mass or mat of hyphae is observed in many species; in others, white or colored strands of hyphae are macroscopically visible and can be followed through the ground or substratum. In the first case, these mycelial formations are called mycelial tomentum, or basal tomentum; in the second case, they are known as rhizomorphs. In both cases, they are frequently useful characters for the systematist, especially in the *Gomphidiaceae*, certain *Agaricaceae*, *Boletaceae* and *Tricholomataceae*. A special form of mycelial tomentum formed in advance and independent of the formation of carpophores is the *Ozonium* of *Coprinus radians* [1].

The mycelial tomentum differs mainly in color, according to the species or variety; also in the degree of development and in consistency.

The rhizomorphs can be subdivided into:

1. True, eventually black, rhizomorphs, and
2. White mycelial strands.

Though admitting that rhizomorphs are usually constant specific and perhaps sectional characters, one will agree with De Bary who says (1887, p. 22) «that the formation of strands is not necessarily found in all the species that belong to the cycles of affinity indicated [by their family and generic names]; on the contrary, it may be wanting in one of two nearly allied species, and be found in the other».

Other formations of the mycelium are: the pseudosclerotia, sclerotia, perennial pseudorrhizae, cryptas, mycorrhizas, and sterile carpophores. The latter will be discussed in subsequent chapters since they are rather a modification of the seasonal basidiocarpous formations, whereas the others are not in any way homologous with the carpophores, and can rather be characterized as special organs where primary functions are either long term resistance, storage of food material exchange of nutrient substances with the rootlets of the

[1] Excepting the *Ozonium*, we can synonymize the mycelial tomentum with Fayod's « mycelium secondaire », or De Bary's secondary mycelium.

mycorrhizal symbiont. In the first category belong : black rhizomorphs, in the second — sclerotia, perennial pseudorrhizae, and in the third — the cryptas and mycorrhizae. The pseudosclerotia are probably without function, and merely a result of processes of extracellular assimilation of substratum with a certain dense hyphal growth in a well circumscribed sphere of the mycelium.

There are, therefore, the following three groups of mycelial formations :

1. *The pseudosclerotium.* This is a mass of substratum (mineral or humus particles, or wood) held together by the mycelium so as to form definitely circumscribed bodies resembling sclerotia. Such formations are characteristic in *Polyporus tuberaster, Phlebopus colossus, Panus velutinus* (Pl. IV).

2. *The sclerotia, perennial pseudorrhizae, and the black rhizomorphs.* The sclerotia differ from the pseudorrhizae and rhizomorphs in shape. The sclerotia are usually bulbous or ovoid-ellipsoid to globose bodies, either immersed or superficial ; the perennial pseudorrhizae are root-like hypogaeous bodies which are vertically elongated ; the black rhizomorphs are horse-hair-like filaments. Sclerotia are found in *Pleurotus tuber-regium* (Pl. III) where they are very large, in *Agrocybe tuberosa* (medium sized), and in three *Collybiae* where they are small.

Sclerotia (myceliums persistants tuberculeux) have been subdivided by De Bary (1884) and Fayod (1889) into mycelial tubercles, exosclerotia, and endosclerotia. The latter has not yet been found in *Agaricales*. However, the first two types are represented in this order. Mycelial tubercles are those sclerotia «where one can oppose to their morphological base, one or several points from which the stipes of the carpophores rise at germination» (Fayod 1889). As an example of this kind, Fayod indicates *Collybia tuberosa*. In the exosclerotium, no such points of germination are present, and any cell or group of cells in the cortical layer of the exosclerotium is apt to produce carpophores ; yet, their morphological base (the «hilar» end) is usually recognizable all through its development. Such a sclerotium is formed by *Coprinus stercorarius, Collybia racemosa,* and *C. Cookei*. It is surprising to find both types represented in one single section of *Collybia*.

Perennial pseudorrhizae have been studied by Buller (1934). They represent the perennial base of the annual pseudorrhizae of the carpophores branching (underneath the earth and close to or inside the substratum) into several individual carpophores. A special term

for the annual pseudorrhiza is necessary since the latter is merely a subterranean (or submerged) part of the carpophore, more precisely of the stipe, and often exists alone, directly rising from the mycelium rather than from the perennial pseudorrhiza. Neither the perennial pseudorrhiza nor the annual pseudorrhiza should be confused with the rhizomes of higher plants with which they are not homologous (though analogous in several respects).

The strange short-lived, soft, sclerotium-like body from which *Tricholoma sclerotoides* Morse is said to arise, is neither quite comparable with any well known type of sclerotium, nor is it comparable with the similar formations known in *Rhodophyllus abortivus*. They may temporarily be kept in a separate group without a definite term.

3. *Cryptas* are sleeve-like formations around tree roots (especially *Coffea, Citrus* and other trees of the evergreen kind) in tropical and subtropical countries. These organs of the fungus provide shelter for certain scale insects between them and the roots and rootlets of these trees. They are the morphological expression of a strange and highly complicated coexistence of various organisms, living partly in epibiosis, partly in symbiosis, and partly in a parasite-host-relationship.

Mycorrhizae are more tender structures consisting of mycelial hyphae enveloping only the thin rootlets and root hairs of certain trees, mainly conifers, *Salicales, Fagales, Urticales, Columniferae* (mainly *Tilia*) and *Ligustrales* (mainly *Fraxinus*) [5]. More precisely, they should be referred to as ectotrophic mycorrhizae. The mycelium of roughly one half of the species of *Agaricales* may be considered as potentially mycorrhiza-forming. This figure is the result of a rough calculation on the basis of syntheses made between fungus and tree in laboratory experiments plus a cautious generalization for the taxonomic groups involved insofar as field observations confirm an ecologic situation similar to that found in experimental studies with closely related species. It is probable that the situation in the tropics differs slightly from that in the temperate zones with the non-mycorrhizal fungi possibly favored under tropical conditions. In ectotrophic mycorrhiza, the phanerogamic symbiont may be furthered in certain phases of its development by the association but it can also be grown — or can grow in nature — without interference of the fungus-sym-

[5] In some other families, mycorrhiza is also found but it is highly doubtful that the fungus species involved belong to the *Agaricales*. At least no data supporting such a relationship are available at present.

biont. The fungi, however, in a large number of cases, do not seem to be able to develop normally unless the connection with the phanerogamic symbiont is established, i. e. unless mycorrhizae are formed. The fungi forming the ectotrophic mycorrhiza of this type are often highly specialized. This specialization of the ectotrophic mycorrhiza gives into the hands of the mycologists an additional character comparable to that available to the student of parasitic fungi of certain groups such as the *Uredinales* and *Exobasidiales* among the *Basidiomycetes*. It must, however, be kept in mind that laboratory experiments neglecting the specific soil conditions and microfloristic features (competition) of the natural habitat tend to obscure rather than elucidate the question of specificity of a given fungus whereas field observations, especially if made with insufficient skill or care, are often inaccurate or inconclusive, or else too limited in their purely regional importance. It has been emphasized by Melin (1936) that field observations are very important and desirable as an indicator for the planning and setting of further experimental study; the same may be said for taxonomy. There is undoubtedly a connection between taxonomic problems and specificity of fungus symbionts as to their mycorrhizal hosts. A temporary tendency to determine this relationship with geobotanical methods (Zinserling, 1922-1924) may be interesting for ecologists but it does not help materially to make field observations on mycorrhiza relationship more precise. A single coniferous tree, even a seedling, in a broad-leaved-stand is apt to alter locally the aspect of the myco-flora as expressed by the population of carpophores within a circle with a radius slightly larger than the spread of the root-system (i. e. up to 30 ft.); herbaceous species of *Salix, Betula* and *Quercus* as well as seedlings of larger trees are often overlooked in stands of more conspicuous but different trees, and wrong conclusions are likely unless a careful survey of all plants of a given locality and a comparative study of other corresponding localities is made.

Agaricales are also involved in another kind of mycorrhiza, the so-called endotrophic mycorrhiza where the hyphae of the fungus enter the tissue of the roots, and are assimilated by the plant. In some tropical orchids, it has been shown that the fungus hyphae belong to agarics such as *Armillariella mellea, Micromphale javanicum* and probably *Gymnopilus aculeatus*. In these cases, the orchids are as dependent on the presence of the fungus as, in ectotrophic mycorrhiza, the fungus is dependent on the higher plant, and ger-

mination can be achieved under ordinary laboratory conditions only after a synthesis of fungus and seed. On the other hand, the fungi named above are by no means dependent on the orchid for their normal development, nor are they in any way specialized as many of the ectotrophic mycorrhizas are. For example, *Armillariella mellea* is almost cosmopolitan and grows abundantly as a wood parasite and also saprophytically in wide boreal areas where none of the mycorrhizal orchids occurs. The same is true for *Micromphale javanicum* and *Gymnopilus aculeatus* which are subtropical-tropical species, yet not specific for orchids but growing on all kinds of *Monocotyledones*, either as symbionts, or as parasites, or as saprophytes, mostly the last.

IV. NON-BASIDIOCARPOUS CARPOPHOROIDS AND ABORTED CARPOPHORES

Sterile bodies that have no visible purpose but are formed the same way and under similar conditions as the normal basidia-bearing carpophores have been observed in *Agaricales* of various groups. They are here called carpophoroids. The only explanation of these strange, apparently function-less bodies is that of an atavistic aberration whereby a gastroid form is occasionally maintained in normally gymnocarpous or hemiangiocarpous or pseudoangiocarpous species, which often leads to sterility. We have such an example in *Boletus rubellus* ssp. *caribaeus* which is evidently comparable to the fertile gastroid forms observed in *Boletinus decipiens* but has never been seen to form basidia or spores. In this case we observe that the carpophore fails to ever achieve the last stage of its individual development after it once, probably exceptionally, reached its angiocarpous phase. These aborted individuals are, however, rather rare and usually constitute only a small percentage of the total local population.

On the other hand, there are several examples, where sterile masses of carpophoroids are formed regularly either by a large percentage of the local population of a species, as in *Rhodophyllus abortivus* (Pl. VI) or the abnormal fruiting bodies completely replacing the normal basidiophorous form on a local scale, as in *Panus tigrinus*. Further investigation of these forms, especially in culture, and a study of their cytology may throw more light on them in the future. The abnormal, sterile forms of the species named above are so distinctive

that apparently they were considered taxonomically different from the normal carpophores by some mycologists. In fact, authentic specimens as well as the type collection of the type (and only) species of the genus *Acurtis* prove that *Acurtis* is the carpophoroid form of the *Rhodophyllus,* not a clavariaceous genus, just as *Lentodium* is the carpophoroid of *Panus tigrinus* rather than an independent genus. In the latter, the hymenophore is transformed into an irregular hyphal mass through which elongate holes run in all directions (reminiscent of the gleba of some gastromycetes). The author has seen only completely sterile forms of this aberration, and in this condition, it may properly be called a carpophoroid. Other authors indicate spore formation in the aborted hymenophore. In this case, the phenomenon appears to belong in the same category as the other common anomalies in agarics such as the pore-bearing forms of *Agaricus, Clitopilus,* etc., the so-called *Ptychella*-forms, and many other aberrations which do not occur regularly with the normal carpophores, nor do they ordinarily form entire populations. The *Lentodium*-form of the *Panus* is in a sense intermediate between an ordinary anomaly and a typical carpophoroid. However, even in typical carpophoroids as in the *Acurtis*-form of *Rhodophyllus abortivus,* transient forms are occasionally observed. These show a pileus and a stipe and a zone that must be considered as hymenophoral. In this zone, sometimes occasional spores can be found, some of them formed on basidia, others directly from hyphae; in both cases, they tend to be thick-walled. The fibrillose, white outer layer of the carpophoroids tends to break off in these intermediate forms, and assumes the character of a «general veil», resembling a rudimentary peridium. These observations seem to leave little doubt but that these carpophoroids or *Acurtis*-forms of *Rhodophyllus* are actually gastromycetoid forms comparable to those of *Boletinus decipiens.*

Carpophoroids of a similar type have been observed by the author in a species of *Marasmiellus* from the Philippines, probably *Marasmius pandanicola* Henn., and closely related to *Marasmiellus semiustus* of the Neo-Tropics. Here, as in the *Acurtis*-form, many carpophores of a population are transformed into brain-shaped or amorphous sterile masses, much like those of the *Rhodophyllus* but less fleshy and smaller.

V. STILBOIDS

In other cases, the sterile, non-basidiocarpous formations with carpophore-like appearance have a definite function as propagula, and are not at all comparable with the aberrations named above. This is the case with the « gemmae » of what is described as *Omphalia flavida* Maublanc and Rangel [6], and has been studied by Buller *(Res. on Fung.* 6 : 387-443. 1934). Here, a sterile carpophore is formed that has a separable capitellum (« pileus ») which is blown by the wind from one leaf to another and thus serves for vegetative propagation, in this case of the epiphyllous phase of the life cycle of the fungus. The « gemmae » do not form any basidia but the capitella attach themselves to the leaf by their gelatinosity and the hyphae start immediately to form new exogenous mycelium parasitic on the leaf. It is especially interesting to remember that certain species of *Mycena* and *Marasmiellus* — as has been shown by the anatomical studies of Kühner (1926, 1938) — have the stipe actually separable from the pileus by an intermediate zone of different structure. The carpophore-like bodies of *Mycena flavida (Omphalia flavida)* were misunderstood by Cooke who described them in a genus otherwise without any relationship with the *Agaricales,* as *Stilbum flavidum* Cooke. The term « gemmae » used by Buller, and the implication of abortion of the fruitbodies found in Maublanc & Rangel's account *(Bull. Soc. Myc. Fr.* 30 : 41. 1914) are both inadequate or misleading in view of the evidence at hand, and therefore the term stilboids is proposed.

VI. CONIDIAL CARPOPHORES — IMPERFECT FORMS

It is well known that conidial carpophores are frequently formed by *Ascomycetes* such as *Xylaria,* and *Aphyllophorales* such as *Ptychogaster.* The controversial genus *Lycoperdellon* may be an example of a conidial fructification of a gastromycete. In the *Agaricales,* no such examples were known until now unless one would see an instance of conidial fruiting bodies in those specimens of *Asterophora (Nyctalis)* where the basidiospore production is (almost) entirely suppressed in

[6] This species, according to the descriptive data available, belongs to the genus *Mycena* although the spores are nonamyloid.

favor of chlamydospores. However, in this case, no modification of the carpophore is observed, and a hymenophore, potentially apt to produce basidia and basidiospores is always formed although sometimes in rudimentary form.

Yet, in recent observations, the author has been able to discover a typical instance of modified conidial carpophores in an agaric of the mountain region west of Tucumán, a new species of the genus *Armillariella, A. ditopa* Sing. ined. (Pl. XXIX). This species, before forming the basidiocarpous fructifications (which do not develop except under optimal temperature and moisture conditions) develops a simple clavarioid carpophore, entirely white and mealy from the innumerable arthrospores formed in palisadic chains on the surface of the clubs. The anatomical and cytological study of the corresponding stages reveals that they belong to the same organism; the arthrospores are binucleate and consequently belong to the dicaryotic phase, as do the carpophores with basidiospores. The arthrospore formation continues on the stipe of the basidiocarps, and the arthrospores formed there are equal, in every regard, with the arthrospores of the conidial fructifications. It appears that the conidial fructifications are homologous to the stipes of the basidiocarpous fructifications. Under certain circumstances, especially after severe changes of the meteorological and microclimatic conditions in certain seasons, it is possible to observe the conidial and basidiocarpous fructifications together, rising from the same dicaryotic mycelium.

The term arthrospores is here used in the sense of Langeron. The chains are radiately arranged when observed in a cross section of the conidial fructification, and consist of hyphae which soon become densely septate and fall apart at the septa in an irregular manner, the resulting spores being consequently what is often called «oidia» of a rectangular to ellipsoid shape, the majority ellipsoid. They are much broader than the basidiospores, and also slightly longer.

Whether the *Sclerostilbum*- form of *Collybia racemosa* is another instance of conidial carpophores, or a young stage, cannot be decided at present.

VII. BULBILLOSIS — RHACOPHYLLUS FORMS

In rare cases, the carpophore of the *Agaricales* is sterile in the sense that sporulation is suppressed, yet the function of the basi-

dium is maintained by the formation of bulbils. These are terminal members of the subhymenium which become more or less isodiametric and more or less sclerotized. The bodies bearing bulbils, are consequently neither carpophoroids nor stilboids, nor true carpophores. They are called *Rhacophyllus*-forms and the phenomenon referred to is known as bulbillosis of the agarics. For more information on bulbillosis, see p. 104, and in the special part.

VIII. DEVELOPMENT OF THE PRIMORDIUM OF THE CARPOPHORE

It was Patouillard's conviction that all the carpophores [7] now usually classed as *Aphyllophorales* — i. e. his Aphyllophoracées — are gymnocarpous, consequently he named them « Gymnocarpes », and opposed them to the «Hemiangiocarpes» (virtually the *Agaricales*) and the « Angiocarpes » (virtually the *Gastromycetes*). It is still true that, with one or very few exceptions, the *Aphyllophorales* must be considered as gymnocarpous. The *Gastromycetes* are mostly angiocarpous and only a few of the species approach the hemiangiocarpous type of development by becoming naked in a comparatively earlier stage, yet the shape of the hymenophore (gleba) is not such as to facilitate the discharge of the spores in the way of the *Agaricales*, and one is justified in letting them pass as basically angiocarpous. In some gastromycetes, the early stage of the hymenium is naked in the primordia and later becomes typically angiocarpous. For this form of ontogenesis, no special term has as yet been proposed. Consequently, the assumptions concerning the development of the *Aphyllophorales* and *Gasteromycetes* as made by Patouillard are still mainly sound. It is in the *Agaricales* where he erred by generalization. It is, as has now been shown by Kühner, and various other authors, not true that all, or even a large majority of the *Agaricales* are hemiangiocarpous. We now distinguish in this group four different types of individual development of the carpophores in regard to the position of their hymenium :

1. *Gymnocarpous.* The common type of development in *Russulaceae*, most of the *Boletaceae*, in numerous genera of the *Tricholomataceae*

[7] The term sporophore, frequently used in the sense of carpophore, is here rejected because it was first used for the basidium by Berkeley.

(Rhodopaxillus, Leucopaxillus, Mycena, etc.). Here, the hymenium is formed on the outside in the very earliest stages and remains so all through the stages of development.

2. *Pseudoangiocarpous.* The primordium has initially naked hymenial surface which later becomes internal by the incurving of the margin, and, at maturity, becomes exposed again by the expanding of the margin. This type of development is found in the veiled *Russulaceae* and *Boletaceae,* also in the veiled *Lentineae* (*Tricholomataceae* etc.).

3. *Hemiangiocarpous.* This type of development is common in the dark spored agarics, especially the *Strophariaceae,* and also in the *Amanitaceae,* probably most or all *Cortinariaceae* and *Agaricaceae.* The primordia have the hymenium formed on the inside, even in the earliest stages, and later become naked, approximately at maturity.

4. *Angiocarpous* (= *endocarpic*). Here, the fruiting bodies are either permanently angiocarpous (e. gr. the gastroid forms of *Boletinus decipiens)* (Pl. XXV, 5) except perhaps for the very earliest stages of the primordia, or they expose the hymenium by longitudinal rather than horizontal scission well after the first spores have attained maturity (as in *Galeropsis).*

The direction and the limitation of the hymenium in the course of development has also been studied by various authors, e. gr. by Kühner (1926), in the small and undoubtedly natural genus *Lentinellus.* The results are thus far not encouraging for the taxonomist; they show two opposed types of development within this same genus.

However, the internal or external development of the hymenium, and the variants of these two types, as described above, seem to have a distinct correlation with other important characters, and should not be neglected. On the other hand, lack of sufficient data on important species, even genera, makes premature conclusions rather dangerous.

IX. STRUCTURE OF THE CONTEXT OF THE CARPOPHORES

In most true *Agaricales* it is possible to distinguish two kinds of tissue which are called (according to Fayod, 1889):

1. The fundamental tissue, and
2. The connective tissue.

Ordinarily, i. e. in the homoiomerous [8] structure, the fundamental tissue consists of hyphae rather than of sphaerocysts; these hyphae are broader, firmer, and straighter than those of the connective hyphae but there is no fundamental difference as to their filamentous character which is the same in both kinds of tissue. This latter structure is typical for all families of the *Agaricales* except for the *Russulaceae*.

In some forms the fundamental hyphae are strongly thick-walled, sclerotic, and tough, thus showing the anatomical basis of the « tough » to « leathery » consistency of the carpophores of certain species, or genera. However, the relative thickness of the wall is not by any means a direct measure of the relative toughness of the carpophores. *Trogia cantharelloides* has rather uniformly but moderately thickened hyphal walls, yet, the carpophores are definitely tough and reviving, whereas *Pleurotus ostreatus* has a majority of rather strongly thickened hyphal walls even in the hymenophoral trama, but is generally described as fleshy and putrescent rather than tough and reviving. The string- or horse-hair-like appearance of the stipes of some species of *Marasmius* and *Micromphale* is due, in addition to the thickness of the walls of the cortical hyphae, to an intimate coherence of the hyphae which are often plugged into each other by means of alternating thorns or spurs fitting into a depression in the neighboring hyphae.

In certain species, the connective tissue is absent, and here we have reason to suspect that we deal with agaricoid forms of lower groups, e. g. in *Cantharellus cupulatus* Fr., as was pointed out by Kühner (1943) who refers to this species as *Omphalia rustica*. It may, however, be wiser to not include the character of differentiation of the tissue into the diagnosis of the *Agaricales* because it is perfectly possible that a reduction of the connective tissue in certain reduced forms does not necessarily mean that these species have no affinity with other *Agaricales*, especially if a well developed subhymenium is present. On the other hand it is often observed that the fundamental

[8] The terms « heteromerous » and « homoiomerous » are also used in lichenology, in a broader sense, yet they have a similar meaning. The alternative terms « heteromorphous » and « homomorphous » have been used for fungous tissues composed of identical and different elements respectively and cannot therefore be employed for this particular case of heterogenity ; they are, at present, used preferably for the characterization of the hymenophoral edges as compared with that of the remaining part of the hymenium (see p. 46).

hyphae are either transformed into a pseudoparenchymatic tissue, or replaced by vascular hyphae.

For instance, in the *Russulaceae,* the fundamental tissue is very well differentiated from the connective tissue because it consists almost entirely or entirely of « nests » of sphaerocysts (Pl. XIX, 5). This structure has also been observed in certain gastromycetes (family *Astrogastraceae,* as it is called by Malençon and Heim). It is called heteromerous by G. Beck (1922).

Generally speaking, the tissue of the stipe is usually somewhat denser and more fibrous or cartilaginous, at least in parts, than that of the pileus and the hymenophoral trama, and a sudden transition from the tighter packed hyphae of the stipe to the looser tissue in the pileus may account for what is often described as « pileus and stipe non-continuous », or « distinct », or « separable ». There is, however, rarely a sharp line between continuous and discontinuous stipes in the *Agaricales,* and this character is neither constant nor particularly helpful for determination in most instances. The only case where the separability of the stipe from the pileus is at present applicable with a definite anatomical meaning, is that discovered by Kühner (1926) in *Mycena and Marasmiellus* where a separation layer consisting of hyphae of a different kind is imbedded between the longitudinally arranged hyphae of the stipe and the larger spreading hyphae of the pileus. The separation layer is probably homologous with the layer separating the stalk and the head of the stilboids in *Omphalia flavida.* It can be used as a specific and sectional character.

In many species we find zones of the context or the entire context gelatinized, i. e. the hyphal walls produce a gelatinous matter into which they are finally imbedded and by which they are separated from each other (Pl. XIX, 1 ; XX, 4). In typically gelatinized tissues, the hyphae of the gelatinous zone are immediately recognizable in 10 % KOH mounts by their strikingly loose arrangement, and as a rule, they are thin, and have thin walls and clamp connections [9]. There is, however, no sharp delimitation between gelatinized and

[9] The mucus forming the gelatinous mass can, in case of doubt, be easily demonstrated by Kühner's method (1933) : Dye the sections during several minutes in watery solution of cresyl blue where the walls of the hyphae take a beautiful vinaceous or mauve color but the mucilage remains colorless ; one differentiates subsequently in absolute alcohol which dehydrates them at the same time and permits the dye to be fixed on the mucus ; to stop the differentiation it is sufficient to pass the sections through xylol ; the mucus is then blue.

non-gelatinous tissues, as can be noticed more readily if the gelatinosity of the surface layers of pileus or stipe are studied. We shall see that there is not just a glutinous pileus (with hyphae scattered in the mucus) and a dry pileus, but all kinds of transitional conditions are seen. The same is naturally true about the gelatinosity of the context. Many tissues consisting of thick-walled hyphae produce a slight amount of gelatinous matter and the hyphae are moderately densely arranged. These tissues are interpreted differently by different observers [10].

One whole tribe in the *Tricholomataceae,* viz. the *Resupinateae,* and several genera such as *Dictyopanus* and *Phaeomycena* have partly gelatinous trama. The tissue of most typical *Boletaceae* is, to a certain degree, gelatinized which accounts for the soft, succulent context characteristic for most representatives of that family as well as related families. A distinct gelatinous layer is also observed in some species of *Crepidotus* (*C. mollis, C. uber,* etc.), so conspicuously so that such an astute observer as Patouillard, misinterpreted a very old specimen of a tropical *Crepidotus* as a tremellaceous species with holo-basidia, and described it as new genus, *Tremellopsis,* belonging allegedly, in the neighborhood of *Sparassis.*

Another interesting structure involving the fundamental and the connective tissue, is that of *Amanita* and related genera of the *Amanitaceae.* Kühner who justly gives credit to Boudier (1866) for having discovered it, describes it as follows : « In the Amanitas (and in the Limacellas)... the connective elements are assembled, end by end, into hyphae, as are the majority of the elements, the fundamental ones as well as the connective ones, in the other *Agaricales ;* the fundamental elements of the Amanitas and the Limacellas, however, especially those in the context of the stipe, are isolated and terminal at the tip of the ramifications of the connective hyphae ». (Kühner, 1945, p. 162).

There are also other than fundamental and connective hyphae in the tissues of the *Agaricales.* They belong to the conducting system, and serve for the secretion and excretion of substances, and in a general way, the transport of substances in the carpophore. It is here as elsewhere impossible to always clearly separate these elements from others on a morphological and physiological basis, as it is

[10] Patouillard, Lloyd, and Singer considered the trama of *Filoboletus gracilis* gelatinized ; R. Heim (1946) thinks it is not.

obvious that in many instances the functions are not limited to the specialized organs, or else the specialized organs have often lost their original function.

Heim (1931) has not maintained Fayod's sharp separation between the « laticiferous » and the «oleiferous» type of conducting elements. Yet, it may be that Fayod's division is basically correct in spite of the fact that, chemically, they seem to intergrade. It would appear that what « laticiferous hyphae» there are in the Russulas, should, according to the Fayodian terminology, be called « oleiferous hyphae », and they are the ones that according to him originate in the connective tissue, and are continued into the «cystidia» of the *Russulae*. In *Lactarius* and *Mycena* the vessels carrying the latex are called laticifers in a narrow sense, yet the resinous substance responsible for the acrid taste of many *Russulaceae* is found localized in the « oleiferous hyphae » of *Russula* as well as in the laticifers of *Lactarius,* as can be demonstrated by the acrid taste of the latex in many *Lactarii*. On the other hand, there are *Lactarii* with mild taste and abundant latex. Fayod believed (though he was not certain about it) that the laticiferous vessels actually originate in elements of the fundamental tissue.

Leaving the morphological aspect aside for the present, we are inclined to admit a temporary classification of the types of vascular bodies on the basis of their function and known chemical and physical differences rather than on their supposed origin.

There would then be the following types to be distinguished :

1. *The laticifers in the narrowest sense.* These carry latex, or are homologous to laticifers that do carry latex ; they do not absorb cresyl blue, do not become deep blue throughout the interior, and they do not necessarily become deep blue in sulfovanilline or brown in formalin. They are not sieve-like on the surface. Example : latex-carrying vessels of *Lactarius volemus,* or *L. nigroviolascens* (Pl. XVIII, 5; XX, 1).

2. *The oleiferous* [11] *hyphae in the sense of Fayod.* These do not carry latex, but often carry resinous substances associated with an acrid

[11] The word is somewhat unfortunate since it specifies the contents, yet the contents are complex and variable, and organic « oils » are certainly a minor factor, if at all. However, terms are only words with a definite scientific meaning, and their derivation should not concern us to the degree of proposing changes. This is also the reason why the author is reluctant to give up the term germ-pore as was proposed by Locquin.

taste of the carpophores, and then they usually turn deep blue in sulfovanilline or brown in sulfoformalin or black in sulfobenzaldehyde. The type of oleiferous hyphae reacting with these aldehydes may turn out to be different from the non-reacting types, yet we take them as being the same, as did Fayod. Examples : 1) of the non-reacting type : *Amanita vaginata* (Pl. XVIII, 2); 2) of the type giving marked color reactions with acidaldehyde combinations : *Russula emetica.*

3. *The gloeo-vessels, in the sense of Singer (1945).* These are vessel-like elements attached to gloeocystidia projected into the trama and staining deep blue. Example : *Favolaschia saccharina.* In the *Agaricales,* they have been observed in *Lactocollybia* (Pl. XVIII, 3). Perhaps, they are also latex-carriers, since in the same genus, a laticiferous species that actually exudes a latex on bruising *(L. lacrimosa)* has been described.

4. *The coscinoids, in the sense of Singer (1947).* These are conducting elements of dark color with a sieve-like surface which is due to winding perforations and holes inside these otherwise solid filaments. The coscinoids (Pl. XVIII, 6) are found running through all parts and organs of the carpophores of *Linderomyces lateritius*, proliferating into cystidia-like bodies which are called coscinocystidia.

The fruiting bodies of some species are composed almost entirely of conductive elements, i.e. the structural function of the fundamental tissue has been taken over by the elements of the vascular system. Examples of this strange and rare condition are found among the *Tricholomataceae (Lactocollybia)* and also in the ochre-spored group *(Phlebonema).*

X. THE HYMENIAL LAYER OF THE CARPOPHORE

The Basidia

As compared with *Aphyllophorales* and the heterobasidial orders of the *Basidiomycetes*, the *Agaricales* are remarkable for the comparative uniformity of shape and development of their basidia. They are all holobasidia, i. e. constantly and persistently unicellular and not divided into what is described as « probasidia » and « epibasidia » (rather inadequate terms). Their position is always [12] in a palisade

[12] Kniep (1927) and others indicate mycelial basidia in *Armillariella mellea ;* very little is known about their occurrence in other species and genera, and about their cytology as compared with that of the hymenial basidium.

characterized by the approximately even level of the basal septum and the acrogenous sterigmata, the former being the wall between the basidiophorous terminal subhymenial cell and the basidium itself, the latter — the connecting link between the basidium and the spore just before discharge of the latter (usually remaining on the old basidium until its collapse). In all *Agaricales*, the basidia are standing side by side (or intermixed with pseudoparaphyses), with their longitudinal axis parallel to the longitudinal axis of the neighboring basidia (provided they cover an approximately plane surface such as the side of a lamella). This special type of palisade is called hymenium. This term is not exclusively used in the *Basidiomycetes,* nor is it exclusively used for spore-bearing surfaces. The apothecia of the *Pezizales* and related *Discomycetes* are covered with a similar layer consisting of asci, and, as will be explained later, the sterile surface of the carpophores of the *Agaricales* are frequently covered by a hymenium in which the basidia are only a small minority of the elements observed.

Most basidia are clavate or almost so, yet some show a strong ventricosity below the apex which is then broadly capitate and constricted beneath the capitellum, or cylindric to attenuate and broadly rounded at the tip. This latter shape would put them in the category of what is now called the *Urnigera*-type of basidia were it not for the number of sterigmata formed which is always 2 to 4 in these *Agaricales* whereas it is up to 8 in the *Aphyllophorales* with typical *Urnigera*-basidia. This false *Urnigera*-type is often found mixed in with normal clavate basidia in the same carpophore, or else some carpophores have the false *Urnigera*-basidia while other individuals have all normal basidia (e. gr. in *Gymnopilus*). According to the author's observations on the nuclear divisions in *Gymnopilus,* this shape is closely connected with the level at which the spindles are formed, and probably a secondary expression of an abnormally low position of the nuclei at the reduction division. A special case of the false *Urnigera*-type is the *Godfrinia*-type, first described by Maire for the two-spored parthenogenetic form of *Hygrocybe conica*.

All *Agaricales* have chiastobasidia (see p. 96), a term mainly based on the cytology of the basidium, but the chiastobasidium is also characterized by its shape and development. It becomes more broadly clavate when mature, but is fusoid or narrowly clavate when young rather than cylindric-filamentous. Besides, the chiastobasidium is, as a rule, less elongate than the stichobasidium. Within the chias-

tobasidial group, there is a large degree of variation as to the relative length of the basidia, this absolute length compared with the absolute length of the spores, their absolute breadth in comparison with the length of ellispoid spores, and the factor (F = length × width) of both dimensions of the basidia compared with the corresponding figure of the spores.

Generally speaking, the basidia of the *Agaricales* with ellipsoid or approximately ellipsoid spores, are about (or often exactly) as broad as the spores are long [13]. They are about two to five times as long as the longer axis of the spores, and if longer, they belong to a type that has some taxonomic importance because of its abnormal length. The abnormal length of the *Hygrophorus* basidium, causing the lamellae to be very thick and waxy in consistency, has been used as one of the characters of the family *Hygrophoraceae.* Some *Tricholomata,* some *Lyophylleae,* the genus *Catathelasma,* some *Mycenae* and *Omphalina umbellifera* along with closely allied species, also many *Amanitae,* are notorious for their longer-than-normal basidia, but they differ from the *Hygrophoraceae* in other regards. The genus *Laccaria* has thick lamellae but the basidia are not too long as compared with the size of the spores, and it is the thicknes of the trama that is responsible for the thickness of the lamellae. The *Strobilomycetaceae,* among the boletes, are noted for their more voluminous hymenial elements, including the basidia, and *Agaricus,* on the other hand, is characterized by the very small size of the basidia as compared with that of the *Strophariaceae* and *Coprinaceae.* Short and thick basidia are characteristic for *Conocybe* (*Bolbitiaceae*).

The walls of the basidia are usually thin, and in old specimens and in poorly dried herbarium specimens, the basidia collapse soon after maturity. This is especially true for the *Coprinaceae,* those with strong autodeliquescent properties, as well as those without them. In the species with tough carpophores, some thickwalled basidia are occasionally found, and even in soft species such as *Armillariella nigropunctata,* occasional basidia with thick walls and a generally sclerotized appearance can be observed. Even if they have distinct

[13] In a paper (published after this manuscript was edited, June 1948) *Studies in the basidium,* E. J. H. Corner points out that, disregarding a few exceptions the volume of the basidia minus that of the initial vacuole equals the volume of the spore multiplied with the number of the spores per basidium.

sterigmata, they always appear empty, and it may be suspected that a cytological investigation of this problem will show them to be pseudoparaphyses (see p. 104). Only one species and genus known at present has typically thick-walled basidia of normal function : *Phaeomycena albidula.*

The order or sequence of maturation of the basidia in the hymenium has been studied by Buller. However, in taxonomy it is not yet accepted usage to rely on more than the two main types of hymenophores named by Buller, viz.

1. *The inaequihymeniiferous type of hymenophore (or carpophore).* Buller who is not the first to have observed this type, is, however, the one who has most thoroughly studied the subject and coined the terms, and therefore his terminology is here accepted (Buller, 1922). In the inaequihymeniiferous type, the hymenophore consists of lamellae which are parallel-sided or almost so, rather thin (trama of small diameter), and they are brought into approximately vertical positions through a negatively geotropic stimulus in the growth of the stipe; the lamellae themselves are not always completely vertical, and one side of the lamella may be turned upward while the other side is turned obliquely downward; the hymenium develops unequally on different parts of the lamellae, generally starting to mature at the edge and continuing slowly upwards along the sides of the lamellae; each small area (0.1 mm^2) does not produce a number of successive generations of spores, but all the basidia on the area mature almost simultaneously. The spores are discharged in succession from below upward, and a zone of autodeliquescence follows, destroying completely those parts of the lamellae where the spores have been discharged (Pl. XII, 1; XX, 3).

2. *The aequihymeniiferous type of hymenophore (or carpophore).* Buller distinguishes this type from the above by the shape of the hymenophore, the development of the hymenium and the manner of discharge of the spores. The hymenophore is lamellate and consists of « gills which are shaped like the blade of a pen-knife ». The thickest part of each lamella is attached to the context of the pileus whereas the more or less sharp edge is turned downward; the sides of lamellae are therefore not parallel; a cross-section of the lamellae is wedge-shaped. The lamellae are positively-geotropic during their development, and their median planes are brought into vertical positions, even if the stipe should not be vertical and straight; the younger the lamellae, and the less the angle of tilt, the greater is the

success which the lamellae attain in bringing their median planes back into vertical position once this has been altered; consequently, the normal lamella has both sides facing outward and slightly downwards at the same angle. The hymenium, in each small area, develops equally, i. e. the basidia do not mature in zones starting from the edge upwards, and the production of basidia takes place in succession. During the spore discharge, the hymenophore is not deliquescent.

Buller has not studied the corresponding types in the boletes, but he has subdivided each of his types into a whole series of subtypes which, at present, are not used in taxonomy. This, however, does not mean that a more complete study of the species belonging to each subtype will never furnish any additional taxonomic characters for the distinction of sections, or perhaps even genera. The most important use of this character was made when the generic position of *Pseudocoprinus disseminatus* was investigated (see under that genus).

The immature basidia, often (perhaps incorrectly) called basidioles) [14], are usually of approximately the same shape as the mature basidia, only often slightly to considerably smaller, or narrower, or rather more fusiform than clavate. Fusiform basidioles are rather characteristic for certain genera, such as *Marasmius, Marasmiellus,* and *Collybia,* also certain smaller tricholomataceous genera, related to these three (Pl. XXVIII, 2, *d-e*).

In most species of the inaequihymeniiferous type and in but a very few of the aequihymeniiferous type of agarics, the basidia are separated by and dispersed in a more or less regular manner, among pseudoparaphyses (see p. 104). Or, as Buller describes this situation

[14] Boudier and Romagnesi use this term for what we call pseudoparaphyses, or aborted basidia; and Petrak uses the word pseudoparaphyses for a certain type of paraphysoids in the *Ascomycetes*. We use the word basidioles in the sense in which it is used by most cytologists and taxonomists in the *Agaricales*, i. e. as term for the young binucleate basidium with meiosis; the following stage, during the nuclear divisions has been called metabasidium by Donk but this term is also used in a somewhat different sense in other groups of fungi. The word pseudoparaphyses is here used exclusively for the consistently sterile, often slightly modified, non-protoplasmatic basidia, since in the *Myrangiales* the term paraphysoids (remainders of the interthecial stroma) is sufficient and satisfactory. — Heim attempts to apply the term basidioles to both young basidia and pseudoparaphyses (« they constitute basidia which are young or arrested in their development »).

for the inaequihymeniiferous type and the *Pasthyrella*-subtype of the aequihymeniiferous type, pseudoparaphyses « are normal constituents of the hymenium. They are very large and are united so as to form a pavement through which the basidia protrude. They not only support the basidia mechanically but act as space-makers so that adjacent basidia are separated from one another by a distance just sufficient to prevent any jostling during spore development and spore discharge » (3 : 122. 1924). This arrangement of the basidia is paralleled by a definite dimorphism, more rarely a trimorphism, or tetramorphism of the basidia, expressed in the distance by which they project above the pseudoparaphyses, their shape and the time at which they develop — the least projecting basidia being the ones that belong to the latest generation. All these characters are included when a hymenial structure is called coprinoid. The coprinoid hymenial structure (Pl. XII, 1) is among the characters that distinguish *Xerocoprinus* from *Coprinus,* and *Pseudocoprinus* from *Psathyrella.*

Of all these characters, Fries concentrated his attention on the only one that is macroscopically visible, viz. the autodeliquescence. However, the autodeliquescence is not understandable unless the shape of the lamellae in *Coprinus* is taken into consideration. The equal diameter of the hymenophoral trama makes spore dissemination difficult. In those species that have angiocarpous development and toughish consistence, the problem is solved by postponement. In the ephemerous species with agaricoid, i. e. non-angiocarpus development, and fragile consistency, the problem can be solved in two ways, either by transformation of the lamellae into wedge-shaped formations — or by autodeliquescence, i. e. by removing the lower part of the lamella that would hinder the free fall of the discharged spores, from the zone immediately above. At the same time, the spores that have accidentally stuck to the hymenium or have not come clear from it, are suspended in a fluid that drops onto the grass, or is taken off by passing animals, hereby receiving a second chance of dissemination. Both the wedge-shaped lamella and the autodeliquescent lamella are realized in the family *Coprinaceae.* Since animals play a certain rôle in the explanation of the latter type of spore dissemination, it is not surprising that the truly inaequihymeniiferous *Coprini* are often found on animal excrements, such as horse manure, rabbit-, deer-, and cow dung, etc., and also on manured fields, white-mushroom beds, and manure heaps.

Cystidia

The word cystidia (cystides, Léveillé, 1837) in its broadest sense designates any sterile bodies that are interspersed in the hymenium or replace the basidia in any part of the hymenophore, or — according to later emendations of the term — occur on one of the usually sterile surfaces of the carpophore but resemble the hymenophoral cystidia which are apparently homologous with them. However, this traditional definition of the cystidia, has recently been — step by step — abandoned in favor of narrower terms. Since the presence or absence of cystidia in the broader sense is not always a constant character, a differentiation between the various types of cystidia is desirable from a taxonomic point of view as well as from a purely morphological, anatomical, and physiological viewpoint.

The main classification of these sterile bodies in the concepts of some authors derives from the distribution of the cystidia on the carpophore. Some authors use the following terminology which we think is rather superficial and not truly morphological though its simplicity has much to recommend it. For this reason we mention it here. It divides the cystidia into the following categories: Cystidia A. on the hymenophore *(a)* on the sides of the lamellae or the interior of the pores: *Pleurocystidia.* — *(b)* on the edge of the lamellae or pores: *Cheilocystidia.* — B. on the sterile surfaces of pileus or stipe *(a)* on the pileus *Pilocystidia.* — *(b)* on the stipe *Caulocystidia.*

This scheme calls pilocystidia the cystidia-like bodies on the epicutis of the pileus in *Russula emetica.* If exactly the same type and subtype of bodies occurs on the stipe of *Russula emetica,* it is called caulocystidia. However, entirely different cystidia-like bodies occurring on the pileus of *Flammulina velutipes* are given the same name as those occuring on the pileus of the *Russula ;* and the elements of cystidia-like appearance found on the stipe of *Leccinum scabrum,* though quite different in shape, chemical characters, and origin from those of the *Russula,* are called caulocystidia in the *Leccinum* as well as in the *Russula.* Actually, the pilo- and caulocystidia of the *Russulae* are homologous and practically identical whereas the pilocystidia of *Flammulina* and the caulocystidia of *Leccinum* belong to very different types.

Those cystidia-like bodies that are found in the hymenophore, on the edge as well as the sides of the lamellae of many *Russulae* and

Lactarii should be put in a category by themselves because of their origin in (or homology with) the conducting system of these genera. By their very nature, they are merely prolongations of the conducting system into the hymenium, or into the epicutis of the pileus or the covering layer of the stipe. This kind of cystidia has been called pseudocystidia by Kühner and Romagnesi. They were first recognized as «false cystidia» («simulant des cystides») by Boudier (1866). We apply the term pseudocystidia as a general name for all cystidia derived from conducting elements, whether they otherwise belong to the laticiferous system, or oleiferous hyphae, or the gloeo-system, or the coscinoids.

Pseudocystidia are common in the *Russulaceae,* in *Lactocollybia,* in *Lentinellus,* and *Linderomyces.* In each of these cases, however, the type of pseudocystidia occurring is different, and has received different names. The subtype found in the *Russulaceae* and *Lentinellus* is known as macrocystidia (Romagnesi, 1944). It is characterized by a chemical feature, viz. the discoloration with acid-aldehyde solutions, and the weak absorption of cresyl blue by its contents. Another subtype has for a long time been known as gloeocystidia; however, the existence of gloeocystidia in *Agaricales* was not known until recently. It is found in *Russula polyphylla* and probably also in some other species of the *Russulaceae,* in *Lactocollybia* (Pl. XXI, 3), etc. The gloeocystidia can be recognized by the oily contents that are often very distinct but sometimes absent, and, more clearly, by the deep blue color they assume when stained with cresyl blue (excepting the walls which remain a pale violet color). This metachromatism is, on the basis of what is known at present, an infallible sign that the bodies showing it are part of the gloeo-system or, more precisely, gloeocystidia. The third subtype is rare, and it is called coscinocystidia because of the sieve-like character of their surface. They are protruding cystidia-like ends of the coscinoids and have been observed only in *Linderomyces.*

The remaining subtypes of pseudocystidia have not been named separately, and are characterized by three negative characters (1) by not darkening with acid aldehydes (2) by not having completely deep blue contents when dyed with brilliant cresyl blue (3) by having an entire rather than a sieve-like surface. These subtypes of pseudocystidia should at present be referred to in a general way, as pseudocystidia or pseudocystidial stages of certain other types of cystidia.

All organs that answer the general description of cystidia in the

wider sense but do not fall into the category of pseudocystidia, are true cystidia.

Not all true cystidia have their origin in the deeper layers of the subhymenium, or in the trama. Some originate at exactly the same level as the basidia, and differ from the basidia and pseudoparaphyses merely in shape. These are called cystidioles. True cystidioles are frequently found on the sides of the lamellae (Pl. XX, 3; XXI, 4; XXVIII, 2*f*) or in the interior of the tubes, and in certain groups they are rather characteristic. If they occur on the edge of the lamellae and lamellulae, excepting the attenuate portion of the latter, or the pores exclusively, they should be referred to as cheilocystidia (Buller, 1924). We cannot believe it necessary or advantageous to differentiate between the cheilocystidia that are, according to the position of their mothercell, localized cystidioles, and those that derive directly from the trama because of the lack of a subhymenial layer at the very edge as is often observed in *Collybia* [15] (Pl. XIX, 4; XXV, 2; XXIII B, 1, 3-4, 6; XXVIII, 2c, g).

Another category of true cystidia has its origin in the tramal hyphae, or, in some cases in the lower part of the subhymenium, at a deeper level than the basidia. Except for their deeper origin they do not essentially differ from the cystidioles in their development or in their resemblance to the basidia. The cystidia of many boletes come into this category (Pl. XIX, 3), and since it appears that Romagnesi's term leptocystidia (1944) belongs here, we shall accept it. However, the leptocystidia sometimes have a tendency to be rather firm and have partly thickened walls (*Gomphidius vinicolor;* Pl. XXVII, 7), or even thickened and colored walls, in which case we call them setuloid cystidia, or for short « setae » [16] (Pl. XV, 2-3).

In certain genera, all transitions between cystidioles and leptocystidia can be found in a single section.

[15] The cheilocystidia of *Collybia peronata* and related species are also remarkable for their development which seems to be retarded; they are much more inconspicuous and scattered in young specimens than in mature carpophores.

[16] Setae of the type observed in the *Aphyllophorales* such as *Phellinus* and *Hymenochaete* are in the opinion of some authors, not observed in the *Agaricales;* however, it is customary to call setae the organs found in *Boletochaete, Marasmius cohaerens,* etc. But they are not always colored, even in the same hymenium, and are variable in color (fulvous, rufous, chestnut, green); in *Chaetocalathus,* the setae-like bodies are hyaline, and become deep brown only after they have been treated with iodine solutions.

Anybody who studies the relationships between the morphology of a given organ and its function will not be surprised to find that the terminology indicated above meets certain difficulties when more single cases, and their different stages of development are analysed. The most intriguing problem is that of the deep-rooted cystidia of the type found in *Panus rudis* and related species. *Pleurotus floridanus,* and perhaps some related Asiatic species, and in all the species of the genus *Hohenbuehelia* (Pl. XXII, 2), also in all cystidiate *Inocybae* (Pl. XVII, 1). Donk has shown (in an unpublished manuscript that the author had the privilege to see) that the same type of cystidia is also found in *Peniophora,* and is called metuloids by Cooke (1879). These bodies start out by being pseudocystidia in the sense that they appear to be proliferations of the conducting system into the hymenium (yet, neither belonging to the subtype of the macrocystidia, nor the gloeocystidia, nor the coscinocystidia), and serving as excretive organs. Later on, they become thick-walled, loose their excretive function, and strongly resemble the leptocystidia, — were it not for the fact that they are uniformly deep-rooted, uniformly thick-walled, and mostly hyaline to straw colored and always non-amyloid. Deposits of coarse crystals are often found even on the old metuloids, especially at the apex, but sometimes all over. This kind of cystidium has often been called «*Peniophora*-cystidium» by mycologists (including this author) but the term metuloids appears to have priority, brevity, precision, and descriptiveness in its favor. Romagnesi (1944) calls them «lamprocystides».

In certain cases, we find that typical macrocystidia, or gloeocystidia, originate not from portions of the conducting system, but become part of it at the very septum that separates them from the next-lower hypha, or in certain cases, they become, theoretically speaking, part of the conducting system from a certain level inside themselves, e. gr. many of the macrocystidia of *Russula nauseosa.* Typical of this kind of pseudocystidia is also the cystidium of *Pholiota astragalina, Stropharia aeruginosa, Naematoloma fasciculare,* and allied species. Few of these cystidia are continued into anything that might be called portions of the conducting system, yet, chemically, they are pseudocystidia. In certain individual cases, they are really cystidioles, or leptocystidia from an anatomical point of view, but the strong absorption of cresyl blue by their contents reveals them to be part of the conducting system even though they may not be directly connected with its internal portion but instead represent a transmu-

tation of ordinary structural hyphae into pseudocystidia at the level where they enter the subhymenium or the hymenium. Romagnesi (1944) has called this type chrysocystidia (Pl. XVII, 3) because of the internal body that is typically colored yellow when ammonia is used as a mounting medium [17]. This term, in the author's opinion, is worthy of being taken up in descriptive mycology, just as well as the term metuloids.

There is nothing unusual in considering macrocystidia as well as the chrysocystidia to be pseudocystidia even if they arise from an ordinary hypha. It can be noticed in most sections of the cortical layer and the context immediately underneath in a large number of agarics, that normal hyphae (of the fundamental system) often, at a certain point, become oleiferous; this is especially true of the so-called « laticiferous hyphae » of *Russula,* which turn deep blue in sulfovanilline; this reaction makes it possible to observe this sudden transition with ease.

It is therefore obvious that an absolutely sharp line between pseudocystidia and true cystidia cannot be drawn because (1) in some cases, the origin of otherwise typical pseudocystidia may be hyphal rather than « vascular »; (2) in other cases, the development of the individual pseudocystidium may include both a pseudocystidial and a cystidial phase.

There are still cases that are not fully investigated chemically, and the function of the cystidia as well as the origin remains unknown. It is a wise policy, in all these cases, to refrain from using any of the above terms, and merely refer to cystidia (in the widest sense).

Only the cystidioles can, from a morphological point of view, be considered as transformed basidia, i. e. basidioles (young basidia) that because of a change in function [18] or by loss of their function as gonotoconts do not turn into normal spore-bearing basidia (Pl. XXVIII, 2 *a*) but rather assume an often characteristic, more or less constant shape in accordance with their function and cytological development.

[17] This internal body can also be stained with ferric acetocarmin as used for the *Lyophyllum*-basidia (see below, p. 103).

[18] In *Coprinus,* the cystidioles have assumed a mechanical function, probably holding the lamellae in equal distance; in *Melanoleuca,* the cystidioles seem to have an excretive function as evidenced by the crystal hood; in *Tylopilus plumbeoviolaceus* and in *Gymnopilus cacaophyllus,* the basidia of young hymenophores are so strongly incrusted with a fulvous resinous matter that they are often retarded or transformed into pseudoparaphyses.

However, even this very differentiation in shape is frequently indicative of their basidial origin. Some of the cystidioles still go through the motion of forming sterigmatoid prongs, but the latter are more irregular in number and shape, often limited to one. In a few cases, the cystidioles even develop an homologon of the spore on the sterigmatoid prong (called mucro) which is then capitate with a stalked, well-delimited capitellum. In extreme cases, the mucro or the capitellum is easily detached from the cystidioles and floats around in preparations of the hymenium. This capitate type of cystidiole is found in the cheilocystidia of *Conocybe* (Pl. XIX, 4) and *Pholiotina septentrionalis*. A good example of transitions between basidia and cystidioles is found in *Omphalotus olearius*.

The term paraphyses, often found in the literature on Basidiomycetes, even in the sense of cystidia, but more commonly to designate the pseudoparaphyses of Kühner and the «basidial cells» of Corda, should be discarded in this class of fungi (see p. 39, foot note 14).

Since the distribution of the cystidia is often different on the edge of the lamellae and pores or contrasted to the sides of the lamellae and interior of the tubes, Maire has proposed to call the edges :

1. *Heteromorphous,* if they are sterile (or predominantly so) because of the presence there of a type of cystidium (cystidiole) that does not occur on the sides of the lamellae (or in the interior of the tubes). We may logically designate as inversely heteromorphous the opposite case where the edge alone is completely free of cystidia.

2. *Subheteromorphous,* if the edges are sterile (or predominantly so) because of the density of the same type of cystidia that is scattered among the basidia on the sides of the lamellae (or the interior of the tubes).

3. *Homomorphous,* the hymenium on the edges is not differentiated from that on the sides.

Romagnesi (1944) has suggested the term «pseudoheteromorphous» for those cases of heteromorphism where the cystidia occur only on the edge without being homologous with any dermatocystidia («hairs»), as is the case in *Psathyrella Candolleana*. The term «pseudoheteromorphous» is based on the somewhat precarious differentiation of two types of what is here called cheilocystidia, viz. those cheilocystidia that are comparable with the «hairs» of the cortical layers (of pileus and stipe) rather than with hymenial cystidia, and those that are not. The fact that the hymenium is, in many primordial and young stages of agarics and boletes extended beyond

the hymenophore proper, makes it very difficult to justify this differentiation on a morphological basis.

Since a variable number of fertile basidia is often found in an otherwise heteromorphous or subheteromorphous edge, it is necessary, in these cases, to refer to « almost heteromorphous » and «almost subheteromorphous» edges.

XI. THE STERILE TISSUE OF THE HYMENOPHORE

The hymenium is only a thin outer layer of an organ usually referred to as hymenophore, i. e. a part of the carpophore, modified and especially adapted to provide a maximum of surface space for the hymenium.

In only a few *Agaricales* the hymenophore is wanting. It is then replaced by a smooth hymenial surface, the basidia either originating from a subhymenial layer or directly from the lower or upper surface of the trama of the pileus or cup or whatever the hymenophorous part of the carpophore may be. This smooth hymenial surface may be a first stage in the development of the carpophore or, in other cases, it may be a permanent reduction of more or less constant occurrence, or, again it may be a primitive form of development. These heterogeneous groups of genera, that have in the past been assembled in the *Thelephoraceae,* partly belong in this last category. In the *Agaricales,* we find this character exceptionally rather than constantly, except for a few genera that may well be interpreted as strongly reduced forms (some «*Cyphellae*», *Physalacria*). In others, we find mature hymenia of the same species, sometimes smooth, sometimes covering a lamellate hymenophore, or a venose hymenonophore (*Marasmiellus*, *Marasmius*, *Mycena*, *Delicatula*). It must now be assumed that some species that where initially described as or considered as *Helotium*, or *Cantharellus* are actually *Agaricales* with either smooth, or venose hymenial surface. However, the so-called veins of such species as *Cantharellula umbonata* (*Cantharellus umbonatus*) or *Hygrophoropsis aurantiaca* (*Cantharellus aurantiacus*) are not true veins of the type encountered in *Cantharellus cibarius* but rather lamellae with more obtuse edges.

In all but the exceptional cases mentioned above, and perhaps in *Geopetalum carbonarium* (A&S ex Fr.) Pat., the hymenophore of the *Agaricales* has either the shape of lamellae or of tubes. The examin-

ation of the internal structure of the hymenophore in these forms, i. e. the anatomy of the hymenophore minus the hymenium, is of great importance in taxonomy. The internal structure of the lamellae and tube walls is studied on longitudinal sections from the plane of attachment to the trama of the pileus down to the edge of the lamellae or the pore edges. In lamellate as well as in tubulose forms, care must be taken to cut exactly at a right angle to the edge of the lamellae [19], and exactly in the direction of the individual tubes rather than obliquely, i. e. in all cases, the section must be exactly vertical; it must also be exactly tangential, i. e. the lamellae should be sectioned at a right angle to their sides. It is also important that these sections are reasonably thin (about 15-20 μ) because otherwise pressure on the cover-glass has to be exercised in order to obtain a preparation transparent enough to show the arrangement of the single elements of the trama and adjacent layers. However, there is always a slight disorganization in such preparations, and if they are taken from old or otherwise poor material, the results will be unreliable. Under no circumstances may the preparations be crushed to the point where its elements are so dislocated that it is impossible to make an analysis of their arrangement. The beginner, and those who have to handle material that is very scanty, brittle, or otherwise difficult to handle, and also those who find it difficult to learn sectioning by hand in the manner described above, are strongly advised to use a microtome. Both freezing and paraffin methods will do.

It appears that the sterile internal portion of the hymenophore consists of one or several layers. If there is only one, it is called the hymenophoral trama, or for short the trama [20]. But more frequently

[19] In ascendant lamellae, the hyphae often do not run to the edge at a right angle, and in this case the section should be oblique in the sense of the direction of the hyphae as otherwise their true proportions may be misinterpreted.

[20] Some authors use the word trama exclusively for the trama of the hymenophore. There is, however, no reason to reserve the term for only a single organ since the trama is not sharply delimited at the plain of attachment of the hymenophore to the pileus in the majority of the species. Only very rarely is there a differentiation between these layers (hymenophoral trama gelatinous, trama of the pileus nongelatinous in *Dictyopanus pusillus*), and even then, the trama of the hymenophore originates in the trama of the pileus. It is therefore more precise and generally preferable to specify as to the part of the trama considered. viz. the hymenophoral trama, etc. If the word trama is used alone, it should either be quite clear from the text or the arrangement that the hymenophoral trama is meant and none other, or else it must be supposed that whatever is said about the trama refers to all parts of the trama in the widest sense.

there are two or more equal layers on both sides of the central hymenophoral trama, more or less easily discernable between the hymenophoral trama and the hymenium proper. If there is only one such layer, it is referred to as subhymenium, always consisting of small elements with numerous septa. If there is another layer between this and the hymenophoral trama, distinguishable from both the former and the latter in structure or characters of the elements composing it, it is called hymenopodium.

The hymenophoral trama occurs in four main types of structure:

1. intermixed to irregular (Pl. XIX, 5; XXI, 1, 5)
1. subregular to regular (Pl. XXII, 3)
3. bilateral (Pl. XXII, 1)
4. inverse (Pl. XX, 2)

The difference between intermixed and irregular trama is secondary; both are characterized by completely or at least predominantly irregular arrangement of the hyphae which are neither parallel (not even approximately so) nor divergent. In the subregular trama and the regular trama, the hyphae run approximately or strictly parallel (approximately in subregular, strictly parallel in the regular trama), i. e. from the plane of attachment to the pileus down to the edges. In the bilateral trama, there is a central strand which is subregular or regular as described above but much thinner in diameter, and an outer layer consisting of approximately parallel hyphae but which are not straight or parallel with the hyphae of the thin central strand but curve outward on both sides, joining the hymenopodium, or subhymenium, at a point farther outwards toward the edge of the pores or lamellae than the point at which each individual hypha departed from the thin central strand. The thin central strand is called the mediostratum, and the divergent portion of the trama on both sides is called lateral stratum. The nature of the hyphae involved may be rather different. Sometimes, the hyphae of the mediostratum and the lateral stratum are of approximately the same type; but in other cases, the average diameter may be different in the hyphae of the mediostratum and the lateral stratum; the pigmentation may also be different, and the gelatinization, and consequently the density, the frequency of septation, etc. may differ in those two layers. Although it is true that it is mostly the hyphae of the fundamental tissue that are primarily responsible for the structure of the hymenophoral trama, in some cases, it appears that at least the more conspicuous part of the elements composing the trama and marking

its arrangement is made up by the conducting elements; e. gr. in *Linderomyces,* where the conscinoids diverge, almost without forming a distinct mediostratum, soon assuming a position perpendicular to the sides of the lamellae, thus making the hymenophoral trama very strongly (yet not quite typically) bilateral. There are also various types of bilaterality insofar as the relative density and diameter of the hyphae are concerned. The bilateral hymenophoral trama of *Catathelasma* consists of very thin hyphal elements whereas that of *Amanita* consists of rather broad and moderately long elements.

If the elements composing the hymenophoral trama differ from each other fundamentally, showing two main types of elements, thin, elongate, hyphal elements and swollen, voluminous, subisodiametric elements (« sphaerocysts »), the trama will logically be neither subregular nor regular; it will also not be bilateral unless the juxtaposition of these two types would coincide with what may be called a mediostratum and a lateral stratum. It is obviously a special case of an irregular trama, and it is called intermixed trama, i. e. a trama where two types of elements are « mixed » with each other.

If the hymenophoral trama consists of a mediostratum and a lateral stratum, the latter consisting of hyphae curving outwards but reaching the subhymenium farther away from the gill edge rather than nearer to it as in the bilateral trama, we may assume with Fayod (who discovered this strange structure) that here the origin of the hymenophoral trama is in the subhymenium rather than vice versa. Perhaps, the isolated manner of development of the fundamental hyphae (here the hyphae of the lateral stratum), often observed in the trama of the carpophores of the *Amanitaceae,* manifests itself in the species with the kind of hymenophoral trama described above in that each subhymenial hyphal ramification produces either a hymenial element (on the outside), or an element of the fundamental tissue, more precisely the lateral stratum (on the inside). Further investigations must show what the origin of the mediostratum is. Whatever its morphological and ontogenetical significance, this type of hymenophoral trama is of as great taxonomic importance as the other types, and has been named inverse trama (trama renversé, Fayod, 1889). Good examples for intermixed trama are the *Russulae;* for irregular trama — *Pleurotus*; for subregular trama — *Hygrocybe* excepting the section *Conicae*; for regular trama — *Hygrocybe*, section *Conicae*; for bilateral trama among the boletes — *Boletus edulis* and all the other *Strobilomycetaceae* and *Boletaceae*, among the agarics —

Amanita caesarea and all the other species of *Amanita*; inverse trama — *Pluteus* (all species known), and related genera.

Less important differences in the structure of the hymenophoral trama can be distinguished as subtypes of the above basic types. These are:

1. *The* Phylloporus-*subtype of the bilateral type :* The lateral stratum is scarcely looser than the mediostratum, hardly less colored, and only slightly more gelatinized, only slightly more divergent, and with the hyphae usually touching each other. Example : *Phylloporus rhodoxanthus* and most species of *Xerocomus.*

2. *The Boletus-subtype* (« *truly bilateral* »): The lateral stratum consists of hyphae that are less colored than the mediostratum, distinctly removed from each other (because of stronger gelatinization), and at first strongly curved. Example : *Boletus edulis,* and most other boletes.

3. *The subbilateral-subregular subtype of the regular type :* The outermost hyphae of the otherwise regular hymenophoral trama show a very slight tendency to diverge but a mediostratum is not differentiated. Example : *Clitocybe dealbata.*

4. *The regular-subcellular subtype of the regular type :* The elements of the otherwise regular trama are so grossly enlarged and broadened that the trama appears almost cellular at places. Example : *Mycena, Psathyrella, Pseudocoprinus.*

In certain genera of the *Tricholomataceae* (many *Resupinateae, Dictyopani,* etc.) and in some *Crepidoti,* the trama is partly or entirely more or less gelatinized. In *Panus, Pleurotus,* also in some species of *Marasmiellus,* in *Heimiomyces, Anthracophyllus,* etc., the trama consists mainly of thick-walled, rather large, rigid, elongate hyphae, and in this type of trama, the thin-walled, thin, small, curved elements of the connective tissue are naturally more conspicuously different from the other elements which belong to the fundamental tissue. This difference may be expressed in calling this type of trama intermixed rather than irregular or subirregular, but it is obvious that this meaning of « intermixed » is not identical with what it is in *Russula.*

In a few cases where the trama proper is strongly reduced in favor of a hymenopodium (rarely a subhymenium), the impression may prevail that the hymenophoral trama itself consists of two layers with the lateral stratum running exactly parallel with the mediostratum instead of diverging. This is the case in *Conocybe* (Pl. XXI, 2). In this

case we may speak of false bilaterality. In *Gomphidius,* especially *Chroogomphus,* the hymenophoral trama is basically bilateral, yet, the divergence of the lateral stratum is obscure by an increasing irregularity of structure as the carpophores mature while the mediostratum is so reduced it is hardly recognizable especially in old specimens. It is consequently easy to mistake the broad hymenopodium that is not sharply delimited from the subhymenium, either for the lateral stratum of the hymenophoral trama, or for an unusually enlarged subhymenium.

This strong development of the hymenopodium is noticeable only in a small minority of the *Agaricales.* The hymenopodium is completely irregular in those *Agaricales* with lamellate hymenophore but otherwise related to the boletes (*Gomphidiaceae, Paxillaceae*), and it is regular and consisting of broad, voluminous hyphae in *Conocybe.* It is also somewhat developed in some species of *Russula, Mycena,* etc., where, however, its taxonomic significance, as far as can be seen now, never goes beyond the species level.

The subhymenium is rather uniform. It is rarely of great taxonomic importance with the exception of the genus *Pleurotus* (Pl. XXI, 1) where it is well developed in contrast to *Panus* (Pl. XXI, 5) where it is almost absent, and *Leucopaxillus* where it is filamentous (ramose), whereas in *Armillaria* it is cellular. This latter difference is not always so sharp as in the case of *Armillaria* and *Leucopaxillus.* This can be seen in some species of *Gomphidius* where the crowded septa shorten the individual cells so much that the whole seems to be a minutely pseudoparenchymatic tissue. Wherever the septa are not so close, the subhymenium assumes a more filamentous character. Wherever the elements become irregular in shape and denser and more intricately interwoven, we have an intermixed subhymenium, as is the case in *Gomphidius*, subgenus *Chroogomphus,* or *Omphalina,* subgenus *Romagnesia,* or some species of *Resupinatus.*

In these species of *Resupinatus,* the trama proper of the lamellae is gelatinized, looser and more regular. In other groups, especially the section *Laetae* of the genus *Hygrocybe,* the hymenophoral trama is non-gelatinized while the subhymenium is strongly gelatinized. This is one of the very few cases where the large diameter and strong differentiation of the subhymenium may lead to the misinterpretation as though the trama proper were bilateral whereas, actually, here again, we have an example of false bilaterality.

In genera with regular trama, the subhymenium is often separable

from the hymenophoral trama, and then lamellae are macroscopically described as fissile, a feature frequently found in agarics but hardly of much taxonomic value.

The subhymenium usually accompanies the hymenium all through the interlamellar zones at the top of the interlamellar space, and in certain cases, the hymenophoral trama or its parts run parallel with it. In this case, a looser layer of differently organized hyphae separates the hymenophore from the trama of the pileus. As a consequence, the hymenophore can be easily separated from the context of the pileus. This is especially remarkable in *Paxillus,* and most *Boletaceae* and *Strobilomycetaceae* where the hymenophoral trama is bilateral and forks above the level of attachment of the hymenophore to the trama ot the pileus — thus facilitating the separation of the tube walls or lamella together with the ceiling of the tubes or the interlamellar zones.

Certain agarics possess a special epiphyllous zone of a structure different from that of the trama of the pileus as well as the trama of the hymenophore. This may also result in an increased separability of the hymenophore as a whole.

The structure of the hymenophoral trama has at present become one of the most important characters in the *Agaricales.* Tribes, genera, even families are based on the structure of the hymenophoral trama wherever this character is correlated with other important features. There can now be no methodical analysis of any representative of the *Agaricales* without a careful study of this particular character.

XII. CORTICAL LAYERS

Cortical layers are formed by a differentiated tissue forming the surface layer of the pileus and stipe of the *Agaricales.* We have already seen (p. 41) that cystidioid bodies, reminiscent of those that occur in the hymenium either on the sides of the lamellae or in the interior of the tubes — or on the pore or lamella-edges, are also found in the cortical layers as relics of an originally indiscriminately expanded hymenium, or as products of a further differentiation of the cortical layers whereby they may have assumed some specific function. The cases where these cystidia are remainders of a primordial hymenium are not rare in the *Boletaceae* and *Strobilomycetaceae,* e. gr. *Suillus,* sect. *Granulati* (Pl. XXV, 8-9); the reticulate *Boleti,* and

Tylopili, all species of *Leccinum* (Pl. XVI, 3), and the alveolate species of *Porphyrellus* and *Boletellus*. Here, the ornamentation of the stipe is still reminiscent of the configuration of the hymenophore. In many of these, even sporulating basidia are found among sterile bodies making up the palisade of the cortical layer covering these ornamentations, especially in the upper portion of the stipe. In rare cases even the whole marginal zone of the pileus is covered by a hymenium, a large portion of which consists of basidia (Pl. XXVI, 5). All this is proof enough that these bodies are of hymenial origin. It is very difficult to state in every single case, whether the elements of the cortical layers are of hymenial origin, or later acquisitions due to an increasing differentiation and division of functions. It is not even certain that, if these elements should have been differentiated in a later stage of the evolutionary development of a genus, they cannot have originated from the hymenium or an extension of it. In such cases as *Russula Mariae* where we find the same type of elements on the edge of the lamellae and on the cortical layers, it may well be that they are both modifications of a degenerated hymenial element.

Considering all this, it does not seem possible at present to distinguish between such cystidioid bodies that have a non-hymenial origin and such that evidently originate from hymenial elements. Consequently, it is, in the author's opinion, correct to refer to all cystidioid bodies of the cortical layer as cystidia or pseudocystidia, if they are in some way comparable with either cystidia or pseudocystidia of the hymenophoral hymenium, with the only difference that those bodies that occur in the cortical layers receive the prefix dermato-. Thus, we have, in the cortical layers:

1, dermatocystidia (Pl. XXV, 9); 2, dermatopseudocystidia (Pl. XV, 1).

Although it is not customary to refer to « dermatogloeocystidia » or «dermatocystidioles» [21] or «dermatometuloids» or «dermatochrysocystidia » or « dermatomacrocystidia », etc. (all these bodies are being called dermatopseudocystidia, or dermatocystidia) it is correct to call the basidia occurring on the stipe or the pileus, outside the area covered by the hymenophoral formations, dermatobasidia, and those of them that remain permanently sterile with slight modifi-

[21] Naturally, with a subhymenium in the proper sense being absent in the cortical layers, it would be difficult to state whether a dermatocystidium has cystidiole character or not.

cations in size or shape, but strongly differ from the cystidia, — as dermatopseudoparaphyses.

Dermatobasidia are found on the pileus of *Boletus subsolitarius* and many *Russulae,* and much more commonly on the apices of the stipes of the boletes and agarics. If fertile dermatobasidia occur in a hymenium-like structure on the surface of the pileus or the stipe, we may then refer to that structure as to an extension of the hymenophoral hymenium, and call it extrahymenophoral hymenium. If there is a hymenium-like structure outside the hymenophore that lacks dermatobasidia and, for that matter, also dermatopseudoparaphyses, we call this structure — hymeniform, and the layer, made up by it, a hymeniform layer (Pl. XIII, 2).

If the cortical layer is formed by hair-like septate hyphae, i e. hyphae inserted more or less perpendicularly to the surface of the organ in question yet not being strictly hymeniform, it is called trichodermium (Lohwag, 1937, 1941 ; Pl. XVII, 3 ; XXVI, 1) ; if the trichodermium is gelatinized as in *Suillus granulatus,* it is an ixotrichodermium (Snell, in Elrod & Blanchard, 1939). These hyphae usually form a velutinous to tomentose surface, but at times, especially when densely interwoven, they are not easily recognizable macroscopically ; in the ixotrichodermia, the surfaces covered by it are, as a rule, merely glutinous. If the hyphae are vertical (erect) and parallel with each other, we speak of a trichodermial palisade (Lohwag, 1937, 1941) which differs from the hymeniform layer in that not necessarily every element originates and ends at the same level as the neighboring elements of the same nature [22]. Trichodermial palisades (Pl. XVIII, 1 ; XX, 1 ; XXV, 1 ; XXVI, 2-4) usually make the surfaces they cover velutinous, or granulose, or pruinose ; they never show a watery, smooth surface, nor are they coarsely tomentose. The rimose or rimulose surface (as in contrast with the rivulose surface) in many boletes is a result of this kind of structure, that easily lends itself to perpendicular cracks that spread tangentially in all directions. Trichodermial palisades are also common in the *Agaricaceae.* The terminal members of the hyphae forming trichodermial palisades frequently are cystidioid, i. e. they are dermatocystidia, probably in most cases of the leptocystidia- and cystidiole-type (example : *Phaeomarasmius*).

If the trichodermium, especially the trichodermial palisade, con-

[22] According to these definitions, the hymeniform layer is a special case of a trichodermial palisade.

sists of shortened hyphal elements that tend to become sphaerocysts (isodiametric hyphae), the result is a mass of subglobose or globose bodies — with or without showing the original catenulate arrangement — that can be characterized as a loose pseudoparenchymatic layer. This type of cortical layer is called epithelium (Lohwag, 1937, 1941), or a cellular layer (Pl. XVII, 4; XXVI, 7). If there is only one stratum of sphaerocysts which are, with their base, directly attached to the hyphae of a lower layer, it is often difficult to differentiate between a hymeniform layer and an epithelium inasmuch as some of the sphaerocysts of the epithelium are often mucronate at the distal end or slightly vertically elongated (short-ellipsoid or short-clavate). The pluristratous epithelium is closer to the trichodermial palisade, especially in such cases where short and long hyphal members alternate, or the shape of the single elements of the chains change from one chain to the other, or in individual carpophores (Pl. XXVI, 3).

If the cortical layer is formed of radially arranged or, at any event, repent hyphae that are parallel to each other, it is called cutis (Lohwag, 1937, 1941) (Pl. XVI, 1).

Typical *Asterostromella*-structure such as described for the genus *Vararia* (*Aphyllophorales*), is not found in the *Agaricales ;* however, a limited *Asterostromella*-structure such as is observed in the cortical layers of such aphyllophoraceous genera as *Campanella* and *Favolaschia,* has been observed in one agaric, *Asterotus dealbatus.* It should be known as a cortical layer of dichophysate structure. It is characterized by hyphae with short branches and secondary, etc., ramifications, all branching off under approximately right angles and at short distances, frequently causing a stellate appearance of the terminal hyphae. These elements are rather stiff, and more or less hyaline (Pl. XVI, 2).

In numerous species, the cortical layer is not or only slightly developed. In some *Russulae,* a dense gazon-like covering of normal, very thin hyphae which are often forking or branching, reach the uppermost layer of the cuticle. They are otherwise not enough organized to call them a trichodermium. A similar, still less differentiated layer is found in the cuticle of some species of *Crepidotus.* In other species, such as *Panus conchatus,* the cuticular layer is merely denser (as that of the stipe is often denser in a « rind ») than the trama of the pileus. Such a structure is very frequent in the *Agaricales,* especially in the white spored families, and cortical layers of this type are called dense.

It should always be taken into consideration that certain types of veil (most conspicuously so the volva of *Amanita*; Pl. XVIII, 2), leave a layer of fragments of not truly cortical origin on top of the cortical layers. When an analysis is made, care should be taken that these velar layers are not misinterpreted as being part of the cuticle. Such precaution can easily be taken by examining the structure of the veil first and substracting any layer of identical structure and origin from the layers of the cuticle proper.

The cortical tissue itself consists of one to three layers. If there is only one, we simply call it cuticle of the pileus (pellicle — it is viscid, and peels easily), and the covering layer on the stipe. If there are more than one, the uppermost layer in a completely developed specimen [23] is called epicutis. The epicutis may be a continuous layer in one of the types of structure named above (hymenium, hymeniform layer, trichodermium, epithelium, cutis, dichophysoid layer, or dense layer), or else it may consist of fragments of such a layer. In this case, the epicutis is the sum of dissociated but identical individual dermatocystidia, dermatopseudocystidia, « hairs », or any other type of bodies characteristic for this particular layer, and its origin in this case must be understood as conditioned by the rapid growth of an elastic (often gelatinized) supporting cuticular layer while the epicuticular layer stops developing at an early age. Such cases are not rare in the *Agaricales,* especially in such groups where the epicutis is the remainder of an early extension of the hymenium beyond the hymenophore, such as the epicutis of the *Russulaceae* (Pl. XV, 1), or at least many species of that family.

The epicutis is followed, downwards, or rather inwards, by a second layer, the hypodermium or subcutis. Though it seems illogical, general custom applied the term hypodermium, as a general term, for any structure between the epicutis and the context in most *Agaricales* (Fayod, 1889). In the species that have a cutis, the term subcutis is preferred by Lohwag (1937). Subcutis thus would become synonymous with hypodermium unless the term is amended to be any layer underneath the epicutis but confined to the cases where the cuticle consists of three layers, and then the upper layer (intermediate between the epicutis and hypodermium) is called subcutis.

[23] In *Agrocybe* and some other genera, the uppermost layer is detersile, and often washed off by rain or handling. It is therefore quite frequently missing in old or carelessly prepared specimens.

e. gr. in *Russula Puiggarii* where, underneath a well-developed epicutis, a layer of hyaline gelatinized hyphae is followed, farther downwards, by a layer of pigmented, non-gelatinized hyphae. In the author's opinion, the term subcutis should not be used in preference to the term hypodermium for any one (or supposedly one, i. e. considered as one by the observer) layer between the epicutis and the context of the pileus or stipe.

Lohwag in his original proposal (in Lohwag & Peringer, 1937) did not pay attention to the fact that he was dealing with two different categories: structures of layers, and layers. If the subcutis is understood in a revised sense, valid only for the naming of a layer, and not descriptive of its structure, the short cells immediately beneath the « hairs » or dermatocystidia of species with *Virescens*-structure (see below), or of species with trichodermial palisade (as *Porphyrellus pseudoscaber*) should be called subcutis [21] (Pl. XVIII, 1, 4).

Another layer that does not necessarily belong to the cortical tissue, must be mentioned here. In some species of *Agaricales,* one can observe a layer of the context of the pileus that is differentiated from the rest of the context not morphologically but merely chemically or physically, i. e. it does not show the difference between the rest of the trama and itself unless it is exposed to a certain kind of radiation, or to certain reagents. This layer has been termed the subhypodermial layer of the context (Singer, 1942), in a discussion of the physical and chemical differentiation of this zone in certain species of the *Gomphidiaceae.* As another example, one may indicate the pigmented upper zone of the flesh of *Mycena iodiolens.* Yet, here it may be questionable whether the pigmentation is a purely chemical or physical character, inasmuch as it is not provoked by any chemical reaction other than the normal chemism of the developing carpophores in nature. It would therefore be better to call the pigmented zone of the context hypodermium, and the next-following (upwards) zone — if any is present except the epicutis — subcutis; this solution, of course is possible only in such cases where the layer between the epicutis and the context is not definitely homologous with what is

[21] The subcutis would then, in many cases at least, be homologous with the subhymenium of the hymenophore. It might be inferred from what Lohwag & Peringer say about Fayod's term « cuticule proprement dite » that the latter was identical with what we call subcutis. This, however, is not the case since Fayod calls by this name either the epicutis or the hypodermium whichever is more developed.

otherwise consistently called hypodermium in the same group. It will therefore be expedient, though perhaps on a temporary basis, to maintain the term subhypodermial layer for a case like that of *Mycena iodiolens*. The same term may also be used for the zones of the context of the *Tricholomataceae, Resupinateae* that become gelatinized.

Gelatinizing of the hyphal walls whereby the hyphae become imbedded in a mucous mass — given enough moisture — is observed very frequently in the main cortical layers of the *Agaricales,* and the macroscopical consequence of such a condition is what is generally called a viscid or glutinous surface (pileus or stipe, or both). If such is the case, the cuticle is often called pellicle because of the easiness with which it can be peeled from the non-gelatinous (or less gelatinous) layers. It must be kept in mind, however, that the separability (the peeling quality) may also hold for a non-gelatinous cuticle that is separated from the lower layers of the trama by a gelatinous subhypodermial layer. The hyphal walls often gelatinize so completely that the walls practically disappear whereby the gluten becomes macroscopically homogeneous. Without the coherence due to the presence of the hyphae, the gluten often drops down, or is washed down, and the anatomical demonstration of such a specimen as having been glutinous or having had a gelatinous layer on or near the surfaces becomes very difficult or impossible. This is apparently the case in certain species of *Hygrocybe,* especially if herbarium material is examined.

A chemical difference between cuticular layers on one hand, and tramal layers of the context on the other hand, is often demonstrated by the iodine stain. This feature will be treated more exhaustively in the chapter on chemical characters.

In the discussion of the layers and elements observed in the cortical tissue of the *Agaricales,* we have not used the word hair so extensively as it is used by some authors. This word, when used as a term, mainly for differentiated terminal formations of hyphae in the epicutis, can often be replaced by the term dermatocystidia, or the more neutral expression « epicuticular elements ». However, if these elements actually resemble hairs — there is no objection to calling them hair-like hyphal ends (Pl. XXVIII, 1), and if the «hair» is actually a strand of hyphae, it may be called a hair-like hyphal strand (Pl. XV, 3), or a pilose agglutination of hyphae, all neutral expressions. The author accepts the term hair only for those epicuticular elements

that are hair-shaped, form a pilose covering or down under a lens, and are not homologous with cystidia, cheilocystidia, pseudoparaphyses, or setae. or any other well-defined bodies. Such true hairs are found in all species of *Flagelloscypha, Lachnella, Crinipellis, Chaetocalathus,* and in some species of *Coprinus, Pseudohiatula, Mycenella,* and the covering that is made up by them is called pilose.

However, if the « hair» is much rather comparable to bodies that, as cystidia or cheilocystidia, or pseudocystidia, also occur in the hymenium of the hymenophore (even if the bodies occurring in the cortical tissue are slightly modified or if the corresponding body in the hymenophoral hymenium is absent in a given species, yet present in a closely allied form), the use of the word dermatocystidium recommends itself much more than the indiscriminate use of the word « hair ». For all these so-called « hairs » the term can only be applied in the case that Romagnesi's (1944) thesis is accepted which differentiates between cystidial and pilose elements in a manner that is at variance with that adopted in the present book [25].

This also refers to the characteristic cells with apical appendages giving them a broom-like appearance occurring on the pileus and sometimes on the edge of the lamellae in *Marasmius,* sect. *Hygrometrici* and some other species. The sterigmalike appendages and the palisadic arrangement as well as their occurrence on the edges of the lamellae in some species may suggest a hymenial origin, and this is also the author's guess. Since there is a good term in French literature (cellules en brosse) which can be adapted to other languages, we shall designate these bodies as broom-cells in a category by themselves at least for the time being (Pl. XII, 2 ; XIII, 2).

In *Russula vesca* and species with similar elements (Pl. XVIII, 1) in the epicutis, we find an elongate erect epicuticular element in palisade that by Maire, Singer, Melzer & Zvára, Romagnesi, and other specialists has been referred to as « hairs ». It consists of a few basal cells which are rather short-cylindric to sphaerocystoid, and the terminal member which is attenuate toward an obtuse or acute tip from a broader basis. More rarely a small appendage, which is usually more or less cylindric, is separated from the elongated cell by a sep-

[25] « The cystidium... is a sterile cell... characteristic for the basidia-bearing part of the hymenium ». « The hair [« poil »] is a sterile cell ... which is originally characteristic for the covering layers [« revêtements »] ». Romagnesi, *Rev. Myc.* **9** (1) : 6. 1944.

tum. With *Russula modesta* as an intermediate, this structure of the epicutis goes back to the so-called *Virescens*-structure found in *Russula virescens, R. crustosa, R. Patouillardii, R. chlorinosma,* and the entire section *Plinthogali* of *Lactarius* (Pl. XVIII, 3-4). Here, the basal cells are more conspicuous, truly made up by erect chains of sphaerocysts, and ending up with a subulate or cylindric, rarely clavate or ventricose « hair ». In both the case of *Russula vesca* as well as in that of the *Virescens*-structure it is probable that the « hairs » are merely modifications of a transformation of some originally hymenial body. In fact, the acute cheilocystidia of some of these species are not basically different from the « hairs », and the short cells from which the latter originate can be compared with the subhymenial elements from which the cheilocystidia originate. Since the edge of the lamellae is not quite sterile, it is not difficult to see that these cheilocystidia have the same origin as the basidia. They gradually turn, however, into macrocystidia, since, for instance in *Russula crustosa,* already near the edge and on the edge many of the cheilocystidia have contents that turn blue in sulfovanillin, and farther upwards on the sides of the lamellae they become very voluminous and deep-rooted true macrocystidia. Since we have a situation similar to that in *Marasmius* with its broom-cells, it is necessary to provide a new term for these bodies, i. e. the terminal « hair » in the *Virescens*-structure as well as the « hair » in the epicutis of *Russula vesca.* This is necessary inasmuch as the use of the plain term dermatocystidia (which would otherwise be correct) may lend itself to confusion with what was formerly called dermatocystidium in the *Russulaceae,* i. e. the dermatopseudocystidium of macrocystidial or perphaps sometimes gloeocystidial origin. The « hairs » in the *Russulaceae* will therefore be called ciliate dermatocystidia in this book, a term that does justice to the homology established by Maire as well as to the rather descriptive name of these bodies, suggested by J. Schäffer (Wimpern, Cilien).

There is another term in *Russula* that must be mentioned here. In the velutinous and flocculose species, the hyphae forming the trichodermium or the trichodermial palisade, are often thickened, as compared with the narrow elements of the connective tissue, quite frequently multi-septate, yet, the single elements still remaining elongate and usually cylindric; they are incrusted, rarely apparently naked, slightly acuminate but rounded, or broadly rounded at the ends; they are colored (usually pale ochraceous). Melzer & Zvára

(1937) called these « hairs » in Czeck « vlákna primordiální » or « hyfy », Singer (1932) in German « Flockenhaare » ; Melzer and Singer agreed later to the term primordial hyphae which Melzer claims is used in the sense of Fayod. These primordial hyphae were later (misleadingly) renamed « Haare » (hairs) or « Fasern » (fibrils) by J. Schäffer (1934).

All these bodies in the *Russulaceae* can be distinguished according to shape and, to a certain degree, origin. However, here again, we find so-called transitions already noticed by R. Maire (1907 and 1910) in which elements that morphologically seem to belong to one type of epicuticular bodies differ chemically, i. e. acquire macrocystidial character. This becomes a specific character in *Russula Peckii* where all so-called « hairs », i. e. the ciliate dermatocystidia reveal bluing granules when treated with sulfovanilline. It would be, in the author's opinion, proper and descriptive to call this ambiguous organ « ciliate dermatopseudocystidia » [26].

For the benefit of those who have no experience with the use of all the terms applied to the cuticle and its elements, it must be emphasized that in enumerating and defining them, we are dealing with three categories and these categories should always be understood and clearly distinguished as such :

1. *Layers :* Velar layer, cuticle (pellicle) ; epicutis, below it the subcutis, below it the hypodermium, below it the subhypodermial layer.

2. *Structures of layers :* Hymenium (mostly in epicutis), or hymeniform layer, or trichodermium, or trichodermial palisade, or ixotrichodermium, or epithelium, or cutis, or dichophysoid structure, or dense structure.

3. *Elements of these layers :* Dermatocystidia, ciliate dermatocystidia, dermatopseudocystidia, ciliate dermatopseudocystidia, dermatopseudoparaphyses, dermatobasidia, differentiated hyphal ends, broom-cells, hyphae of the fundamental tissue, hyphae of the connective tissue, dichophysoid hyphae, primordial hyphae, sphaerocysts, structure-less mucilaginous masses.

[26] These facts, taken from the anatomy of the *Russulaceae*, are especially instructive because of the thorough study that has been devoted to them by several authors whereby the knowledge of the anatomy of the *Russulaceae* was temporarily extended beyond our general knowledge in the *Agaricales*.

XIII. SPORES

In the *Agaricales,* uni-nucleate and binucleate oidia, conidia, and chlamydospores are comparatively less common than in most other orders of fungi. Only the chlamydospores of *Asterophora* have taxonomic importance as a generic character whereas the presence of conidia in other groups has ordinarily not more than the value of an auxiliary specific character inasmuch as the conditions under which conidia are formed in nature, their constance, and even their existence in many species are unknown (see Brefeld, Vandendries and others on *Coprinus*).

In *Asterophora,* the chlamydospores (Pl. X) arise from the binucleate phase of the fungus, more precisely from the upper portion of the pileus or the hymenophore and also from the binucleate mycelial hyphae. These portions of the fungus become pulverulent, and, at the same time, the hymenophore and the production of basidiospores appears to be suppressed to a certain degree. However, basidia and basidial spores are formed in both the known species, and they have even been brought to germination by Brefeld (1889). The resulting mycelia often disintegrate into oidiachains. The chlamydospores can also be obtained in culture; they are formed predominantly intercalarly in *A. parasitica* where they are smooth, and predominantly terminally in *A. lycoperdoides* where they are coarsely stellate-echinate; the chlamydospores have been made to germinate by various authors, and even carpophores have been obtained in culture (Thompson, 1936). It is now amusing to look back at the classical controversy between those who attributed the chlamydospores to the agaric and those who wanted to see in them an ever-present parasitic Fungus Imperfectus.

As for the arthrospores of *Armillariella ditopa*, see p. 28.

The main form of propagation in the *Agaricales* is by the way of anemochoric basidiospores [27] which are formed by the basidia of the hymenophoral hymenium; a small minority in a few species is formed by dermatobasidia and these spores are, in all cases investigated thus far, identical in all respects with the spores formed by the basidia of the hymenophoral hymenia. The hymenia sporulate through-

[27] In papers on *Agaricales*, the word spores customarily refers to the basidiospores; the latter, more exact term is very rarely used.

out the mature life of the carpophore in the fleshy forms but are frequently found in a non-sporulating stage (inaccurately, these carpophores are usually referred to as sterile). This is much like the conditions in the tough and leathery *Aphyllophorales*, especially the *Microporus-Daedalea* group, and the *Stereaceae*. These long-lived (though in the *Agaricales* always annual) carpophores «time» the sporulation period or periods in accordance with the weather conditions and the seasons. For the practical purposes of spore study, the genera of the *Tricholomataceae Lentineae,* the genus *Trogia,* and the genera *Marasmiellus, Marasmius, Crinipellis, Chaetocalathus,* and *Collybia,* are most annoying. Otherwise, the spores are always present in smaller or larger number though often not in sufficient number to produce a spore print. The examination of the spores from spore prints is preferable to the examination of the spores found in fragments or sections of the hymenophore. The spore print contains only mature spores, and it is then not necessary to fall into the habit of measuring only the largest spores (as was done by Bresadola) in order to be sure to exclude immature spores, and also of measuring all spores, excluding the very smallest and the very largest (as was done by Lange). These methods will invariably, in an average, yield too large, or too small measurements which can be demonstrated if the measurements obtained by these methods are compared with those obtained by measuring all sizes of spores from a spore print[28]. Not only the measurements will be exact, it will also make it impossible to create nomina ambigua by studing the hyphae of one species and the spores of another as has sometimes happened when a large amount of foreign spores (even mould spores—incredibile dictu) was blown on the hymenophore of the specimen under examination while it was in the basket or in situ.

In shape, the basidiospores vary from almost perfectly globose (Pl. XIX, 2; XXIV, 1) to strongly elongated, from round to nodose (Pl. XIV, 2), stellate, or angular (Pl. XII, 5-6) in circumference, and from terete to laterally compressed (Pl. XI, 4) or angular (polyedric) when seen from one end (the longitudinal axis toward the objective of the microscope) (Pl. IX). They are never perfectly orthotropic (Corda

[28] R. Maire also enumerates other sources of errors in spore measuring (comparison of measurements in different media, faulty use of the ocular micrometer, etc.) in a paper that will be very helpful for those in need of more elementary advice in techniques (*Bull. Soc. Myc. Fr.* **42** : 43-50. 1926).

1842) and equilateral, a feature common to almost all spores produced on the outer surface of carpophores in the *Basidiomycetes,* and often put in contrast to the symmetry of spores produced by angiocarpous (endocarpic) forms. This can be explained by the manner in which the spores are produced and discharged at the tip of the sterigmata. A study of the spore discharge in non-angiocarpous *Basidiomycetes* shows that the inaequilateral spore is advantageous in spore discharge, or at least a logical by-product of the exogenous discharge [29] whereas in angiocarpous *Basidiomycetes,* the spores are disseminated through a final disintegration (partial or entire) of the peridium, or by other devices, after the basidia themselves have collapsed and disappeared in the gleba. The spores are consequently freed from the sterigmata by the disintegration of the basidia rather than by forceful discharge, and they are not in need of any lever-action or any other advantage gained by the asymmetry (or heterotropism, Corda, 1842) of the spores so general in the non-angiocarpous forms. While all this is obviously basically true, the further statement that, therefore, all *Agaricales* have heterotropic spores, and all *Gastromycetes* orthotropic spores is not a law without exceptions. In the first place, the spores of most *Gastromycetes* (the author has studied in this regard *Secotium* and *Torrendia*) are not all perfectly orthotropic, but some spores are always heterotropic because of the lower or more oblique position of the sterigma on which they were produced. Furthermore, the gastroid form of *Boletinus decipiens* which, biologically speaking, is a Gastromycete rather than a bolete, has truly heterotropic spores. Some *Russulaceae* that are otherwise close to certain *Gastromycetes* of the group called *Astrogastraceae* by French authors, and form their spores either gymnocarpously or pseudoangiocarpously, have spores so close to truly globose that it is very difficult to establish whether they are inaequilateral while still being slightly obliquely attached by their hilar appendage which makes for a certain degree of heterotropism (Pl. XIX, 2). This «almost orthotropic» manner of spore formation cannot otherwise be explained than by the affinity of these species with true *Gastromycetes.* Orthotropism and heterotropism of basidiospores are, consequently, not a character of immediate adaptation to either angiocarpous development of the

[29] See the various theories given on spore discharge in *Basidiomycetes* by Buller (1924), Ingold (1939), Lohwag (1941), Prince (1943), Corner (1948). It appears that the mechanism (septation, etc.) varies in the different groups.

carpophores or to non-angiocarpous spore production even though, historically and evolutionally, the manner of development appears to be the source of this divergence of spore development.

The strong inaequilaterality of the spores of many *Agaricales* makes it easier to differentiate between an inner (often flatter or even depressed) and an outer (often more ventricose) side of the spore; in elongate spores, these sides are distinguishable to the right and left of the longer axis. The hilar end (base) of the spores is oblique and the hilar appendage (part of the spore that joins the tip of the sterigma) is nearer the inner side than the outer side. It lies in the direction of the geometric axis of the spore only if seen from the inner or the outer side. If the spore is turned around the geometric axis by 90°, i. e. when seen in profile, the hilum becomes somewhat removed from the geometric axis. On the other hand, the apex of the spore is always on the distal end of the geometric axis of the spore [39]. In spite of its inaequilaterality, the spore has in most cases approximately the same breadth whether it is measured from the inner side to the outer side, or seen in profile (i. e. tangentially — if the 4 spores are thought as four points in a circle). This means that the smaller diameter is almost identical in all positions the spore may take when it turns around its axis as often happens when the spore moves in the medium of a temporary preparation. There are, however, exceptions to this rule. The genus *Deconica* is especially notorious for its spores being narrower in profile, and broader (about 1-2 μ) when turned around on their axis by about 90° (i. e. to a point where the position of the hilum coincides with a continuation of the geometrical axis and the spore is seemingly symmetric; Pl. XI, 4). Such spores are called lentiform in spite of the fact that they are not subcircular in circumference but rather oval. Lentiform spores also occur in *Conocybe* and in *Coprinus,* but here, the character has no more than specific value though Fayod proposed a separate genus for those *Coprini* that show it. It is remarkable that most lentiform spores are slightly to distinctly rhomboid, i. e. they have an inaequilaterally hexagonal outline (shape of benzene ring formula) in frontal view.

The inner side is either convex, or flattened (Pl. XXV, 4, 11); or

[39] This is one safe way, for the beginner, to make sure which end of the spore is the apical, and which the basal end when the spore is detached from the basidium.

depressed, especially in the region just above the hilum, or in the lower half. We therefore speak of spores as having (or lacking) a suprahilar applanation, or a suprahilar depression.

The elongate spores are called ellipsoid (Pl. XXVIII, 3 *h-i*) or ovoid if their Q (length divided by breadth) is smaller than 2; otherwise, they are called ellipsoid-oblong (Pl. XXVIII, 3, 6), fusoid (Pl. XXVII, 3-4) or cylindric (Pl. XVI, 8), more rarely (especially in *Marasmius* and *Tylopilus,* sometimes *Boletus*) clavate with the club-end beneath. Cylindric (rarely fusoid or ellipsoid-oblong), white or pale-colored spores are characteristic for wood-inhabiting species (yet, of course, by far not all xylophilous species have cylindric spores), and even more for a certain tribus in the *Tricholomataceae* (*Lentineae*) where this shape is a tribus-character.

Among the species with angular spores, Romagnesi distinguished two types, a symmetric and an asymmetric type. Usually a good indication of symmetry (Pl. XI, 5-6) is the presence of a right (90°) angle at the lower end of the spores when the spores are seen frontally (i. e. with the hilum in line with the geometric axis), whereas in asymmetric spores, the lower end forms a larger angle [31]. Since these two main types are known to exist — along with a series of subtypes — only in a single genus, *Rhodophyllus*, we refrain from a more detailed discussion of this problem.

In two genera the spores are visibly angular only in «upright» position, i. e. if seen from one end, with the longer axis of the spore vertically pointing at the objective. The sides between the angles are, in this view, either plane or slightly concave, and the number of angles varies from 5 to 10 (it is most frequently either 6, 8, or 10). When seen in profile or in frontal view with the long axis being horizontal, these spores hardly show much unevenness and will easily pass as smooth (*Clitopilus*, Pl. IX; XXVII, 8-9) or warty-rough (*Rhodocybe)* unless the angles are slightly projecting into subalate striae. This character is of an undeniable importance in the taxonomy of the *Rhodophyllaceae,* and has also been observed in spores with «removed» [32] ornamentation of *Melanoleuca* (*Tricholomataceae*).

[31] For a more thorough understanding of Romagnesi's spore types, it is necessary to study his paper, *Bull. Soc. Myc. Fr.* **53**: 319-338. 1937.

[32] This means with the amyloid exosporium dissolved according to the method employed by Josserand (1941).

The walls of the spores are either smooth or ornamented. Locquin (1942) distinguishes 3 types of ornamentation, viz.

1. *The primitive ornamentation (ornementation primitive).* It disappears soon because of the growth and the further differentiation of the wall, and leaves usually no traces on the mature spore. Locquin who is inclined to think that it may be interpreted as phylogenetic reminiscence, suspects that possibly certain ornamentations of little developed species might go back to this origin. If so — and the thoughts of Locquin are theoretically not incorrect —, the author cannot see why the primitive ornamentation in this case does not become identical with the secondary ornamentation which is the persistent and final one. If only one persistent ornamentation exists, there seems to be little to gain by calling it any more technical names than simply — ornamentation. The primitive ornamentation has been discovered in *Macrolepiota procera* where the mature spores are completely smooth; it does not exist in the majority of the species of *Agaricales.*

2. *The secondary ornamentation (ornementation secondaire, définitive).* This is said to be the final persistent ornamentation originating in the episporium, the exosporium, or the endosporium, i. e. in any of the layers of the spore wall proper (not in the perisporium). However, Josserand, two years earlier, has distinguished the fundamental ornamentation in the *Russulaceae,* which excludes the exosporial ornamentation that is of later origin and should be known as secondary ornamentation if this latter term were applied at all (the author prefers the term «exosporial ornamentation»).

3. *The perisporial ornamentation (ornementation perisporique, evanescente).* This is of perisporial origin (for the term perisporium, see below, p. 70, 71), and is fugacious, becoming ruptured into patches and warts much in the manner the volva of an *Amanita* is ruptured and finally obliterated by dissolution or lack of elasticity.

This classification of the ornamentations cannot at present be applied in all cases because it requires very exact studies of the fine structure of the walls and their metachromatic properties against a series of dyes and reagents, as well as a study of the development of the spore from its first appearance at the tip of the sterigma till maturity. Consequently, in many cases, it is wise to speak of ornamentation in the general sense of the word. In contrast to the anatomical-ontogenetic classification it is always possible to apply the classification of typical configurations of the spore ornamentation in the

Agaricales [33]; these configurations are marked with Roman numbers.

Type I. Coarse banded ridges form a reticulated surface *(Strobilomyces floccopus, Boletellus retisporus, Lactarius lilacinus).* (Pl. XIX, 2; XXIV, 2, lower spore).

Type II. Ridges and fine lines and warts form a reticulated surface *(Russula Mariae).*

Type III. Warts or spines connected to form a reticulation.

Type III a, which signifies a complete network as in *Russula emetica.*

Type III b, which signifies an incomplete network.

Type IV. Warts or spines connected by scattered thin lines, not forming a reticulation or a fragment of a reticulation. (Pl. XXIV, 6, lower spore).

Type V. Warts or spines from which short, thin lines run over the surface of the spore wall but do not reach the nearest wart or spine. (Pl. XXIII, A, 1).

Type VI. Warts or spines completely isolated *(Russula Schiffneri, Laccaria echinospora).* (Pl. XXIII, A, 2, 4, 5).

Type VII. Punctations and fine, short lines, sometimes touching or crossing each other *(Russula melliolens).*

Type VIII. Catenulate warts usually crowded into or connected to chain-like rows *(Russula elephantina).*

Type IX. Ornamentation continuous, a smooth surface resulting (young spores of *Russulaceae, Fayodia bisphaerigera).* (Pl. XI, 1).

Type X. Longitudinal ridges, often slightly spiralling, often somewhat anastomosing *(Bolëtellus Russellii,* Pl. XXIV, 9; anastomosing : *B. ananas,* Pl. XXIV, 7).

Type XI. Short warts or cylinders perforating a heterogeneous wall but scarcely projecting *(Porphyrellus gracilis, Boletellus betula. Crepidotus,* sect. *Echinosporae, Tubaria thermophila)* (Pl. XI, 2; XXIV, 4-5).

Type XII. Surface irregularly warty-roughened (*Lepista nuda : Linderomyces lateritius).*

In descriptions, the use of the figures designating the type of ornamentations, or a number of these figures combined (the unusual ornamentation in a species given in parentheses) shortens the descriptions considerably while still maintaining a high degree of pre-

[33] In other fungus spores, more types have been distinguished (short-ridged, loculate, etc.).

cision, and is generally recommended, especially for those groups that, like *Russula* and *Lactarius,* have a great variability in spore ornamentation according to species, subspecies, varieties, individuals and individual spores. This scheme of ornamentation types is not concerned with the fundamental nature of the ornamentations, i. e. with the questions by which layer and by which process in the development the ornamentation of the spore is formed. Isolated warts in a spore layer beneath the outermost layer in *Fayodia bisphaerigera*, isolated spines in *Russula Schiffneri,* and innate (fundamental) spines in *Laccaria echinospora*, all correspond to the definition of type VI, yet the chemical character, the development, and the homologies of these ornamentations are by no means identical [34].

The spore wall is in many cases simple or seemingly simple (Pl. XXVIII, 3) i. e. under the prevailing method of investigation, it cannot be recognized as double or complex. In the *Agaricaceae*, and in the related dark-spored families, the spores often consist of two or three layers, easily distinguishable in ammonia, KOH, or Melzer's reagent, and in cresyl blue solutions. These layers have a varying relative diameter. The inner layer is called the endosporium [35] (De Bary, 1881) (Pl. XIII, 1; XXIII, B, 2, 5); the external layer is called the episporium (De Bary, 1884) (Pl. XIII, 1; XXIII, B, 2, 5). Sometimes there is an intermembranal layer or what appears to be an intermembranal space *(Chlorophyllum molybdites),* and in some species *(Macrolepiota procera),* the endosporium has two layers, the internal and the external endosporium (Locquin, 1942). In other instances, there is a third, often ruptured or saccate structure present that envelops the whole spore like a bag, or fragments of a hyaline covering. This part of the spore is called the perisporium (Pl. XI, 1). It is very evident in such forms as *Galerina Hypnorum* forma *montana* or *Strobilomyces floccopus* (Pl. XXIV, 1), in the latter case strongly reminiscent of what is known in *Scleroderma cepa* and other species of that gastromycetaceous genus. Even in *Russula* (e. gr. *Russula archaea*) such enveloping layers have been noticed. Lohwag (1937) thinks that they result from the outer part of the

[34] More data on the spore development and the micro-structure of the spore walls and ornamentations can be found in Locquin's papers on this subject (see literature).

[35] It is important to distinguish between two similar terms: endosporium — the innermost wall of the spores, and endospore — a spore formed endogenously, inside an ascus or sporangium.

basidial-sterigmatic-wall which is usually either so closely agglutinated, or so fugacious that no trace of it can be seen in mature spores.

In the literature, there is also indicated another term, exosporium. The exosporium was first given by the pre-Fayodian authors, e. gr. De Bary, as a synonym of episporium. However, the word episporium was preferred. Fayod himself, unfortunately mixed up the terminology as has later been shown by R. Heim (1931) [36]. What Fayod called exosporium is a layer outside the episporium. In some cases, Fayod may have taken the optical halo as an outer layer, as was suspected by Heim but Locquin has proved that a true exosporium in the sense of Fayod actually exists in many cases, and that this exosporium had thus far escaped the attention of all authors except Fayod. There can be no valid reason to prefer Fayod's terminology to the use of the term episporium in the sense of De Bary and the French authors (starting with Patouillard), and the term exosporium in the sense of Fayod and Locquin.

Consequently, in the most complex spores known, we have to distinguish between the following layers of the wall and its outer envelopes (from inside outward) :

1. The internal endosporium.

2. The external endosporium : both colorless, usually thinner than the episporium, or equally thick, sometimes absent (in monostratous spore walls).

3. The episporium : in the colored spores, this is the pigmented portion of the spore wall ; in hyaline spores, it is always the thickest layer of all, and frequently shows an ultrafiltering capacity for cresyl blue in watery solution. It is doubtful but possible that the episporium may also be composed of two layers in a few species (see Heim, 1931, and Locquin, 1943).

4. The exosporium : this is colorless and consistently thinner than the episporium, usually delayed in its dissociation from or deposition on the primordial episporium, often of different chemical structure as compared with the neighboring strata (perisporium and episporium).

5. The perisporium : a loosely attached non-pigmented layer that envelops the spore as a bag, or a closely attached but fugacious layer

[36] What Fayod calls endosporium is not the endosporium of De Bary and of the modern anatomists but the episporium of De Bary and the French mycologists, a term adopted in this book. The true endosporium is called « la couche membraneuse du protoplasma » (Primordialschlauch of the German authors) by Fayod.

that is destroyed by dissolution or fragmentation in an early stage of the spore development. It is doubtful but possible that there are occasionally two sub-strata composing the perisporium (see Locquin, 1943).

These anatomical facts and discussions of terminology are not of a remote significance for the taxonomist but of primary importance. The spore, with all its characters, has become, more and more, one of the most important characters on which the taxonomy of the *Agaricales* is based. The descriptive data become simpler and more definite when they are based on exact anatomical observations, and the homologies become more evident. Comparison between the spores of different species must be based on the comparison of homologous parts of the spore. The consecutive observations of Malençon (1929, 1931), Josserand (1941) and Locquin (1943) have shown that the *Russulaceae* have a fundamental ornamentation formed by the episporium which is slightly colorable with Melzer's reagent; the zone above the hilum and the larger portion of the surfaces of the fundamental ornamentation are covered with a thin exosporial ornamentation which is disrupted and clings to the episporium by a certain adhesiveness of its own and an increased readiness for humectation of at least parts of the episporial surface, and it responds chemically to most tests for amidon. It can be dissolved by several chemical substances, such as concentrated HNO_3, and less uniformly by NaOH, KOH, etc. in a heated concentrated solution. Thus, Josserand first bared the fundamental ornamentation of the *Russulae* and *Lactarii;* but in *Leucopaxillus pulcherrimus,* after dissolution of the amylon-layer, he did not find any fundamental ornamentation; neither did the author on the related *L. albissimus*, using concentrated nitric acid. This means that the entire ornamentation of *Leucopaxillus* (at least those two species) is superficial, heterogeneous, and exosporial. Josserand indicated for *Melanoleuca* that the fundamental ornamentation is likewise either absent or insignificant. This marks a difference between the *Russulaceae* and the *Melanoleuca-Leucopaxillus* complex which is most important considering the elongated spores of such species as *Russula heterospora* and *R. ventricosipes*, and their similarity with those of *Melanoleuca.*

It may be noted here that the ornamentation of the *Russulaceae* has been thought to be, in its entirety, of destructive origin, i. e. a layer, at first continuous that because of the growth of the episporium breaks into more or less regular fragments. Josserand exempted the

fundamental ornamentation from this rule but thought it still applicable for the exosporial ornamentation. However, Locquin offers a new hypothesis on the development that is a physical one : As the exosporium solidifies at a certain point in the maturation of the spore, it cannot cling to certain areas because of the physical differences which, according to Locquin are determined by the different organization of the micelles of the surface of the episporium. Whatever the fate of this explanation may be, it must be admitted that it is the only one that is in full agreement with all the facts available. It does not by any means invalidate Malençon's now classical series of spore configurations in the « Asterosporées » i. e. the bridge between some *Gastromycetes* and some *Agaricales ;* leading from the *Hydnangium-carneum* group to the *Russulaceae.*

The warty spores of the *Cortinariaceae* have not been studied equally carefully and by an equal number of observers in recent years, and one might assume that here the development of the ornamentation is, in the great lines, similar to that of the *Russulaceae.* However, unless more comparative anatomical ontogenetic and microbiochemical work supports this analogy by more facts, it would be a wise course to refrain from taking the obvious homologies for granted. Yet, the spore ornamentation of *Galerina* (*Cortinariaceae*) has a character in common with the *Russulaceae* that is of great importance in any discussion of the ornamentation in these genera, and also of a significance in taxonomy that can hardly be exaggerated. This is the round smooth (or comparatively smoother) area just above the hilar appendage on the inner side of the spores which we may name suprahilar disc. If this disc is amyloid, it is termed hilar spot (tâche, Heim, 1938), so in *Melanoleuca* and the *Russulaceae ;* if it is not amyloid and merely stands out by its smoothness (well visible in NH_4OH-preparations imbedded in Shear's mounting fluid or with the ammonia replaced by a 50 p. c. watery solution of chloral hydrate) it has been called plage (Pl. XII, 3) by Kühner (1926), and this term has been adopted without change in other languages than the French. The plage is the most important character distinguishing the typical *Galerinae* among the *Cortinariaceae,* and the hilar spot is one of the most important characters separating *Melanoleuca* from *Leucopaxillus.*

The wall and its layers are continuous in many species, in others, especially those with complex walls, the spore wall is partly or entirely interrupted or modified at the apex. This apical interruption or modi-

fication is either (1) a germ pore (Pl. XI, 2; XI, 4; XIII, 1; XXIII B, 2, 5), i. e. an interruption of the outer layers of the wall with the endosporium either intact or also modified to interrupted (examples: *Macrolepiota, Kuehneromyces, Bolbitius,* etc.) whereby the apex of the spores often becomes truncate if the interruption is broad enough, or (2) a callus, i. e. a thinner-walled apical region that is more or less convex, or even callously protracted rather than truncate (example: *Galerina* spp.). The callus has been named by Heim (1931) who first distinguished it from the germ pore with which it was often confused before (Fayod, 1889) provided it was noticed at all. The germ pore has been known for a long time but its taxonomic importance has been stressed only by Fayod and Patouillard.

The germ pore of light colored spores is not always easy to recognize under dry objectives, and sometimes even under immersion lenses. It should be studied after an initial treatment with 10 p. c. KOH which is subsequently removed, and replaced by cresyl blue solution (see p. 77) or aceto-carmine.

The microscopical basis of the macroscopical difference in spore print color is usually the pigmentation of the spore wall (see p. 16, 105); most of the dark spored families can be recognized from the spore color under the microscope; however, the cream color, greenish and pink shades in light spored agarics and boletes are not always clearly reflected in the color seen under the microscope. Sometimes, the pigmentation of the spores under the microscope is of independent value in the taxonomy of certain groups (*Xanthoconium stramineum; Callistosporium,* etc.).

The size of the basidiospores ranges from 2 to 40 μ in length and accordingly in volume. All spores are unicellular, except for a few isolated cases where the old spores have been seen to become septate (*Crinipellis mirabilis*, etc., Pl. XXVIII, 3 *d-e*) after discharge. Heim (1948) interprets this as a direct transformation of the spore into a binucleate chlamydospore.

The spores are never sessile on the basidia in the *Agaricales*, in spite of Fayod's indication of sessile spores in his genus *Astylospora* which seems to be based on faulty observation. They are always borne on the apex of sterigmata that are apical and half-sickel-shaped or horn-shaped (Pl. XII, 1; XXIV; XXV, 3; XXVI, 5; XXVII, 2, 6, 10) in the *Agaricales* (very rarely lateral, a feature that has no taxonomic significance since it is an individual irregularity in an occasional basidium).

The protoplasmatic interior [37] of the spores is usually colorless; it often includes one to several oil-droplets [38] (Pl. XXVIII, 3 *c*) which are of much less taxonomic value than in the *Discomycetes*.

XIII. STAINS, MACROCHEMICAL COLOR REACTIONS AND CHEMICAL ANALYSIS

Absorption of specific dyes is not a direct expression of the chemical constitution of the various parts of the plant tissue, yet in certain cases, the absorption of the dye is different in different organs and different in different parts of hyphal or sporal walls, etc. This so-called metachromatism is not the same in the same organs of all *Agaricales*, and Kühner, Singer. and Heim have recently used this fact as the basis of taxonomic as well as organographic differentiations, i. e. for the characterization of groups in the classification of the *Agaricales* and for the characterization of certain specific types of organs. These metachromatic colorations like the chemical reactions which also have been introduced into agaricology in recent years, are only characters, and are not pretended to be more than that. Some authors speculated on the chemical nature, and the physico-chemical conditions under which these selective colorations and color reactions take place; in some instances, the substances involved have been studied to a certain degree, from a chemical point of view, or else the type of reaction taking place was too obvious to be overlooked by the mycologists, yet, as a rule, no systematic attempt has been made to identifiy the reacting substances by a standard method of chemical analysis, and to explain the reactions taking place in a biochemically proper manner. The notable exceptions that might be mentioned here are the poisonous agents in a very small number of poisonous fungi, especially *Amanita*.

This, in the opinion of most modern mycologists employing chemical characters, does not render them any less valuable from the taxonomic point of view. The only requirements of a good character are its constancy and correlation with other characters. Those who have in the last 20 years systematically introduced new chemical characters had only two preocupations: (1) are the reactions obtained genotypical — or phaenotypical and accidental, i. e. are they reactions typical for

[37] « nucleus » of Corda (1842).

[38] « nuclei » of some authors.

the form under consideration, or dependent of factors such as temperature, substratum, or host, and consequently irrelevant for taxonomic purposes; (2) are these reactions correlated with morphological characters ?

Even if the chemical substances involved in the reaction are unknown, or the modus of their transformation hypothetical, their taxonomic value may thus still be considerable, and arguments in questions of systematics based on chemical characters may still be valid provided that the chemical character is both genotypical and correlated with morphological characters.

This does not mean that an investigation of such reactions from a purely chemical point of view be omitted in the future. It is quite obvious that work of this order is highly desirable. An attempt has been made to correlate both taxonomic and chemical research in lichenology especially as far as the isolation and identification of lichen acids were concerned, and the results have been interesting and valuable both from the chemical as from the taxonomic point of view.

The only objection that may be made to the taxonomic use of « good » chemical reactions as characters in spite of the lack of a chemical explanation of the changes observed, is that an equal external effect may be obtained by using the same method with different species even though the substances involved may be different. The error in our interpretation would then be the application of a term such as « positive » or « amyloid » for a reaction of a certain order in all cases whether they are due to the presence of an identical substance or merely of a substance with identical or similar reaction in contact with a given reagent. It is, of course, probable, or almost certain, that the amyloidity of spore ornamentations or spore walls is not based on the presence of the same substances in all cases where a «positive» reaction with an iodine reagent is obtained. In fact, the apices of the asci of certain *Ascomycetes* (pure blue reaction with Melzer's reagent), the ornamentation of the spores of the genera *Melanoleuca* and *Leucopaxillus* (blackish violet), the hyphae of *Marasmius* and *Mycena* (vinaceous to vinaceous brown), and the hairs of *Crinipellis, Chaetocalathus, Lachnella* and *Merismodes* (brownish violet to deep reddish brown), the spore walls of *Neohygrophorus angelesianus* (pale greyish livid) and perhaps some more « amyloid » walls, are probably of a different chemical composition, and the amyloid reaction is not caused by the same substance, or the same groups of substances.

All this may be true. Yet, if an argument concerning a taxonomic question, and based on chemical characters, is only one part of a series of reasons that support, for example, the affinity of two groups, the chemical character should not be disregarded on theoretical grounds. The overemphasis put on a chemical character alone (e. gr. in Melzer & Zvára's monograph of the *Russulae*) is not justifiable even if the chemical identity of the reactions in each case could be demonstrated by analytical means. This does not make the discoveries of Melzer & Zvára any less valuable for the use by an experienced taxonomist. Those who reject chemical characters must also reject color, odor, taste, and gelatinosity which are likewise characters without a fully explored chemical basis in most cases. We would then have to rely on morphology alone — and morphology, at present, does not provide complete guidance either. It is therefore necessary to use, with the utmost caution, but without blind reluctance, all available characters, the more — the better. Biology is not yet a strictly exact science, and asking to consider it as such without allowing for a large number of working hypotheses, is equivalent to stopping its development.

These observations are necessary in order to introduce the use of chemical characters to a broader public than has ever been done before. The acceptance of chemical characters as a valuable contribution to the factual material available for the determination of affinity is not in danger since even the critics of this new method make widest use of it in their own papers. However, unreasonable criticism is merely another factor in delaying the broader application of chemical characters among the mycologists, including collectors data and routine determinations.

Metachromatism with cresyl blue

Cresyl blue mounts of spores of *Macrolepiota*, *Leucoagaricus* and *Leucocoprinus* allow the observation of the endosporia because of a selective coloration that results from ultrafiltration of the dye solution by the episporium in such a manner that the endosporium is dyed reddish, and therefore stands out enough to be rather conspicuous even in cases where it is not very strongly developed. In other genera of the *Agaricaceae*, the endosporium — whether strongly developed or not — does not show such an effect in cresyl blue mounts. This differentiation has been shown to be of great help in the subdivision

of this family, as an additional spore character to be used together with the iodine reaction and the germ pore.

In the hyphae of the stipe, cresyl blue often provides a similar character based on metachromatism which is somewhat but not quite parallel with the Melzer reagent's metachromatism (see p. 79). Kühner who has discovered both the sporal and the hyphal metachromatism in cresyl-blue-sections enumerates several important differences between the positive reaction with the Melzer on one hand and the metachromatism with cresyl blue on the other hand (1933). This makes the use of cresyl blue in addition to that of the Melzer even more important. The sections can be treated with ammonia or KOH at first in order to separate the hyphae, but the alkaline solution must be removed entirely afterwards; the section is then colored with a watery solution of cresyl blue, the excess dye is removed with filter paper, and replaced by water. In certain species such as most *Mycenae*, many *Marasmii*, etc., the hyphae of the stipe become red, thus contrasting with the normal coloration of hyphal walls obtained with cresyl blue which is a pale violet, pale blue, or practically nil. The cortical layer of the stipe should be disregarded for this purpose.

A strong deep blue stain is obtained by the use of cresyl blue on the interior of all gloeocystidia, in the *Corticiaceae*, *Cantharellaceae*, *Leptotaceae*, and in the agarics (Pl. XXI, 3); this characteristic metachromatism is, as we have seen above, a perfect means for recognizing these bodies in dubious cases (Singer, 1945; Heim, 1946). The technique is the same as described above.

Kühner has also indicated (1934) « very numerous precipitations of a bright red color» in several Hygrocybes (especially the species with viscid stipe) which is not generally observed in other fungi; the walls of the cystidia are colored either metachromatically (deep lilac or violet, or the same color but very pale, Pl. XXI, 4), or blue (*Inocybe*, Pl. XIV, 2; XVII, 1); reddening (metachromatic) trama is found in most of *Agrocybe*, *Hygrophorus*, all *Mycena*, *Lepiota*, and *Hebeloma* species studied by Kühner, never in *Cortinarius*, *Inocybe* or *Naematoloma*; strong reddening of the trama of the *Amanitaceae* is observed in the subgenus *Eu-Amanita* (excepting the *Phalloides*-group), and in the *Emetica*-group of *Russula*, the basidia are distinctly metachromatic in many agarics (*Tricholoma*, *Cortinarius*, and others) but never in the *Boletaceae*.

Cresyl blue can also be used in order to study the ornamentation

the same way as is done in the *Russulaceae* with Melzer's reagent; cresyl blue has given a picture surprisingly similar to an exosporial ornamentation of the type III b-IV-(V) in *Russula,* when used on spores of *Neopaxillus echinospermus.* The exosporial ornamentation of the *Cortinariaceae* is also deep violet colored but usually less well differentiated from the episporium, at least optically.

Cresyl blue is not the only dye that provides the anatomist, working on *Agaricales,* with metachromatic colorations. Several more (cotton blue, diamine blue, alkaline methylene blue, various violets, carmines, etc.) metachromatic stains are known, but their use has not yet become taxonomic routine.

This brings up the question which dyes are recommended for general use in *Agaricales.* For routine preparations of an unknown agaric or bolete, it is customary to use ammonia-mounts [39] first without any dye; it is not wise to start the study of a species with stained material. Only as a second step, in order to get clearer pictures, this same preparation may be dyed with phloxine (now generally used by American specialists of *Aphyllophorales*), 2 p. c. alcoholic solution, which is stable in ammonia or even KOH mounts. Phloxine is, however, taken up by the interior of the hyphae rather than by the walls, and for the walls, cresyl blue is as good as any other dye for a first try. As a rule, every fungus and every organ, and even every part of this organ require individual stains, according to their chemical constitution and physical properties. In many cases, chemical reactions will be used, such as Melzer's reagent, in preference to organic dyes.

Micro-chemical reactions

It is difficult to differentiate between dyes and reagents since many reagents, among others the most important reagent for the study of *Agaricales,* Melzer's reagent, act rather by absorption than by fully measurable chemical transformations of the treated material. However, even though the Melzer reagent is, in a certain sense, an inorganic dye causing metachromatisms of the same order as cresyl

[39] KOH is preferred in tough species or in species with dense tissue that are not mercerized easily by NH_4OH, also in preparations that are intended to show the general structure of an organ, as in KOH strong pressure on the cover glass can be avoided. However, for a study of fine structures such as diverticulation of epicuticular hyphae, pigment incrustations, and spore ornamentations, KOH is definitely inferior to NH_4OH.

blue, it is currently considered as a reagent rather than a dye. It was unavoidable to mention the Melzer reagent in the preceding chapters, but we shall now attempt to give a resumé of the reactions that can be obtained with it.

The reagent has completely replaced the use of any alcoholic iodine solution and the classical use of Zn $Cl_2 \cdot J_2$. Its composition though slightly altered in one sense or another (without much difference in effect) by some mycologists is still the original indicated by Melzer (1924).

KI	1.5 gr
Iodine	0.5 »
Water	20.0 »
Chloral hydrate	22.0 »
	44.0 gr

It has first been used on the exosporial ornamentation of *Russula*, and this was its original purpose. However, the mycologists who later searched for all kinds of positive iodine reactions in the tissues and spores of the *Agaricales*, found in Melzer's solution a convenient standard solution that would always give identical results if applied in an exactly circumscribed manner. It cannot be emphasized too strongly that any deviation from the formula and the following procedure, may (not must) cause a discrepancy between the results obtained and those described by the authors. In the first place, it makes no difference when the material has been collected and in what manner it has been dried. Material about 120 years old still reacts nearly as well as freshly dried material. The preparation must first be wetted for a few seconds in ammonia (NH_4OH-concentrated), then the ammonia must be completely removed with filter paper, and a large excess of Melzer's reagent must be added in order to compensate for any alkaline reaction still prevalent immediately around the fragment examined. Usually, a positive result can be seen without prolonged action of the iodine, yet if the result seems to be negative or inequal or doubtful at first, it is recommended to warm up the slide after about 20 minutes waiting, and then examine it.

The reaction is called amyloid or pseudoamyloid — if positive — and nonamyloid, if negative. The amyloid reaction is nearly black in some cases, in others it is a slight pallid grayish with a livid shade, with many intermediate shades between the two; pseudoamyloid (Singer, 1938) is a positive reaction if the final color obtained is brown to purplish brown. Nonamyloid walls are yellow to nearly

hyaline. Naturally, the reactions of strongly pigmented spores and hyphae, at least the strongly pigmented layers of their walls, cannot be inserted in this scheme since the reaction, one way or another, would be obscured, and covered up by the pigment, and treatment of these walls with substances that in the end would extract or destroy the pigment, would also alter the initial reaction of the wall. Consequently the question of amyloidity and therefore the use of Melzer's reagent is confined to hyaline or light colored (stramineous, palest melleous) tissues, and spores, and to the colorless endosporia of pigmented spores.

Amyloid reactions, obtained in the asci of the *Ascomycetes*, in the spore walls of the smooth-spored *Leucopaxilli*, in the exosporial ornamentation of *Leucopaxillus* (Pl. XXIII A; 1-6), *Melanoleuca, Russula* (Pl. XIX, 2) and *Lactarius* (*Bondarzewia* among the polypores), and in the hyphae of the agarics are by no means equivalent to each other, or suggesting a similar chemical composition of these walls. Not only is the color obtained dissimilar (pure blue in the asci — pale livid gray to almost subhyaline in the smooth-spored *Leucopaxilli* — blackish violet in the exosporial ornamentation — amethyst to deep red-brown, i. e. reaching a tone usually associated with pseudoamyloid reaction, in the hyphal walls) but solvents and dyes act in a different manner. Amyloid and pseudoamyloid reactions are clearly different in color in the spores of the *Basidiomycetes*, yet they intergrade almost unnoticeably in the hyphal walls. Amyloid reaction that is not of the amylon (starch) type becomes almost invariably pseudoamyloid in thick-walled hyphal walls, and more amyloid in thin-walled hyphae or such hyphae where only a thin layer is iodine-positive. Locquin thinks that the exosporial ornamentation of the spores of the *Russulaceae* contains amylon which would almost certainly be true also for *Leucopaxillus* (warty-spored species) and *Melanoleuca*. Another chemical composition is probable in other amyloid spore walls, and in pseudoamyloid walls.

Amyloid spores and hyphae were discovered at approximately the same time (1887) by Patouillard (*Aleurodiscus vitellinus*) and Rolland (*Mycena tenerrima*) in the *Basidiomycetes*, and in other fungi and lichens, amyloid reactions were known even before that. It was later found by Kühner (1938) that not only the hyphae of the stipe of *Mycena tenerrima* but all hyphae of the trama of most *Mycenae* are amyloid; he has later defined some sections in the genus *Marasmius*, and some of these sections, or parts of them, are characterized by

amyloid hyphae. More species with amyloid trama were found later by Singer (*Pseudobaeospora oligophylla; Poromycena anastomosans*, etc.). The author has also (1942) discovered epicuticular hairs that are somewhat intermediate between amyloid and pseudoamyloid, more frequently closer to the latter (*Crinipellis, Chaetocalathus; Lachnella, Merismodes*).

Kühner and Maire have first indicated a large number of Lepiotas with an unusual red-brown reaction with Melzer's reagent which they interpreted as nonamyloid, but were later designated as pseudoamyloid by Singer, who also indicated that, aside from certain genera related to *Lepiota,* especially those with spores that have a germ pore, and the genus *Pseudobaeospora,* pseudoamyloid spores also occur in the genus *Chaetocalathus* (1942).

One species of *Marasmius* has cystidia which turn olive gray in Melzer's reagent.

All students of agarics will readily admit, after they have given the iodine tests a fair trial, that this microchemical method provides characters that are of enormous importance in taxonomy if evaluated critically, and used with discrimination. This may not always be the case in other groups of *Basidiomycetes* though it is certain that in the complex *Scutiger - Bondarzewia - Diacanthodes - Abortiporus,* in the group *Hericium* (and related *Corticiaceae*) - *Steccherinum - Dentinum,* etc., and also in the family *Leptotaceae* Maire emend. Sing. 1945, the amyloidity of the spores is very important — and in *Amyloporia* and *Amylocystis* among the polypores, the amyloidity of hyphae and cystidia also seems to have some taxonomic importance. In the agarics, this character must be used just as all other characters — cautiously, applying it as a specific character in the beginning stages of the investigation, and eventually — after enough data have become available — the amyloidity may or may not turn out to be a sectional (*Marasmius, Cystoderma*), a subgeneric (*Amanita*), generic (*Mycena, Pseudohygrophorus*), tribal (*Panelleae*), or even family character (*Russulaceae*). The same is true for pseudoamyloidity (generic character in *Lepiota* and all *Leucocoprineae, Crinipellis* and allied genera). There can be no methodical determination of agarics without Melzer's reagent and a careful study of its action upon the walls of the spores, hyphae and epicuticular elements. As has been said before, it may well be that the term «amyloid» should be supplemented with a few more terms, indicating more clearly the quantitative and qualitative composition of the mixture of amylaceous

and « amyloid » substances that make up the walls of the fungi. A first step in this direction is perhaps the introduction of the term pseudoamyloid which — without a chemical analysis — is based on a difference in color. Perhaps, the reaction of the exosporial ornamentation of certain white-spored groups, in the first place the *Russulaceae*, should be described as amylaceous rather than amyloid. It is felt, however, that a hasty introduction of new words for chemically and physicochemically unknown or halfknown phenomena is premature unless the optical effect is different enough to warrant such a distinction. It is also hoped that the examination of the amyloid substances in the fungi will become clearer in detail when each iodine stain is accompanied by a series of other microchemical reactions and metachromatic colorations with a large variety of dyes. The positive and negative result for every single one of these will then serve as a further modification of the amyloidity as it is now understood in a very general way.

It was Josserand's (1942) idea to remove the amyloid exosporial ornamentation with certain organic or inorganic solvents of amylaceous and amyloid substances or mixtures containing such. Locquin was more methodical about this (1943); the author refers to Locquin's paper on the subject because it appears that this method may have some influence on a future subdivision of the amyloid reactions on one hand, and on the introduction of the fundamental ornamentation in taxonomic mycology on the other.

Locquin, for this purpose, used nitric acid, zinc chloride (solvents of starch-containing spore walls), ammonium oxalate (for walls containing pectine compounds), NaOH and KOH (for walls containing hemicelluloses), and sodium hypochlorite (for chitin). Some of these reagents are also used in other micro-and macroscopical tests newly introduced into taxonomic mycology, especially in the *Agaricales* (but also in the *Aphyllophorales*, and with a great potential importance in the *Gastromycetes*, and perhaps the *Ascomycetes*). We shall first review the microchemical tests:

Potassium hydroxyde, KOH (which can be substituted by sodium hydroxyde, NaOH), is used in *Cystoderma* (Smith & Singer, 1945) since it darkens certain layers of the covering ot the pileus in certain species whereas this reaction is not noted in others. It has been found a valuable additional character. In fact, the main classification of the genus, in the new monograph, is rightly based on two microchemical characters, amyloidity and KOH reaction. In *Crinipellis mira-*

bilis, the epicuticular hairs become gray in KOH (Singer, 1942). KOH also causes a green discoloration of the carbonaceous articles in the trama of *Anthracophyllum* (Singer, 1944). In concentrated H_2SO_4, the spores of certain *Coprinaceae* change from black to pale livid whereas in others the same black or fuscous membranal pigment is resistent (Kühner, 1929); this reaction has been used for the classification of the *Coprinaceae* by Singer (1936). Another taxonomically important reaction was that obtained with ammonia (NH_4OH) on the internal body of the cystidia of *Stropharia, Naematoloma, Pholiota* (Kühner, 1936); these cystidia were later distinguished from other (pleuro) cystidia of the *Strophariaceae* and the *Agaricales* as a whole by Romagnesi, under the name chrysocystidia (Pl. XVII, 3). The author has found deep blue contents in cresyl blue mounts, and therefore thinks that the chrysocystidia are chemically — if not otherwise — related with the gloeocystidia. The trama of *Xeromphalina caulicinalis* and closely related forms turns red with ammonia (NH_4OH) according to Singer (1936); this reaction is due to a transformation of the brown, incrusting pigment of the hymenophoral trama. Another group of species in this small genus, does not show this reaction. Kühner (1935) has first noticed and used as a character in his *Galera* monograph, the needle-like crystals that are formed in preparation of the hymenium in various species of *Conocybe* whereas other species of the same genus do not form them. Singer (1937) reported the same long, colorless needles in ammonia preparations of the hymenophore of *Phaeomarasmius Wieslandri.* This is the first chemical micro-reaction that is not a color reaction.

Several other microchemical characters have since then been added to the above, viz. the reddish pigment in the *Gomphidii* that shows in a formaldehyde-acid solution, and other characters based mainly on the solubility of fungus pigments (see. p. 105). The widest application of microchemical reactions is now made in the *Russulaceae* where a mixture of aldehydes with strong acids is known to provoke a darkening of the contents of the macrocystidia, dermatopseudocystidia, and some oleiferous hyphae and laticiferous vessels. The reagents used are:

Sulfovanillin:

Chemically pure vanillin	0.5 gr
Distilled water	2.0 »
Pure sulphuric acid	4.0 »
Sulfovanillin	6.5 gr

The resulting solution is of a deep rich yellow. It should be filtered through glass wool, or handled very carefully as the unsolved crystals and those that form after a while when the solution begins to disintegrate, often cover the section studied, and, under pressure, the cover glass may easily be broken. Sulfovanillin must be used on fresh material. It is true that the results are sometimes satisfactory with well dried material during a period of several months after collecting but they are no more conclusive if the reaction is negative.

Sulfoformol :

Formaldehyde 40 p. c. watery solution	6 ccm
Distilled water	3 »
Pure sulphuric acid	10 »
Sulfoformol	19 ccm

The resulting solution is colorless. It must be used on fresh material or on material that has been in formalin for not more than 6 months.

Sulfobenzaldehyde :

Same as sulfoformol, the formalin replaced by benzaldehyde.

Chlorovanillin :

Same as sulfovanillin, but the sulphuric acid is replaced by concentrated hydrochloric acid.

All four reagents give parallel reactions :

Organs of the *Russulaceae*	Sulfovanillin	Sulfoformol	Sulfobenzaldehyde (or sulfo-paradimethyl - amino benzaldehyde).	Chlorovanillin
Macrocystidia, Dermatopseudocystidia, some oleiferous and laticiferous vessels.	blue	brown	black	blue
Cystidia, gloeocystidia, basidia, hyphae, ciliate dermatocystidia, primordial hyphae.	(hyaline to) rose color	hyaline	hyaline	(hyaline to) rose color

Sulfoformol has also been tested in the pseudocystidia of *Lentinellus* where it gives the same results as in *Russula* and *Lactarius*. All these reactions have been used on a large scale by R. Maire (since 1907). Sulfovanillin was introduced into lichenology by Lindt (1885), and in mycology by Arnould & Goris (1907). They are now generally used in *Russula, Lactarius,* and *Lentinellus*.

Macrochemical color reactions

Macrochemical color reactions were first used for the determination of certain polypores. Müller (1872) discovered the violet discoloration taking place when *Hapalopilus nidulans* is exposed to ammoniac vapors. Harlay (1896) discovered the deep violet discoloration of the pigment of *Lactarius necator* with alkali which can be used as an indicator — in acid solutions, the same substance turns pale pink. This reaction is unique among the *Lactarii*, only *L. necator* and, according to the experience of the author, *L. atroviridis* show it. In the same year, Bourquelot & Bertrand introduced guaiac, whose reaction with fungous tissues had been discovered by Schönbein (1856), into general use in the *Agaricales*, and in 1907 Arnould & Goris initiated the use of sulfovanillin in *Russula*. Since then, some authors continued to study the action of chemical reagents on the various parts of *Basidiomycetes*, especially agarics (Bataille, R. Maire, Barlot, Kühner). But reagents did not become routine tests in any group of *Agaricales* until Melzer & Zvára (1927) introduced a whole series of chemical reactions, and, at the same time used the reactions for taxonomic purposes; in fact, the subdivision of *Russula* in Melzer & Zvará's monograph is almost too much based on chemical reactions. In 1938, J. Schaeffer & Möller introduced the use of several chemical characters in the taxonomy of the genus *Agaricus,* and in the same year, chemical characters were first used in the *Boletaceae* and *Gomphidiaceae* by Singer, and new reagents were added to those already used in *Russula* and *Lactarius* by Heim. Also in 1938, the first general survey of the reaction of guiacol was made by Singer, and it was shown that even in *Russula,* the genus for which it was first introduced, the reactions are constant in some species, and inconstant and unreliable in others. At the same time, the author used chemical characters for the delimitation of genera, and in phylogenetic problems, and so did other authors (Heim, Romagnesi). In 1939, Bousset recommended the use of monomethylparamidophenol for chemical tests of

Basidiomycetes, and his findings were supplemented by numerous tests of this particular reagent in the author's papers, and he as well as other authors widened the scope of application of Melzer & Zvára's reagents beyond the genus *Russula*. Especially $FeSO_4$ and Fe_2Cl_6, phenol and formaldehyde have become standard reagents for the laboratory and even for extended field trips. Henry makes use of these and other chemicals in his work on *Cortinarius;* Singer on *Paxillus;* Konrad & Josserand on *Collybia*, etc.

This is in short the history of the macrochemical color reactions up to the war. The tendency to use these characters has rather increased than decreased since then.

The following is a list of the most important chemical reagents, the reactions obtained and the genera in which they are used most frequently:

1. *Reagents of oxidases:*

Guaiac. Ordinary guaiac tincture; the oxydases present in fungi act on the guaiaconic acid present in the resin, if atmospheric oxygen is present. A blue (or green) to purple spot is formed at the surface of the section through the stipe in all those agarics and boletes that react positively. The reaction can be used in all genera. The time necessary to obtain the first result should be noted. Indispensable in *Inocybe.*

Guaiacol, watery solution, slightly below the saturation point. Reaction, if positive, from salmon-color-orange to rose color or bluish pink, slowly darkening to dark copper or chocolate color in most cases; the base of the stipe is always most sensitive; the reaction is useful in the *Russulaceae, Tricholomataceae, Amanitaceae.*

Pyramidon in saturated, watery solution. In species with positive reaction, the context of the stipe becomes light lilac color. It is used only in *Russula* and *Tricholoma.*

2. *Other organic reagents:*

Phenol (carbolic acid), 2 p. c. watery solution; reaction either negative or positive; if positive, it is chocolate color, or deep purplish violet after 20 minutes; in some cases the reaction is more sordid grayish vinaceous, reddish, etc. If after 20 minutes no distinct reaction has taken place, the reaction is called negative, even though it may show up after an hour (Amanitas of the phalloides group). Indispensable in *Russula* especially the mild tasting species, *Amanita,* especially the exannulate and the amyloid-spored groups, in *Leccinum,* also used in the *Tricholomataceae* generally, and in *Lactarius.*

Formaldehyde (formalin, formol), 40 p. c. watery solution; reaction varies; it usually is positive in such species that have a tendency to change the color of the context by autoxydation, yet, at times it may act in the opposite way, inhibiting the autoxydation. This is also a slow reaction, and sections treated with formaldehyde should be observed at least 20 minutes. This reaction is indispensable in *Russula, Tricholoma* (the clampless group), *Gomphidius Leccinum* and other boletes.

Aniline (aniline oil and aniline water). This is either pure aniline oil, or the «oil» mixed with an equal volume of distilled water. Since 1932, aniline oil is used almost exclusively. It becomes red to copper red on wounds of the stipe of mature *Russula xerampelina* and allied species, and is more or less parallel in its reaction with that of ferric sulfate. It is also specific in certain cases with the lamellae of the *Russulaceae* where it causes a central stained spot and then a characteristic gray or bright colored zone around it (especially important for *R. emetica*). Also occasionally used in other groups, e. gr. boletes, *Agaricus,* and, among aphyllophoraceous genera: *Scutiger ovinus.* In *Russulaceae*, especially on the lamellae, the reaction is slow.

Cross reaction: This was described by J. Schäffer and Möller, and consists in a test made on the surface of the pileus of the species of *Agaricus* whereby a transversal streak with HNO_3 is made, and then crosswise, another streak with aniline oil. The result, if positive is an orange red to fire red discoloration. It must be considered, however, that the two substances often react with each other without interference of the *Agaricus* whereby a colored crystallized mass is formed that may be misleading.

Phenol-aniline. This consists in the mixture of a few drops of aniline in phenol (2 p. c. watery solution). While all the preceding reactions have to be performed with fresh material, never with dried or otherwise prepared material, this reaction is recommended by J. Schäffer for dried material of *Russula.* The reaction is from nil to nearly black after prolonged exposure.

Sulfovanillin, sulfoformol, sulfobenzaldehyde and chlorovanillin. The formulas are the same as those given under microchemical reactions. For macrochemical color reactions, the first and the last of these reagents are preferred. They are used mainly for the identification of certain *Russulae* (*R. rosea, R. minutula, R. albida,* etc.) in which the context of the stipe and, especially, the surface of the stipe, with sulfovanillin immediately turn very bright red, and

remain that color (Pl. 1, L-6, becoming Pl. 2, L-6, then Pl. 3, L 6, finally Pl. 4, L-6); any stain less bright red, such as « Tommy red », « Red Cross », or even more purple or carmine, or tending to brown or blue, and soon disappearing or becoming very deep colored, is considered as negative. With chlorovanillin, the difference between positive and negative is even more conspicuous (the negative reaction being not deeper than « baby rose », « candy pink », « coral », « confetti »). The reaction with *R. albida* is not quite as striking as that with the two red species. All color indications are in Maerz & Paul terms. Sulfovanillin has also been used (by Kallenbach and Romagnesi) for *Boletaceae* but its use in that family is hardly justified since the reactions are those of sulphuric acid. Sulfoformol is used for these same *Russulae*, that turn red with vanillin, and also, according to Bataille, with *R. luteotacta, R. rosacea, R. Queletii,* and according to Singer, with *R. subalbidula.*

Alpha-naphthol. A scalpel tip full of the reagent is dissolved in about 2 ccm of 90 p. c. alcohol, and then 4 ccm water are added. The solution reacts almost uniformly with the context of the stipe of *Russula* causing a deep indigo to violet blue discoloration after a few minutes. Some species react very slow, or perhaps not at all. It would be interesting to know what results — if any — can be obtained in other genera.

Pyrogallol. A 5 p. c. watery solution is said to give richly colored (yellow to brownish yellow) reactions with the context of the *Russulae.*

Monomethylparamidophenol (« methol »). The crystallized reagent, often used in photography, is dissolved in about 20 times its weight of distilled water, the solution is used immediately since it is unstable. The positive reaction varies from a pale sordid lilac or lilac (« vinaceous purple » of Ridgway, or more sordid), finally reaching « dark nigrosin violet », « deep naphthalene violet », « blackish purple », « taupe brown », etc. The reaction sometimes passes through pink or salmon, and sometimes through blue *(Lactarius volemus),* and at times becomes arrested at these colors. In other cases a more yellow reaction is observed which is probably of another chemical nature than the violet one which is obviously due to the fact that the fungus tissue contains some substance that yields oxygene to the reagent. The same capacity as reducer may also prevent the autoxidation that takes place in the bluing *Boletaceae* when monomethylparamidophenol is added before the discoloration of the context starts.

The negative reaction is neither preventing any natural discolorations, nor does it show any pink, salmon, blue, violet, or yellow discoloration provoked by the reagent itself, and the darkening that is often seen after a very long time is rather due to a transformation of a different order than to the reaction called «positive» here. The reaction is variable with a large number of species, but with others it is quite constant, and a variable amount of time (1—30 minutes) is required to reach the different stages of the reaction. This difference in time is perhaps more important than the differences in shade which do not seem to be very constant. The reagent must be applied on fresh, mature, not watersoaked material. It gives good results in many groups, almost uniformly — as far as limited experiences allow to state — in *Russulaceae* (weak reaction in *Russula fellea*) and *Lyophylleae*. It is, generally speaking, more valuable in white-spored agarics than in dark spored groups and in the boletes.

Methylchlorantimoniate (in methylalcohol-solution) is a reaction designed to translate the acrid taste of the *Russulaceae* into an optical character. The positive reaction is lead gray, the negative reaction is unchanging (or belatedly becoming slightly bluish) context.

Ethylchlorostannate (alcoholic solution) is said to give a yellow-brown reaction with *Amanita gemmata* (under the cuticle) whereas all other species examined in this genus are completely negative.

3. *Iron salts.*

$FeSO_4$, Fe_2Cl_6, and ferric alum can all be used for the same purpose, the first of them being the most commonly used, in 10 p. c. watery solution, on fresh mature specimens. The discolorations are of several categories (1) none, i. e. negative reaction — no color change, or color change indistinct; (2) some kind of olive, green, blue-green, blackish-green discoloration of the context of the stipe — often also the surfaces; this reaction is widely distributed in agarics and boletes, especially in *Russula xerampelina* and related species, *Lactarius volemus* and related species, some *Russulae*, *Compactae*, some species of *Tricholoma* and *Tricholomopsis*, many *Cortinarii* and other dark-spored agarics, many *Boletaceae*, *Gomphidius*, etc. In the genus *Gomphidius*, the reagent differentiates the presence of a chemically distinct subhypodermial layer. (3) All gradations from a rather pure pink or salmon color to sordid gray with or without a slight mixture of reddish. This is the ordinary reaction with the *Russulaceae*, with *Tricholoma albobrunneum*, and other agarics. (4) Blue or green-blue to slate gray. This reaction is commonly found in *Leccinum* where the

gradation between blue and gray is of taxonomic importance; also in other boletes. (5) A variable color effect on the cuticle of the pileus (e. gr. *Russula ferrotincta).*

4. *Ammoniac :*

Ammoniac vapors (NH_3) and ammonium hydroxyde, concentrated solution (NH_4OH), are both used. They must be used on strictly fresh specimens, on all organs separately. The color effects are very varied, and often differ in different organs as well as with the age of the carpophore and temperature (specimens that had been exposed to freezing temperatures sometimes react differently). The most valuable results were obtained in the *Strobilomycetaceae, Boletaceae, Gomphidiaceae, Paxillaceae, Cortinariaceae,* and *Agaricaceae,* but also with some genera of the *Tricholomataceae,* with *Russula* and *Lactarius.*

5. *Strong alkalis :*

Potassium hydroxide (KOH), 15 p. c. solution in water (some use 10 p. c.), and sodium hydroxide (NaOH), same concentration, act in the same way in all cases known to the author. KOH is a standard reagent for all groups of *Agaricales* used in fresh and in dried specimens separately, on all organs separately. The action can often be reverted at a given pH by application of a diluted acid, and certain pigments of *Agaricales (Lactarius turpis, L. atroviridis, Collybia iocephala)* are good indicators. KOH as a reagent, specific for certain species or groups of species, is indispensable in the *Strobilomycetaceae, Boletaceae, Agaricus, Amanita, Leucoagaricus,* and *Cortinarius.* The action is almost instantaneous.

6. *Strong acids :*

Sulphuric acid (H_2SO_4), concentrated. Used on fresh specimens of *Amanita,* also on boletes, some *Tricholomataceae, Gomphidius, Cortinarius, Agaricus, Lepiota, Leucoagaricus,* etc. Less important than KOH, this reagent must be used on fresh specimens. The action is instantaneous or almost so.

Hydrochloric acid (HCl), concentrated, used as above.

Nitric acid (HNO_3), concentrated, used as above.

Those who go beyond the verification of data already available, by testing thus far untested species, or species whose reactions have not yet been published, will do well to adhere closely and consistently to the formulas, and also to constant and equal conditions and methods. It is also extremely important to avoid painstakingly interference between different reagents. Phenol and anilin can never be used on the same carpophore, and without utmost cleanliness.

Ammoniac vapors should be kept from other reagents, especially $FeSO_4$. Young specimens should not be taken into consideration, or only for the sake of comparison with adult specimens. Generalizations should be made only after a long experience with the species in various ecological conditions, and with the behavior of the reagent under various chemical influences. The colors obtained should be indicated in color chart terms wherever this appears to be advantageous.

Chemical analysis of the carpophores

The use of chemical analysis of the carpophores of the *Agaricales* is merely in the beginning stages as far as their taxonomic value is concerned. However, some of the possibilities will be mentioned here because even the fragmentary data now available show that results of taxonomic importance might well be expected.

In this category belong the demonstration of the formation of cyanic acid by certain agarics. In order to become more conclusive, the list of agarics known to produce HCN beyond a certain minimum amount (according to the sensitivity of the picric acid method), should be supplemented with a list of the species that under these circumstances do not show any appreciable formation of HCN. More than half of the species indicated by various French authors [40] have

[40] The last complete list published is that of Josserand (*Rev. Myc.* 3 : 29, 1938). Several more species were indicated later :

Cantharellula obbata, C. cyathiformis ;
Clitocybe Alexandri, C. fragrans, C. infundibuliformis, C. parilis, C. geotropa ;
Collybia dryophila ;
Lepista nuda (this has not been verified by other authors) ;
Pleurocybella porrigens ;
Leucopaxillus giganteus ;
Marasmius globularis, M. oreades ;
Rozites caperata.

The tests have been made with the picric acid method which consists of the following procedure : The specimens are, in strictly fresh condition, cut into fragments, and inserted in a glass vessel that can be closed nearly airtight (exsiccator). A piece of filter paper (2 × 20 cm) is immerged in a solution of picric acid (1 p. c.). After the paper has dried the same paper is immerged in a 5-10 p. c. solution of sodium carbonate (repeat this operation several times, leaving the paper in the $NaCO_3$ solution several seconds each time). The paper is then hung riding on the rim, and the vessel closed firmly. The paper outside remains yellow ; the paper inside becomes slowly (over night) dull red if the fungus exhalates HCN.

been checked by this author on material from the United States, and the result was positive in every case. This points to a strong specific constancy of this character, and the comparative simplicity of the method of qualitative demonstration of cyanic acid in agarics makes it possible to use it more extensively than is done at present.

Quantitative analyses of certain specific carbohydrates, acids, etc. are also useful in taxonomy though they cannot be expected to become routine tests for determination. For instance, Heim & Romagnesi (1934) referred to the analyses that were made on a rather large (yet still insufficient) number of *Agaricales* in regard to allantoic acid. Heim & Romagnesi found that the high percentage of this acid present in *Coprinus* and *Leucocoprinus*, as against a low percentage in *Macrolepiota*, shows a certain chemical affinity between *Coprinus* and *Leucocoprinus* and increases the hiatus between *Leucocoprinus* and *Macrolepiota* on the other hand. These data are based on a paper by Fosse & Brunel (1933).

Frerejacque (1939) published a list of species which he had studied as to their contents in mannitol. He states that the list is not complete enough to make final conclusions. But it is obvious that the figures representing the weight of mannitol per 100 gr of the dried carpophores, keep in definite limits characteristic for certain groups of fungi. So we find a medium to high percentage of mannitol in *Gomphidius* (which is thus chemically separated from the other black spored agarics), *Paxillus*, and boletes; in the natural group of *Lactarius* and *Russula*, he indicates a medium to usually high percentage of mannitol, with *Russula delica* showing a more than twice as high percentage than *Lactarius vellereus*. There is also a rather high percentage in *Agaricus* and *Leucoagaricus* whereas in *Lepiota* it is abruptly very low. This would tend to show chemical affinity between the *Agaricaceae* with germ pore; in the *Tricholomataceae*, the figures are low to rather high (up to 10.0 in *Armillariella mellea*), and in all other groups consistently low (to zero in *Inocybe maculata*).

A large number of facts, many of them concerning the *Agaricales*, have been assembled on the coloring matter in fungi by I. A. Pastac (1942) but this interesting survey that is recommended to those concerned with fungus-chemistry, shows clearly enough that the accumulation of facts has not arrived at a level where data of taxonomic value can be derived with safety. Especially promising aspects are the data available on atrotomentine, boletol, dermocybin, muscarine and others.

XIV. PHYSICAL CHARACTERS

It has been suggested (but never realized in experiments) to compare the specific weight of dried carpophores and make tests on their elasticity. These tests are almost impossible to translate from speculation into reality. The specimens vary too much in different ages and under different climatic conditions, habitat conditions, and by intraspecific variation — as every mushroom grower will readily confirm. Another approach is that of provocation of luminescence by application of polarized light and Wood's light on various fungi, and the conclusions are though neither too encouraging nor too disillusioning, in any event worth the attention of the taxonomist. Josserand and Nétien think they have found another difference between *Russula* and *Lactarius* in the behavior of the carpophores in Wood's light, and this recalls a similar attempt, still unpublished, I believe, by Zuderell, Cernohorsky and Singer, with polarized light, where the most striking effects of luminescence were obtained with *Russula*, whereas the *Lactarii* remained almost dead. For more detailed evaluation of these results the reader is referred to the authors of the paper cited above *(Bull. mens. Soc. Linn.,* Lyon, reprint, p. 1-20).

XV. CYTOLOGICAL CHARACTERS

Nuclear cytology

Cytology in the wider sense is now frequently applied in the taxonomy of the *Agaricales;* it has even found its way into the basic keys for determination in monographs as well as in surveys of genera.

The number of chromosomes has not yet been used by systematists; it seems to be generally rather low, and differences in shape apparently do not exist, or have not been brought to the attention of the mycologists.

The nuclei, as a rule, are small to very small, and their number in the mycelium, the hyphae of the carpophore, cystidia, basidia, and spores differs according to races, species, or larger groups of species or genera. This whole problem cannot be studied without due consideration of the whole life cycle and sexuality of the *Agaricales* whereby certain types and aberrations from the normal form will

be considered separately, with their taxonomic application in view.

The most important contributions were made by Maire (1900-1902) and later Kühner (1926-1945). Many other authors have contributed important details without, however, attempting to evaluate them for taxonomic purposes.

Summing up what is generally considered as the « normal » life cycle of an agaric and bolete, we shall start with a uninuclear spore that after germination gives rise to a haploid (monocaryotic) mycelium (also called primary mycelium, a term that should be abandoned). The septa between the hyphae of the haploid mycelia are clampless (except for a very few reported cases of « autodiploidization »).

The spores as well as the haploid mycelia resulting from them are all morphologically different, therefore the « normal » type of *Agaricales* comes under the group of so-called heterothallic thallophyta. Heterothallism in fungi was discovered by Blakeslee (1908) and in the *Agaricales* by Bensaude (1918). The sexuality of the heterothallic *Agaricales* appears in two forms, one of which is called bipolarity, and represents the usual bipolar isogamy among the representatives of this group, and another that was discovered by Kniep (1922) in which the mycelial descendants of a carpophore are physiologically divided in four instead of two groups, according to the schema :

	M1	M2	M3	M4
M1.....	—	+	—	—
M2.....	+	—	—	—
M3.....	—	—	—	+
M4.....	—	—	+	—

This means that in the bipolar forms, of the two physiologically different types of mycelium, each can copulate with the other type, whereas in the tetrapolar type, a mycelium of the type 1 can copulate only with a mycelium of the type 2, and a mycelium of the type 3 can copulate only with a mycelium of the type 4. In other words, we have here a form of sexuality with four sexes instead of two, a fact that made it necessary to emend the conception of sexuality (this is Quintanilha's opinion — but compare H. S. Jackson, *Trans. R. Soc. Canada* 38 : 4-5, 1944).

The study of the copulations is technically achieved by single spore cultures [41].

[41] As for methods, we refer to special papers, especially by Vandendries.

After the copulation of two mycelia of the haploid generation, the second generation, normally the more important one in the *Basidiomycetes* (because the carpophores are usually produced by it), begins with the formation of the dicaryotic (sometimes called secondary) mycelium, or the mycelial phase of the dicaryophyte. The dicaryophyte immediately starts the formation of clamp connections and the cells consistently contain two nuclei which divide at the same time and pass into the new cell in a rather complicated way that is reminiscent of (and according to most contemporaneous authors homologous with) the similar hook-formation of the ascogeneous hyphae (*Ascomycetes*). One nucleus of the pair resulting from the division of one nucleus of the original dicaryon enters a bulge that points outwards and backwards at the place where the new septum will be formed. The bulge — called clamp now — fuses with the parent cell, the double wall becoming dissolved, and the nucleus that was in the bulge enters the parent cell. At the same time, between the two nuclei of the second pair resulting from the division of the second nucleus of the original dicaryon, a new septum is formed inside the old hyphal cell, separating the new cell from the old one and thus leaving one nucleus of each pair in each cell. The two in the old portion and two in the new cell are now separated from the clamp by the laying down of an additional septum. The resulting structure, characteristic for the *Basidiomycetes*, is called a clamp connection (Pl. XI, 3; XXIII A, 7). These clamp connections are normally present on all or almost all hyphae of the whole dicaryophyte, including the carpophore. The dicaryotic mycelium contains two nuclei in each cell because, after the fusion of two haploid mycelia with opposite polarity, the nuclei — though entering the same hyphae and remaining in pairs all through the dicaryophytephase — do not fuse to form a diploid single nucleus. Thus reduction division is postponed throughout this generation and finally takes place in the basidia of the carpophore [42]. Normally, only the dicaryotic mycelium is able to form carpophores. The hyphae of the carpophore and also the basidiole are typically binucleate (Dangeard, 1895). The reduction division is usually followed by one or two more divisions which take place in the upper part (club) of the basidium, and the spindles of the first two, or at least the second division are in an obliquely subhorizontal or in an almost horizontal

[42] Falck (1902) calls the carpophore phase of the dicaryophyte — tertiary mycelium, an unnecessary and misleading term that must be abandoned.

position, the spindles of the second division often forming an X-shaped (chiastic) figure. This is in contrast to the stichobasidial type in which the figures of this division are found one beneath the other in a more nearly vertical position and at a lower level of the basidium. There are probably what may be termed as transitions between the basic types — chiasto — and stichobasidia — but not normally on the level of the *Agaricales*, nor, for that matter in the holobasidial *Aphyllophorales* (excluding the *Exobasidiales*), where both types occur in otherwise rather closely related forms such as the *Clavariaceae* sensu lato and the *Cantharellaceae* sensu lato, and in the *Gastromycetes* where only chiastic basidia are known. After the second division, there will be four nuclei in the basidium, and in the simplest case, these four nuclei ascend to the sterigmata which by this time have been formed, and the sterigmata bulge out at their apices where the uni-nucleate spore is formed. This completes the life cycle of a «normal» representative of the *Agaricales*.

This life cycle can, consequently, be expressed by the following scheme :

1. *Bipolar species:*

Basidiospore + → haploid mycelium + →
Basidiospore — → haploid mycelium — → } dicaryotic mycelium →

→ Carpophore → binucleate basidiole → Basidium { o { o, o }, o { o, o } } four basidiospores

2 [43]. *Tetrapolar species* (Aa, Ab, Ba, ab, AB : pairs of Mendelian factors):

Basidiospore Ab → haploid mycelium Ab →
Basidiospore Ba → haploid mycelium Ba → } dicaryotic mycelium → carpophore Ab . Ba →

→ Binucleate basidiole Ab . Ba — Basidium { o { o basidiospore AB or AB or Ab, o basidiospore ab or ab or Ba }, o { o basidiospore Ab or AB or Ab, o basidiospore Ba or ab or Ba } }

For taxonomic purposes, only the aberrations from this scheme are of interest, and as far as they present constant features, they can be used.

[43] Sec. QUINTANILHA, A., *Le Problème de la Sexualité chez les Champignons. — Bol. Soc. Brot.* **8** (11), 1933.

As for techniques of cytological investigations in the *Agaricales*, we cannot go into detail. However, it is recommended to start with an organism which is easy to collect in all stages, easy to fix and dye and uncomplicated in its development. Such a species is, for example, *Collybia dryophila*. It is advantageous to carry the fixative on collecting trips and insert the fragments, properly labeled, right in the field. Every genus, and every tissue, the mycelium, the basidium, and the spores, require an individual treatment as far as fixation and coloration are concerned, and there is no never-failing method that works with all cells of all fungi. However, Kühner (1938, 1945) has published repeatedly on the subject, and the chapter on cytology in his *Mycena*-monograph as well as a later article on the study of the distribution of the nuclei in the mycelia of the *Basidiomycetes* is recommended.

In 1934, Chow stated that in certain *Coprini* the mature spores are binucleate; in 1933 Kühner reported the spores of *Marasmius rotula* uninucleate. Later (1945) he indicates that in the *Amanitaceae*, *Bolbitiaceae*, *Cortinariaceae*, *Strophariaceae*, and most *Agaricaceae*, the spores are binucleate at the moment of discharge and afterwards. This is explained by the fact that the third division of the nuclei after meiosis (comparable with the third division of the asci of the *Ascomycetes*, resulting in eight uninucleate ascospores) usually takes place in the spores rather than in the sterigmata or basidia and must result in binucleate spores. In most *Hygrophoraceae* and most *Tricholomataceae*, however, the third division takes place in the sterigma and only one nucleus ascends to the spore while the other descends back to the basidium where it degenerates. Thus, only one nucleus is present in the spore at discharge and immediately afterwards; however, the author has found this single nucleus dividing later on while still in the spore, and consequently some of the spores are then found to be binucleate and some uninucleate. The number of nuclei in the spore is easy to establish, and has undoubtedly a great taxonomic importance.

The bi- and tetrapolar forms, the germ-tube and the whole initial stage of the mycelium is usually multinucleate and later becomes septate and uninucleate until copulation, whether it starts from a binucleate or a uninucleate spore. It is known, however, that in many cases, the mycelium resulting from germination of the spores is immediately binucleate, i. e. the haploid phase is not at all represented, and the life cycle of these species starts out with the dica-

ryotic mycelium. These forms are called homothallic (Blakeslee, 1904) because the thallus does not show any change of generations. Homothallism is obviously a characteristic of the genus in *Clitopilus* (Kühner & Vandendries, 1937). Many species are known in which homothallism is either the rule, or is found in special races of the main « normal » form. The latter case is frequent in such groups where 2-spored forms and 4-spored forms are known in a species (such as *Mycena, Mycenella, Marasmiellus, Conocybe,* etc.) whereby the 4-spored form usually represents the normal form, and the bisporous form the homothallic form. Clamp connections are sometimes absent in homothallic forms but, of course, not necessarily so.

The life cycle of homothallic dicaryophytes can be shortly described as follows:

1. *Bisporous form:*

Binucleate basidiospore — dicaryotic mycelium — dicaryotic carpophore — binucleate basidiole — basidium o{o o}{o o} two binucleate basidiospores

2. *Tetrasporous form:*

Binucleate basidiospore — dicaryotic mycelium — dicaryotic carpophore — binucleate basidiole — basidium o{o{o o}{o o}}{o{o o}{o o}} four binucleate basidiospores

In other forms, the haploid mycelium is able te form carpophores without previously forming a dicaryotic mycelium, i. e. every single spore (as in the homothallic-dicaryotic forms) is apt, theoretically, to form carpophores and another generation of spores without interference of another mycelium. In spite of the fact that these carpophores are necessarily composed of uninucleate hyphae, and there is no reduction in the basidium, the formality of the formation of uninucleate basidioles is nevertheless conserved. The single basidial nucleus divides as in any other cell, and the resulting two nuclei move into the spores, one into each ot the two spores. This is the case, for example in *Mycena galericulata* forma *bispora,* and the fructification

is then called parthenogenetic. Parthenogenetic carpophores, naturally, never have clamp connections [44].

The parthenogenetic forms are, as far as we know, not characteristic for larger taxonomic groups but merely for certain hereditary races, with « normal » and sometimes dicaryotic-homothallic parallel races. Consequently, the number of sterigmata on the basidia is not necessarily the expression of a certain type of life cycle, i. e. it is impossible to say whether it belongs to a dicaryotic-homothallic or a parthenogenetic form unless the nuclear divisions are carefully studied from the basidiole to the spore. It is probable, and, in the author's opinion, logical to expect that some of the normal (bipolar or tetrapolar) forms have died out, and the bisporous homothallic-dicaryotic or parthenogenetic form alone has survived. Such seems to be the case, according to all taxonomic evidence, in certain species of *Laccaria,* and, if so, these species posses bisporous basidia as a specific character. Though, on the basis of the data available, it must be assumed, that these bisporous *Laccariae* actually are species, this represents the exception rather than the rule, and we can now say that Lange (1914) overestimated the importance of the number of the sterigmata. Besides, the situation is not always as clear-cut as it may appear on a scheme. In many specimens with basidia developing sterigmata of a number lower than 4, the 2-spored basidia are inter-

[44] Even if the absence of clamp connections in the carpophore and the presence of but one nucleus in the hyphae can be demonstrated, the specimen studied is not a priori parthenogenetic, for one of the following two reasons :

1. It may be that the sole nucleus is diploid whereby the fusion takes place immediately after copulation of the hyphae which may have been overlooked, or without any copulation, whereby the species would be homothallic-diploid. This explanation is contrary to all we know in the *Agaricales,* and, aside from that, highly improbable since parallel races of wholly uninucleate forms are binucleate and bipolar or tetrapolar in the manner described as « normal ».

2. It may also be that the sole nucleus is haploid until, by now unknown means, the basidioles become binucleate, yet the four spores are again uninucleate. Here we have a life cycle in which the dicaryotic phase is shortened to the limit — something similar to the correspondent phase in the *Ascomycetes.* Yet, this type of sexuality, the so-called *Typhula*-type, has been observed in *Agaricales* only once (Chow, 1934, in *Coprinus fimetarius*), and it remains to be seen whether this latter observation is correct, and if so, how common it is under normal conditions of culture and in the field. It is probably at most a rare exception in the *Agaricales.*

This shows, that theoretically at least, in all cases, a complete cytological study is needed in order to arrive at exact results.

mixed with 1-, 3-, and 4-spored basidia which usually results in a marked polymorphism of the spores which vary between widely separated extremes of length and breadth, the volume of the spores from 1-, 2-, and 3-spored basidia decreasing (in this order), and the 4-spored basidia developing the smallest spores. These facts can be explained by cytological irregularities — very frequent in fungi — which do not interest us here since their taxonomic value is close to nil.

The absence of clamp connections (Pl. XXIV ; XXVII) can also by no means be linked with parthenogenesis exclusively. Clamps are often absent on the septa of binucleate hyphae, and there are rare, thus far not fully explained cases where clamps have been observed on the haploid mycelium. For taxonomic purposes, we may neglect the latter case, but if the presence or absence of clamp connections is used as a character in taxonomy, it is essential to make sure that the specimen studied is not merely a parthenogenetic form of a normally bipolar or tetrapolar species. If this possibility is excluded, we have further to deal only with species with normal sexuality that have lost their ability to form clamp connections, and homothallic forms, species, or genera, that find themselves in the same condition. Under these circumstances, the presence and absence of clamp connections must be accepted as a valuable character. Vandendries was the first to emphasize that a defined species has constantly clamps or is constantly clampless (i. e. in the non-parthenogenetic form). This statement is, as we shall see later, somewhat too exclusive but it foreshadows the use of the clamp connections in systematics. As a taxonomic character, they were first used by Singer (1942) and Kühner (in a foot note on *Tricholoma* in 1937, and again in 1945).

The presence or absence of clamps is a very good and usually constant character that can be used for units as large as families (*Gomphidiaceae*, *Strobilomycetaceae*, *Russulaceae* — all three without clamp connections ; *Paxillaceae*, *Hygrophoraceae* — both with clamp connections), and for genera (*Melanoleuca*, etc.), sections (*Omphalina*, *Lepiota*), species (*Pluteus atromarginatus* [Sing.] Kühner) and forms. In only very few species, the clamp connections are completely inconstant as well as scarce. This is the case in certain species of *Boletinus*, and in *Phylloporus rhodoxanthus*. Here, as in all characters, even the most useful ones, one can easily see that their value varies according to the group with which one is working. It often appears that the observer is not patient enough to search for clamp con-

nections, or not experienced enough to search for them at the right place. If there are clamp connections, even in small number, anywhere in the carpophore but between hyphae cells exclusively (not at the base of the basidia), we may state that clamp connections are present. The best place for the search for clamps is a layer consisting of filamentous, thin, thin-walled, not too densely interwoven hyphae; these are found, depending on the species, either in one of the covering layers — more commonly on the surface of the stipe than on the pileus, or in the basal tomentum, or in the hymenophoral trama, or in the tissue of the veil. A certain flexibility in the methods of the observer will be very advantageous. It should also be made a rule that a negative statement (clamp connections absent) should not be made unless at least several specimens from different locations have been patiently searched for clamps, and all septa observed have been found to be clampless. Doubtful (because of the early stage of the clamp formation or because of optical conditions) clamps should not be taken into consideration. The clamps are either well developed at some septa, or not at all.

It is also important to keep in mind that occasionally, the clamps are formed in one tissue and not in another. This is especially true for densely interwoven layers consisting of thick-walled hyphae, and in intricately agglutinated tissues of cortical layers. Here formation of clamps may be actually supressed rather than difficult to observe. In *Armillariella mellea,* a form is known that does not form any clamp connections in the carpophore up to the septum between the last subhymenial cell and the basidium, where a distinct clamp is formed. In *Cantharellula cyathiformis,* the mycelium has been observed to have numerous clamp connections, yet the carpophores are so constantly devoid of clamp connections, that this feature is used as one of the best characters for the distinction of this species. The opposite case (clamps present in carpophore — absent in mycelium) has also been observed in *Basidiomycetes.* It may well be assumed that those species with inconstant clamp formation as well as those where clamp formation has been abandoned except for a specific organ, can be considered as being in the evolutionary process of loosing the clamp connections as an unnecessary and uneconomical [45] way of cell division. It is therefore by no means surprising to find the transient species always in groups that, also according to the

[45] It is only fair to state that some cytophysiologists hold the opposite opinion.

sum of their other characters, are intermediate between constantly clamped forms and completely clampless forms.

A further use of cytological characters derives from the fact that not all carpophores of the dicaryophyte have actually all single hyphae (i. e. the space delimited by wall and septa) binucleate. Hirmer, Brunswick and Kühner have shown that many hyphae and cystidia, especially the hyphae of the interior of the stipe, and the cystidia of such genera as *Pseudohiatula* often contain more than two nuclei, i. e. they are actually coenobial cells where the septum between the single dicaryons has failed to form. The number of the nuclei, in such cells, varies from 3 to 54. Kühner (1945) attributes considerable taxonomic importance to these multinucleate hyphae and cystidia, at least he uses it in phylogenetic arguments. Counting of the nuclei in these cells has not yet become a routine of the systematist but this may not always be so.

The so-called *Godfrinia*-basidium, characterized by its development from a uninucleate basidiole, by the nuclear division taking place in the middle of the basidium (the basidium therefore attenuate above from a ventricose middle portion), and by the number of the sterigmata being two instead of four, with two uninucleate spores resulting, is not as marked a type as had initially (Maire, 1901) been suspected. It is merely the basidium of a parthenogenetic haploid of the genus *Hygrocybe*. The genus *Godfrinia* based on it by Maire, has been abandoned by all mycologists.

Another basidium-type, the *Lyophyllum*-basidium, has, in contrast with the *Godfrinia*-basidium, great taxonomic importance. It is characterized by the fact that — everything else being normal — the nuclei are not readily seen because of a dense granulation inside the basidia if aceto-carmine is used for staining. This kind of content is called carminophilous granulation (Pl. VIII). *Lyophyllum*-basidia, i. e. basidia with carminophilous granulation are found in all representatives of the tribus *Lyophylleae* in the *Tricholomataceae*, and according to Kühner, in some *Rhodophyllaceae*. A fragment of a not too young hymenophore is heated on a slide and kept moving in the medium which is the ordinary acetocarmine as used in cytological laboratories. When the first drop begins to evaporate and a film is beginning to form, the fragment is removed onto another slide; this is repeated twice, and the preparation is finally cooled off abruptly by putting the slide on a cold metal plate (microscope table); for stirring the fluid and for moving the fragment, a microscope needle is used

whereby enough iron is dissolved by the concentrated acetic acid of the acetocarmine to deepen the coloration of the contents of the basidia sufficiently, as far as the carminophilous granulation is concerned. This granulation is then blackish purple to violet-black and rather dense. The method results either in distinctly granular basidia or in non-granular basidia; intermediate cases are not known. Only *Lyophyllum connatum* does not show a very dense (yet satisfactorily distinct) granulation in adult basidia which is, however, absent in the basidioles. This method has the advantage of being applicable not only on fresh material but on well-dried herbarium material.

The author found that the basidia with carminophilous granulation can easily be studied with morcein replacing acetocarmine, whereby the nuclei are colored in much the same way as with acetocarmine, yet the carminophilous granulation is invisible, the interior of the basidium is homogenized, and the nuclei and spindles are clearly distinguishable.

Still another aberration from the normal can be observed in some basidioles that remain sterile. The fusion and the divisions in those bodies do not take place in the ordinary manner; their contents are visibly non-protoplasmatic (hence their « empty » appearance), and at maturity, instead of forming spores, these bodies become slightly larger or otherwise insignificantly different in size or shape from the normal basidia. These bodies are called pseudoparaphyses (Pl. XII, 1; XIII, 1; XXVIII, XXIII). Their presence or absence, number, and distribution in the hymenium or on the edge of the hymenophore have a certain importance in systematics.

In very rare instances, concerning mainly tropical agarics, the last-formed subhymenial cell, instead of becoming a basidium, transforms itself into a more or less isodiametric, often more or less sclerotized organ which cytologically corresponds to the basidium (see genus *Rhacophyllus* Berk.) yet, morphologically, differs in not forming sterigmata. Together with a more or less sclerotized cortical layer, it causes the carpophores to be more resistent to desiccation and postpones normal sporulation in favor of a higher degree of security for the organs in which reduction division takes place. This completely atypical behavior of some agarics is known as bulbillosis.

The indications given above show clearly that the cytological characters as such are either useless or of thus far unknown use for the purposes of the systematist. At the same time, some characters that are closely connected with the study of the life cycle, sexuality,

etc., yet not direct indications of any particular type of reproduction but rather « by-products » of the investigations on the latter, turn out to be of invaluable importance in taxonomy. The characters that are a direct expression of the sexuality of the *Agaricales* have not been studied in large enough number to allow any definite conclusions. It is not impossible (according to recent data by Quintanilha and others, 1941) that the future will give the two categories of spore polarity the standing of a character in specific or even generic taxonomy, but in the only genera where extensive studies have been made, viz. *Mycena* and *Coprinus* (the former genus was investigated by A. H. Smith, 1934, and Kühner, 1938, the latter is since 1918 the favorite genus for sexuality research in *Basidiomycetes* because of easy culture methods and a wide variety of different behavior), the n-polarity of the spores, homothallism, parthenogenesis, etc. did not show more than intraspecific constancy, and seem to be due to minor physiological mutations.

On the other hand, minor details of the main types of life cycles, prove to be of enormous taxonomic interest, e. gr. the location of the third division (sterigma or spore); the presence or absence of clamp connections in cases where they have no or little connection with the sexuality of the species; number of the nuclei in the voluminous coenobial hyphae and cystidia that cytologically function as merely another part of the dicaryotic system; presence of a granulation in the basidia that is colored by the same dye that colors the nuclei, incomplete or aberrant divisions in the basidiole leading to the formation of pseudoparaphyses.

Pigmentation of the cells

As an appendix rather than as an integral part of cytology, we shall now investigate another character that has to do with the anatomy of the interior of the cell and with cell physiology, i. e. the types and distribution of the pigments.

The rich and varied pigmentation of the *Agaricales* which surpasses by far that of the flowering plants implies the presence, in that group, of a large number of pigments, differing in regard to their chemical and physical particularities as well as their distribution on or in the hyphal (sporal, basidial, cystidial) wall or in the cell sap. Kühner has made a special study (1934) of the topography of the colored substances (as he expresses himself in the title) of the agarics and boletes.

We shall here reproduce, in the outline, his classification of the pigments, and indicate examples for each type and subtype:

I. Intracellular pigments.
  a. Present in the living cell.
    1. Localized in the cytoplasma. *Cytoplasmatic Pigments.*
      (*Leucocoprinus luteus* — yellow globules; *Inocybe geophylla* var. *lilacea* — uniform).
    2. Vacuolar. *Vacuolar Pigment* (Pl. XVIII, 4).
      (*Amanita muscaria, Bolbitius* [yellow species], *Leccinum aurantiacum*).
  b. Appearing after death of the cell.
    (*Callistosporium*, all species).
II. Membrana-pigment.
    (Elements of the cuticle of *Panaeolus sphinctrinus*).
III. Intercellular pigment.
    (*Naematoloma fasciculare; Lactarius griseus* and related species; *Paxillus involutus* [46]; *Suillus granulatus*; Pl. XXV, 9; XXVII, 5).

In a special chapter, Kühner shows that the topography of the pigments in the *Agaricales* has taxonomic value. It will become a more important factor in systematics, as soon as the number of single data, now accumulated (since Kühner's advice to taxonomists to describe the pigments observed) has grown sufficiently. Even now, in many genera, species can be most clearly distinguished by the type and location of the pigment. Naturally, in many cases, two or more different types of pigments are combined either in the interior of the cells, or in the wall, or intercellularly. For instance, the reaction with H_2SO_4 observed on the spores of certain *Coprinaceae*, and indicated above under « microchemical reactions », shows that there are two different kinds of pigment in these spores, one soluble and one insoluble in sulphuric acid. The same is true with pigment combinations in the cuticle of certain *Russulae* (*Russula*-red and *Russula*-yellow often combined). There may also be combinations of vacuolar and membrana pigment, and vacuolar and intercellular pigment, and membrana- and intercellular pigment (e. gr. in the boletes). It is often difficult to decide whether a pigment is membranal or intercellular-incrusting (« epicellular »). It is a feature of the intercellular pigment to be easily dissolved (either after decoloration, or with a change of color when dissolving, or without any color change) in alcohol, ammonium hydroxide, even in water. Only few epicellular pigments are

[46] Atrotomentine, a 2-5-di-para-oxy-phenyl-3-6-di-oxybenzochinone has been analyzed and later synthesized by Kögl. It is the intercellular pigment of *Paxillus atrotomentosus*.

insoluble in these solvants, and these are readily recognizable as superficial (e. gr. the resinous crust responsible for the colored crust on top of the colorless wall of the cystidia in some boletes). On the other hand, the true membrana pigments even though they have the appearance of epicellular pigments because of the lack of elasticity of the outermost, strongly pigmented layer of the wall which then breaks off into fragments (spiral or areolate ornamentations), are always insoluble except in such rude solvants as concentrated sulphuric acid.

XVI. PLANT GEOGRAPHY AND ECOLOGY

Plant geography and ecology of the fungi, and especially the *Agaricales* are so enormous in their theoretical and practical significance, so wide and ramified in spite of the superficiality of most of the data available, they can not really be treated here. However, the influence of data of this order on problems of taxonomy is too obvious to be ignored. There are all shades of opinions on the question whether or not the *Agaricales* have definite areas determined by the climate and its changes in history of plant life as admitted for *Cormophyta*. It shall not be denied here that the average geographic area of a representative of the *Agaricales* may be larger than the average area of an angiospermous plant. (It should likewise not be denied that the average area of an angiosperm is larger than that of an insect). But we have, in the *Agaricales*, everything from pantropical species and pantropical genera to endemics on tiny islands; we have typical vicariants, geographic races (which we call subspecies) that are fully the same as the geographic races of the phanerogams. The larger spores of the European *Suillus granulatus* showed it to be the type subspecies of a « circle of races » that was determined not merely by geography but also by mycorrhizal relationship: The American form was connected with 5-needle pines, and the European one with 2-needle pines.

Here, we have a characteristic correlation between the fungus-host-relationship and the climatic factor. A form that differs from the other only in the host, not in geography, is called a mycoecotype (Singer, 1940), if, of course morphological differences are also present. Otherwise, the distinction is based on experimental transplantation exclusively which would not be conclusive for *Agaricales* as much as

it is for *Uredinales* (where the mycoecotype without morphological differentiation is known as ecological form).

It is quite obvious that the host-relationship, often taking the form of mycorrhiza partnership, is an important taxonomic factor since it often caused a regional if not geographic separation of the races involved and an independent evolution of both ramifications of the system in many cases. There is evidently a basic difference between the *Gymnopili* on frondose trees, on *Monocotyledones*, and on conifers. There is also a significant difference between the primitive *Russulae* and *Lactarii* that are non-mycorrhizal and the higher forms that are mycorrhizal and even specialized. The *Sphagnum*-Galerinas appear to form a definite group, and the constancy with which the *Suilloideae* confine themselves to mycorrhiza with conifers is undoubtedly no less impressive than the near-unanimity with which the *Leccina* favor the *Fagales* and *Salicales*. No less striking as a constant conifer-mycorrhiza, is the entire family *Gomphidiaceae*. Other ecological groups distinguish themselves by a prominence of forms preferring open places (outside the woods), gardens, greenhouses, lawns (e. gr. most of the *Bolbitiaceae*, which, even where entering the woods, never were found to form mycorrhiza). It is undeniable that all these ecological groups are at the same time taxonomic groups. Consequently, we feel safe to cite geographic and ecologic differences and similarities as auxiliary characters, supplementing and sometimes explaining the morphological and chemical characters.

It is too early to be very precise about the geographic areas and the ecologic characteristics of all the groups. The data available are though by no means too scattered, yet, unfortunately, too unreliable. A citation of an agaric or bolete, without study of the specimen, by anybody less than a first rate specialist, is not a scientific document of any weight. Reducing our material by elimination of the doubtful, we finally arrive at a point where the material begins to become so scarce that, in some cases, conclusions can no more be drawn, and even in the remaining cases this can be done only in the three or four best herbaria of the world.

Under these circumstances, speculative theories, area maps, and conclusions reaching far beyond the available evidence have often been published, recently even on boletes. An improvement on the taxonomic methods, more collections, and less reliance on literature sources will eventually show that the boletes are an excellent field

for those who are interested in the mycological aspect of historical plant geography, and the evolution of the species in fungi. Only the richness of a large herbarium, with a few genera worked out according to the standards of modern taxonomy, will circumscribe clear areas, and even these will be corrected by further planned collecting in the border regions.

PHYLOGENETIC THEORIES CONCERNING THE ORIGIN OF THE AGARICALES

The phylogeny of the *Agaricales* is a strongly controversial field. The history of phylogenetic systematics of the *Agaricales* has been analyzed at length in a previous paper by the author [47]. It is intended to give, in the present chapter, an account of the arguments used and the views expressed in accordance with the facts now available. The accumulation of facts, found in a search for supporting data for one's own hypothesis, or for the purpose of invalidating an opposing argumentation, would in itself be justification enough for the serious discussion of this subject — a subject that seems to be so utterly « theoretical » for some scientists. It is generally acknowledged that only paleobotany can ultimately prove the direction of progress and regression, yet all the other available data taken together often give a rather convincing picture of the evolutionary trends in certain groups, and only those who refuse to recognize it because of prejudice against evolutionary theories in general, will deny the high degree of probability in certain parts of the phylogenetic schemes proposed.

Among the facts brought to light in comparatively recent times, we have to mention the connection existing between certain *Gastromycetes* on one hand and certain *Agaricales* on the other hand, and between certain *Agaricales* on one hand and certain *Aphyllophorales* on the other hand. It will be enough to study the whole series of forms between the extreme *Astrogastraceae* and the extreme *Russulaceae* as has been done by Buchholtz (1902), Lohwag (1924), Malençon (1931), Heim (1938) and Singer (1936-1939), or the series from *Cyttarophyllum* to *Galeropsis* (Singer, 1936), or from *Truncocolumella* to *Gastroboletus* and *Boletinus decipiens* (Malençon, 1938, Zeller, 1939,

[47] R. SINGER, *Das System der* Agaricales, *Ann. Myc.* 34 : 286-378, 1936.

Singer, 1942-1945), or from *Montagnites* to the *Coprinaceae*, in order to lose all illusion about the sharpness of the key-characters allegedly distinguishing the *Gastromycetes* from the *Agaricales*.

On the other hand, real or apparent transitions from the *Aphyllophorales* to the *Agaricales* were suggested in large number in order to satisfy the hypotheses — dominant at times — of derivation of the *Agaricales* from the *Aphyllophorales*. The collapse of all the speculation about a relationship between the *Boletaceae* and the *Polyporaceae*, based by Neuhoff & Ziegenspeck on a *Gyrodon* with allegedly white spores, and by others on Höhnel's white-spored *Filoboletus*, is now complete. The *Gyrodon* turns out to be *Boletus edulis*, and its spores are not white but — absent, and the *Filoboletus* turns out to be a poroid form of the marasmioid *Tricholomataceae*. A careful revision of the tramal structure of all *Strobilomycetaceae* and *Boletaceae* (Singer, 1945) has established the fact that all boletes have more or less bilateral hymenophoral trama, a structure unknown in the *Polyporaceae*. White spore print also does not exist in the *Boletaceae* and *Strobilomycetaceae*, and the genus *Leucogyroporus* was based on an erroneous observation by Murrill, while *Polyporoletus* Snell turned out to be a *Scutiger*.

However, other connections between the *Aphyllophorales* and the *Agaricales* have been uncovered recently. The author does not enter the argument about an alleged affinity between *Cantharellus* and *Hygrophorus*. It may be enough to say that a collective group, an assemblance of notoriously unrelated species, such as Fries's genus *Cantharellus*, can be used to prove the affinity with numerous other groups, exactly as many as there are represented in the collective genus in the first place. While there are elements of *Clitocybe, Hygrophoropsis, Leptotus, Geopetalum, Cantharellus, Gomphus*, to name only a few — there is, as far as is known to the author, no representative of the *Hygrophoraceae* hidden in *Cantharellus*. Should it have been the bright yellow-orange or red color of some *Cantharelli* and some *Hygrophori* that first suggested the affinity?

But there is an affinity between *Lentinus cyathiformis* and the genus *Polyporus* (sensu stricto). Kühner (1929) gave several valid reasons, and Bondarzew & Singer (1941) added more. Donk stated (1933) that the whole genus *Lentinus* should be treated taxonomically in continuation of *Polyporus*; but since he did not at the time offer any additional proof, it seemed possible to think that only *Lentinus cyathiformis* was affected by Kühner's comparison. Donk's statement, how-

ever, proved correct in another sense. A detailed anatomical study of the trama and subhymenium of *Pleurotus, Panus,* and *Lentinus* reveals that each of these genera has its counterpart, anatomically, in the genus *Polyporus* (sensu stricto) (*Favolus, Pseudofavolus*). Some of the species of the genus *Polyporus* are distinguishable from the corresponding *Pleurotus, Panus,* or *Lentinus,* mainly by the configuration of the hymenophore. The latter, however, has ceased to be considered as of great weight since the close relationship between *Xerocomus* sect. *Pseudophyllopori* and *Phylloporus* has been established on the basis of anatomical and chemical data (Singer, 1945), since a more detailed study of the false, agaricoid *Laschiae* by Singer (1945) and Heim (1946) revealed that Van Overeem (1926) was right in attributing to some tropical agarics a tendency to transform the configuration of their hymenophore, step by step, from lamellate to tubulose. In the light of these data, it appears that there is actually no appreciable gap between *Lentinus, Panus, Pleurotus* on one hand, and *Polyporus, Favolus, Pseudofavolus* on the other hand.

In the same investigation of the types once referred to the so called *Laschiae*, Singer (1945) attempted to draw a line between the true *Agaricales* (*Dictyopanus, Filoboletus,* and especially *Poromycena*) and the other laschioid *Basidiomycetes* (excluding the original *Laschia* which belongs to the *Auriculariaceae*) which were considered as belonging in the suborder *Cyphellineae* in a wide sense. It was also said, in the same paper, that certain *Tricholomataceae* with always lamellate hymenophore, such as *Panellus, Hohenbuehelia, Asterotus,* and perhaps *Schizophyllum* might perhaps be close to a group deriving from these cyphellaceous genera rather than from any *Gastromycetes.* Since it now appears that the *Cyphellineae* themselves are a rather artificially mixed group (Donk, *ined.*), they have lost their phylogenetic importance as a starting point, and *Favolaschia* becomes solely allied (though not closely) to *Aleurodiscus* unless more facts supporting the connection between *Favolaschia* and *Dictyopanus* become available in order to make the bridge between *Favolaschia* and the *Agaricales* something more than speculative. By the same token, *Campanella* and *Leptotus* are rather isolated agaricoid branches of aphyllophoraceous groups, and unless more evidence is brought to light, to substantiate the speculative bridge one may be tempted to construct between *Leptotus* and *Omphalina* on one hand, and between *Campanella* and the *Resupinateae* (*Tricholomataceae,* genera *Resupinatus* and *Hohenbuehelia*) on the other hand — such

connections between the *Aphyllophorales* and *Agaricales* must be considered as possible but not as probable in the same degree as the bridge *Polyporus-Lentineae*, or the bridges indicated between *Agaricales* and *Gastromycetes*.

All these affinities, assumed or otherwise, make the question timely again that has been asked before: What exactly are the limits between the *Agaricales* and the neighboring orders of *Basidiomycetes*? It has come to the point where the answer to this question cannot be given by an agaricologist alone but it is a problem that must and will seriously concern those working on *Aphyllophorales* and *Gastromycetes*. We agaricologists want to have help in the important decision that lies in the answer to the questions : Is there (and where) a sufficient gap between the genus *Polyporus* and the remaining polypores? Is there (and where) a line between the *Secotiaceae* of the *Galeropsis* group and the « true » *Gastromycetes ;* is there (and where) a line that can be laid between *Rhizopogon* and the other boletoid *Hymenogastrineae* on one side, and the « true » *Gastromycetes* on the other; is there (and where) a sufficient gap between the *Astrogastraceae* on one hand and the remaining *Gastromycetes* on the other hand?

There are those who doubt that there is such a gap inside of what was formerly considered a solid group — the *Aphyllophorales*.

There are those who doubt that there is such a gap in what was formerly considered — if not a natural group — a strongly convergent group of strictly parallel lines, the *Gastromycetes*.

If both are right, i. e. if there are no gaps in either case, and the three groups intergrade with each other without so much as an appreciable hiatus, there are only three alternatives for the phylogeny of the *Agaricales :*

1. The *Agaricales* are interpreted as an intermediate group between the *Aphyllophorales* and the *Gastromycetes*, with the *Aphyllophorales* the starting point, and the *Gastromycetes* the summit.

2. The *Agaricales* are an intermediate group between the *Gastromycetes* and the *Aphyllophorales*, whereby the former are considered as the starting point (or points), and the latter as the « summit ».

3. The *Agaricales* are a genuinely polyphyletic group with one part derived from the *Aphyllophorales*, the other from the *Gastromycetes*.

The hypotheses (1) and (2) have the disadvantage of suggesting that the evolution supposed to have taken place, runs in an immense

circle. In fact, starting as we do, from the assumption that no convincing dividing lines between *Agaricales*, *Aphyllophorales*, and *Gastromycetes* exist, we have to admit that the only reasonable derivation of the *Gastromycetes* as a whole is that outlined in rare concordance by nearly all specialists of the *Gastromycetes*, i. e. an evolution starting at a low point of the *Aphyllophorales*-system, and running parallel with the *Tuberales* of the *Ascomycetes*, finally reaching the most highly developed, unipilous forms of the *Phallineae* and the agaric-like *Secotiaceae*, *Hymenogastrineae*, etc. If, then, no gap is allowed between the *Agaricales* which would be derived from the *Gastromycetes*, and the *Aphyllophorales*, the latter would become merely strongly reduced agarics, step by step sinking backwards and downwards to the level where the *Gastromycetes* were supposed to have started. The same (vicious) cycle results if the direction is reversed.

Hypotheses (1) and (2) are therefore not popular at present, and it would take the discovery of a whole series of entirely new and unexpected facts to ever revitalize them.

This leaves more or less intact only the theory of polyphyletic derivation of the *Agaricales* — always assuming that there are no gaps either in the *Aphyllophorales* versus *Polyporus,* nor in the *Gastromycetes* versus *Galeropsis, Hydnangium, Truncocolumella,* etc. To this theory, we may add the two other possible theories, one based on the conviction that a gap between the *Agaricales* and the true *Aphyllophorales* does exist, and the other based on the conviction that a gap between the agaricoid *Gastromycetes* and the true *Gastromycetes* does exist.

Consequently, the three logically possible, and actually important theories, of today, each of them defended or favored by a group of systematists, are the following:

I. [(3) of our previous scheme] Derivation of the *Agaricales* from the *Gastromycetes* and from the *Aphyllophorales.*

II. Derivation of the *Agaricales* from the *Gastromycetes* alone.

III. Derivation of the *Agaricales* from the *Aphyllophorales* alone.

It cannot be stated at present that the probability of one of these theories is overwhelming as compared with the others. A taxonomist, after enough practical experience, can only give one a slight edge over the others, expressing a preference. In spite of the author's preference for theory II, the attempt will be made to state the case for each of them.

We shall start with the theory that has a slight majority of mycologists on its side because it is the oldest and most deeply rooted in the mind of mycologists, i. e. theory III which, it seems, can be linked with the name of Fayod (among many others in his generation), Neuhoff and Gäumann.

DERIVATION OF THE AGARICALES FROM THE APHYLLOPHORALES

The author has the strange task to revitalize a theory that was originally based on the faulty assumption of a bridge between *Polyporus* and *Boletus*, and another between *Cantharellus* and *Hygrophorus*. However, it seems that the same result will be obtained if more reasonable suggestions are followed up. For example, one may assume that the line leading to the higher tropical polypores of the genus *Microporus* continues by the way of *Microporellus* Murr. and finally reaches into the genus *Polyporus* sensu stricto, whereby the turning of a poroid hymenophore into a lamellate hymenophore would be merely a repetition of an analogous development in the *Daedalea-Daedaleopsis-Xerotus (Gloeophyllum)*-group and in the *Coriolus-Lenzites*-group. It may also be assumed that *Leptotus* (that may be derived from a corticiaceous or meruliaceous source), by a growing differentiation of its trama finally achieves an elevation to a level that makes it comparable with *Omphalina* (especially its pleurotoid representatives with which it has some external similarity). Finally, it may be assumed that *Favolaschia* is something like a halfway mark between *Aleurodiscus* and the *Tricholomataceae*, and it may then be considered as possible that the direction of the evolution is from *Aleurodiscus* to the *Tricholomataceae*. These three potential bridges do not necessarily exclude each other; they may be parallel.

This manner of seeing the interrelationship between certain borderline *Aphyllophorales* and the *Tricholomataceae* — only these are concerned — recognizes and explains the similarity between the structure of the cortical layers in *Favolaschia* and *Mycena, Campanella* and *Asterotus;* it also explains the presence of gloeocystidia in *Agaricales* such as *Lactocollybia,* and the gelatinous strata in the *Resupinateae*. It explains furthermore the presence of forms with tubulose hymenophore in the *Tricholomataceae*. The round dendrophyses of *Favolaschia,* especially those with vacuolar pigmentation, and the diverticulate hyphae of some of its species are found

again in *Mycena* which also has the amyloid spores of that genus.

The other families of the *Agaricales* must all branch out from the *Tricholomataceae*. There is no other choice in this scheme. The thicker walls in spores like those of *Phaeomycena* would perhaps lead to the *Crepidotaceae*, and the genus *Ripartites* may also be considered as transitional between *Tricholomataceae* and *Crepidotaceae*. From the *Crepidotaceae* one line would lead to the *Cortinariaceae*, and one to the *Paxillaceae*. This necessitates the assumption that the *Boletaceae* derive from lamellate families. The line from the *Tricholomataceae* to the *Cortinariaceae* may also lead by the way of *Ripartitella* and *Cystoderma* to the *Agaricaceae* and from *Cystoderma* to the *Cortinariaceae* (via *Phaeolepiota*), or by the way of *Leucocortinarius*. The *Amanitaceae* would be the terminal of a branch leading from *Armillaria* to *Catathelasma*, and from there to *Amanita* and/or from certain pleurotoid groups to *Rhodocybe* and further to the non-volvate *Amanitaceae*. The insertion of the *Hygrophoraceae*, *Rhodophyllaceae*, *Bolbitiaceae*, *Coprinaceae*, and *Strophariaceae* would perhaps cause certain difficulties but this can be considered as a minor problem.

All the possible ramifications of the descendants of the *Aphyllophorales* are possible on the presumption that a progressive development with a tendency to complication of the gross structure and the anatomy of the *Agaricales* takes the lead. The spores become gradually pigmented (mostly with a membrana-pigment which is still absent in the early stages of spore development and arrives at its peak at maturity), the layers of the wall become more and more complex, and the originally smooth spores become ornamented. The originally inconspicuous carpophore becomes larger in size (at least where the *Campanellae* and *Favolaschiae* are envisaged as ascendants of *Agaricales*), or at least more regularly stipitate, or the pseudostipe of *Microporus* becomes a true stipe in *Polyporus*, or is replaced by a stipe. The stipe in the soil-inhabiting forms becomes central, the veil develops gradually from simple and rudimentary to double and well developed.

At a certain stage of development, a tendency of the agaricoid carpophores toward angiocarpy begins that ends up in making them gastromycetoid. Under the influence of arid climates, the pileus remains closed until after the maturity of the spores, and this, automatically relieves the hilar attachment from its functions of forceful spore discharge and the spores become orthotropic. This leads to

strong convergence with another series of true *Gastromycetes* which have become angiocarpous at a much lower level of development. A similar convergence must explain the russuloid and the boletoid *Hymenogastrineae.*

The advantages of this scheme are: Easy placement of the *Leptotaceae,* elegant disposal of progressive lines leading towards groups with more complex structure; ecological explanation of the genera *Montagnea, Galeropsis,* etc.

The disadvantages are: Difficulties to explain the derivation of the *Russulaceae.* If hard-pressed, one may indicate *Melanoleuca* as starting point of that family but the characteristic heteromerous structure of the trama, the macrocystidia, and the bright pigmentation would remain unexplained (as well as the absence of lamellulae in the higher *Russulae,* an otherwise unheard-of development). This situation would become worse if any hymenogastraceous forms are admitted as further ramifications of the *Agaricales*-system. This would lead to the assumption that forms with clamp connections are derived from forms without clamp connections, and the beautiful structure of progressive development within the *Agaricales* proper would appear to be gained at the expense of an unlikely line of «degradation» (loss of the amyloidity of the spores, loss of the stipe, loss of the regularity of the hymenophore, etc.) as soon as the gastroid field is entered. Another disadvantage of this scheme must be seen in the fact that the progressive development toward more complex structures as expressed in the formation of one or more layers of veil formations is left without a biological background. If the biological explanation of the veil is — a preliminary stage to angiocarpous development, then, nobody will understand why this should be started in a slow way — long before the level is reached at which the actual transition into gastroid forms can take place. This reasoning is not only un-Darwinistic but contrary to any kind of logic. The alternative is to assume that veil formations, volva, cortina, pellicular veil, and marginal veil are all of advantage to the conservation of the species in one way or another. How that could be the case — we do not know.

DERIVATION OF THE AGARICALES FROM THE GASTROMYCETES

In the *Russulaceae*, the nests of sphaerocysts can be interpreted by the fact that in the process of transferring the fertile zone of the hymenophore downwards and carving out lamellate instead of loculate hymenophores, the hollow spaces would be filled in by «overgrowing» of the hymenial covering, the elements pushing against each other and thus forming a pseudoparenchymatic tissue-enclosure according to the rule explaining the formation of the sphaerocysts in the annulus superus of *Amanita* and certain organs of the Phalloids. The pseudoangiocarpous development of some *Russulae* and *Lactarii* is explained as the reproduction in the primordial stage of the development of some hymenogastraceous fungi which have a gymnocarpous earliest stage which is followed by a prolonged angiocarpous stage and a post-maturity stage with naked gleba or partly exposed gleba. The spores, originally orthotropic and mostly globose with prominent fundamental ornamentation, become at first slightly heterotropic but still remain so strictly globose that it is difficult to see their axial asymmetry; their exosporial ornamentation becomes comparatively more and more important; eventually, the spores become somewhat elongate, and truly heterotropic with the ornamentation so covered up with the exosporial ornamentation that the latter is no more recognizable without dissolution of the amyloid portion. At the same time, the spores become more yellow, the mycorrhiza relationship more constant and more selective. Veils that in the first stage have appeared in consequence of the pseudoangiocarpous development are lost in the higher forms because of abandonment of the angiocarpous phase as a reminiscence of the angiocarpous development of the *Astrogastraceae*. The clamp connections, still occurring in some of the gastroid forms, are entirely lost in the genus *Russula* as well as in *Lactarius*.

Some of the characteristic features of the gastroid group are retained in the *Russulaceae*. These are, among others, the fleshy-granulose consistency, the presence of pseudocystidia, the white to yellow color of the short, ornamented spores, the bright pigments of the peridium and the presence of a latex in many forms. In lower groups (*Russula delica*) «poroid» — actually gastroid — aberrations of the hymenophore are still common, in higher groups they are rare.

Another line running in the same general direction (gastroid-agari-

coid), has been found in the *Rhizopogon-Chamonixia-Truncocolumella-Gastroboletus-Boletinus decipiens* (gastroid condition) line. Here, too, the spores are basically similar in both groups, the banded spores of *Chamonixia* (finding their counterpart in some *Strobilomycetaceae*) and *Truncocolumella* coming so close to gastroid conditions of *Boletinus decipiens* that their only principal difference consists in orthotropic spores in *Truncocolumella,* and heterotropic spore formation in the *Boletinus.* In this case, the arrangement of the cavities has been changed into a regular hymenophore rather than abandoned and filled out as in the *Russulaceae.* Consequently, there is no heteromerous tissue here, but the tendency to bluing by autoxidation *(Chamonixia, Porphyrellus)* or reddening by wounding *(Rhizopogon, Strobilomyces)* is common in both groups. Clamp connections are still found in the agaricoid group but they evidently soon disappear in the higher forms. On the other hand, the formation of obligatory and specific mycorrhiza goes further back in the gastroid line, and is probably typical for all *Strobilomycetaceae* and *Boletaceae.* The trama of the walls between the loculi in *Rhizopogon* is distinctly bilateral (as in many *Gastromycetes*), yet with the medio-and lateral stratum not as well differentiated as in the higher *Boletaceae* and *Strobilomycetaceae,* the outer layer not being gelatinized. The subhymenium is very similar to that of the *Boletaceae* with catenulate short-rectangular to almost cubic cells predominating. The pigments are membrana pigments and epimembranal pigments in the peridium and in the cuticle of the pileus in most Rhizopogons and boletes, and these pigments are not easily dissolved in either case. We find that the host range of certain parasites is limited to *Rhizopogon,* boletes, and *Paxillus* (Heim, 1934).

In both cases, i. e. in the *Astrogastraceae-Russulaceae* line and in the *Rhizopogonaceae-Boletaceae* line, by far the more numerous, more varied, and as a whole more widely distributed group is the agaricoid group. This is considered as an indication that the direction from gastroid to agaricoid forms is more likely to be true than the reverse, if such an additional indication is still necessary. This direction is very important because — if it is accepted as probable in one case, it becomes very suggestive as a general principle, even in groups where the derivation from the *Gastromycetes* is still entirely speculative, e. gr. in the connection suggested by Romagnesi between *Richoniella* and *Rhodophyllaceae,* in the connection suggested by Maublanc between *Battaraea* and *Agaricaceae,* in the connection suggested as a

possibility by Singer between *Torrendia* and *Amanita,* and in the undoubtedly existing connections (but without a clear indication of the direction of the phylogenetic trend) between *Galeropsis* and *Bolbitius,* and between *Montagnea* and *Coprinus.*

The remaining *Agaricales* may be derived from any of these *Gastromycetes,* or from the bolete branch which shows some tendency at certain levels to form lamellate hymenophores (or perhaps, the original hymenophore, as in *Russula,* was lamellate, and the evolution of the tubes was secondary) and these lamellate groups though still distinctly showing their affinity with boletoid forms (*Paxillus-Gyrodon; Phylloporus-Xerocomus; Gomphidius-Suillus)* begin to abandon the mycorrhizal specificity in favor of lignicolous habitats and saprophytic nutrition, or to complicate their dependence on mycorrhiza by acquiring a double dependence *(Gomphidius).* Another step would lead to the *Crepidotaceae,* and from here to the *Cortinariaceae, Tricholomataceae* and *Rhodophyllaceae.*

A separate derivation of certain other groups, such as the *Agaricaceae, Amanitaceae,* and *Coprinaceae,* is also warranted because of the volva which is in this scheme not considered as a useful acquisition by highly developed agarics but rather as a reminiscence from gastromycetous ancestors for which the volva evidently was an additional adaptation. This becomes clear when (1) the fact of the existence of many transitions between a compact volva and volva rudiments is considered which can much easier be explained by reduction of the volva than by progressive acquisition; (2) when it is understood that the truly volvate species, even in *Amanita* are a small minority, and that *Amanita muscaria,* occurring far to the north under tundra conditions, as well as *Amanita nana,* occurring in the steppes and semideserts of Asia, both have reduced volvae whereas the truly volvate *Amanita caesarea* occurs in warmer temperate regions not only in the comparatively dry mediterranean zone but in the humid zones of the Colchic district and in Eastern North America. The number of truly volvate *Amanitae* is higher in ancient floras that have remained undisturbed during a long period such as many tropical regions in southeastern Asia. The fact that *Amanita* shows a rather high development in other regards than the volva can only be explained by the fact that the *Amanitae* originate in a series of genera other than the nonvolvate agarics with a lower organization in other regards. These indications of a higher level are (1) absence of clamp connections in many species (2), binucleate spores in all

species studied, (3) amyloid spores in more than half of the species — though rarely in species with a complete unreduced volva of the *caesarea* type. It is, however, impossible to link all these characters with the presence of a volva. Another genus with double veil, *Catathelasma,* has numerous clamp connections and uninucleate spores in all species known.

If the search for gastromycetous ancestors of the families of *Agaricales* leads to desert species, it is not assumed that they themselves, highly organized and rather specialized as they are, represent the very ancestral forms from which our recent *Agaricales* have immediately derived. But we assume that it was from forms of the same general type as those deserticolous *Gastromycetes* that they may have derived, viz. from non-mycorrhizal forms becoming mycorrhizal, or from non-coprophilous forms becoming coprophilous. It is not pretended that parts of a desert flora have given rise to a typical forest flora.

Advantages: This theory is able to explain all the facts known in the *Russulaceae* and the boletes. In this regard it is perfect — as far as any theory based on non-paleobotanical material exclusively can be perfect. The scheme for the remaining families, though essentially hypothetical, has the advantage of pointing at a possible solution that is, in its general tendency from angiocarpous to gymnocarpous forms as admitted in the *Russulaceae* and boletes, completely conform with that in the latter two families. It also explains the veils more satisfactorily than the other theories.

Disadvantages: It cannot in detail explain the derivation of the *Amanitaceae* and a few other families, and is handicapped by the complicated manner by which it attempts to explain the xerophilous character of some of the supposedly ancestral forms of the *Coprinaceae* and *Bolbitiaceae.* It leaves unexplained the similarities between the genera *Campanella* and *Favolaschia* on one hand and certain *Tricholomataceae* on the other hand unless the latter two genera are considered as reduced and atypical members of the *Tricholomataceae* (as has virtually been suggested by Patouillard).

The derivation of the *Agaricales* from the *Gastromycetes* has been first indicated by Brefeld, on the basis of general considerations of the structure of both these groups; later it was adopted by Buchholtz on the basis of his data on *Elasmomyces* and *Archangeliella.* Höhnel accepted Brefeld's view, and Lohwag accepted and elaborated on Buchholtz's theory. Singer (1936) brought his new classification in

agreement with this theory and accepted it in detail for the whole order. It would, however, be incorrect to say that a classification is entirely based on a phylogenetic theory. Neither the classification of 1936 nor the modifications admitted in this book, are the result of phylogenetic deliberations but, vice versa, the phylogenetic theory is the result of taxonomic data.

While the theories of a derivation of the *Agaricales* from the *Aphyllophorales*, and the derivation of the *Agaricales* from the *Gastromycetes* have often been linked with a specific complete classification of the *Agaricales*, this has never been the case in regard to the last, remaining theory, that of a derivation of the *Agaricales* from both the *Aphyllophorales* and the *Gastromycetes*. In fact it is rather difficult to cite authors who have expressed their views in exactly this way. While a certain degree of polyphyletism is admitted by many mycologists, it has never been said, except by implication, that part of the *Agaricales* were derived from the *Aphyllophorales*, and part from the *Gastromycetes*. Yet, if it is allowed to piece together various statements by C. Dodge, perhaps also R. Maire at certain periods, and R. Heim p. p., it will be correct to consider these authors as in favor of a polyphyletic derivation of the *Agaricales* as specified above.

DERIVATION OF THE AGARICALES FROM BOTH GASTROMYCETES AND APHYLLOPHORALES

Assuming that the derivation of the *Russulaceae* and boletes from hypogeous *Gastromycetes* is sufficiently substantiated, it would here be considered as possible to separate completely these two families and any such groups that may have derived from them immediately (e. gr. *Strobilomycetaceae*, *Paxillaceae*, *Gomphidiaceae*), from the rest of the *Agaricales*, especially the white spored group (*Tricholomataceae*) which would then be derived from the *Polyporaceae* and or the *Leptotaceae* or parts of these families as has been pointed out above.

Advantages: At the present stage of our knowledge, this scheme presents all the advantages of both preceding theories, and avoids some of their disadvantages. It reflects the wise and conservative attitude under the given circumstances.

Disadvantages: As all « eclectic » theories, it is somewhat inco-

herent and inconsistent. It appears to be objectionable to allow an evolution from angiocarpous to gymnocarpous in one case, and from gymnocarpous to angiocarpous in another case. Though it may be argued that it is undoubtedly true that both these developments have taken place in the evolution of the fungi at one place or other, and nothing forbids a priori to believe that this has also happened within the *Agaricales*, it is nevertheless bewildering to find the veil assuming one rôle and designating one direction of evolution while — almost simultaneously — the same organ plays the opposite rôle and the general trend seems to run in the opposite direction. Besides, a more practical disadvantage would arise, one that has probably prevented those in favor of this theory to ever link it with an elaborate classification of the *Agaricales :* this practical disadvantage is the necessity, in any classification, to draw a dividing line between the two main groups of *Agaricales*, those derived from the *Aphyllophorales*, and those derived from *Gastromycetes*. Since the taxonomic data at hand do not warrant such a sharp dividing line, it would be necessary, probably for a long time to come, to maintain the division into fifteen families rather than to attempt a new bipartition of the *Agaricales*. If this bipartition should have removed only the *Russulaceae* from the bulk of the *Agaricales*, it would have been easy to do so on the basis of a large number of excellent facts. But there is, in systematics of the present day, absolutely no way to find a common denominator for a group that is left over after both *Russulaceae* and the boletes with « appendages » have been removed.

Future research will perhaps give more weight to one of the three schemes outlined in this chapter — or possibly advance a fourth.

PROBLEMS OF NOMENCLATURE

The problem of nomenclature is not a subject in itself as far as the present work is concerned but it becomes an important factor in the choice of the correct names to be adopted. The author is fully aware of the fact that here, more than in any special paper intended primarily for specialists, the correct choice of the fungus names is a great responsibility. The bibliographical work involved will only be appreciated by those who are familiar with the difficulties of the strict application of the International Rules of Nomenclature in mycology. The difficulties lie not so much in the following of the

rules as in the handling of cases that are not foreseen in the Rules of Nomenclature. It is necessary in all cases to follow the rules and even the recommendations have been followed to the letter. Certain provisions of the Rules, however, are in need of further clarification by an International Congress and some articles, especially those referring to the starting points of mycological nomenclature are subject to a difference in interpretation that causes more divergence of nomenclature in specific cases than is generally realized.

Two very important problems, those concerning the typification of the genera (« nomina lectotypica ») and the conservation of generic names are practical problems that require a thorough examination of the cases from all angles and, afterwards, the proposition of a list of lectotypes and « nomina conservanda » for acception by an International Botanical Congress. Such lists have been proposed for adoption by Singer, R. & A. H. Smith (1946), and since these are the only approximately complete lists that can be used in the *Agaricales*, the nomenclature of this book is based on these lists. The genera that are not mentioned in these lists are either typified by their authors, or they are monotypic, or else do not constitute a taxonomic problem at present. The « genera conservanda proposita » by Singer & Smith are here treated as if they had been accepted already since this seemed to be the only possible consistent policy.

A third problem is very difficult. It concerns the habit of all modern taxonomists who follow the rules at all, to consider a pre-Friesian name, validated according to Art. 20, *e.f* by a post-Friesian author as based on the specimen or description of the latter. It is, as has been pointed out to me by M. A. Donk in a very interesting discussion on the subject, rather questionable whether or not this customary procedure conforms with the intention of those who voted the original rules in 1910. Nevertheless, it would be grossly unfair and detrimental to the general acceptance of nomenclatorial rules, if those who have adhered to them as they best understood them, were now penalized by a revision of the interpretation or, if one wants to express it so, by a reconstitution of the original intentions. From a practical point of view, the admission of the original pre-Friesian author's concept as the type of a (re-) validated name would contribute toward a better documentation only in the case of Persoon (and even here not in all cases); in the case of other authors, especially Scopoli, Schaeffer, Bulliard, Withering, Linnaeus, Batsch, Bolton, etc., it would open the door for futile discussions and a variance of inter-

pretation which would be especially dangerous and detrimental in cases where the Friesian name is already fixed by a more methodical description, or by unanimous tradition. This should be avoided, and can be avoided; for, the decisive factor is Art. 5 which says that « in the absence of a relevant rule, or where the consequences of rules are doubtful, established custom must be followed » [48]. Since it is obviously established custom, at least among those who follow the rules at all, and the consequences of Art. 20, *e* and *f*, are doubtful in the light of Art. 18 (Type Method) which has been added later, it must be assumed that Art. 5 applies here. Consequently, the author does not admit pre-Friesian types even if cited in the (re-)validating diagnosis, if this diagnosis is in contradiction to the pre-Friesian concept. There is only one complication which, by the tacit consensus of those concerned, has thus far been handled in a way suggesting the existence of an explanatory note supplementing Art. 20 saying that « transfers made after the starting data in the different groups (or : in « Fungi caeteri ») but regarding pre-Friesian names revalidated in the sense of a post-Friesian author must be understood as transfers of the unit concerned in the post-Friesian concept rather than in the original pre-Friesian concept, unless the transferring author makes a definite statement excluding the Friesian or post-Friesian concept such as « non Fries », and « nec Fries » [49]. Since such a note does not exist at present, it would appear that there is a definite need for it, and it should rather be accepted before the first differences of interpretation break out into monographs than afterwards. The nomenclatorial problems in which this tentative provision

[48] *International Rules of Botanical Nomenclature adopted at the International Botanical Congresses of Vienna, Brussels... Cambridge,* Jena, 1935.

[49] *Example :* Gray's description of *Leccinum scabrum* goes back to some author that probably had a species in view that is not identical with *Boletus scaber* Bull. or *Boletus scaber* Fr. However, since he does not explicitly exclude Fries's concept, *Boletus scaber* Bull. ex Fr. can be correctly transferred to *Leccinum* under the binomial *Leccinum scabrum* (Bull. ex Fr.) S. F. Gray. Without a note of explanation as suggested above, Gray's name would not be valid for the Friesian *B. scaber* since it does not refer to Fries. When the Friesian species is transferred to *Leccinum*, this would not be possible under the epithet *scabrum* because by now *Leccinum scabrum* would be a homonym of Gray's binomial. As a result, two « nomina nova » would be necessary. Under the provision of the explanatory note, however, only one new name would be necessary, and even that new name has been taken care of by the publication of other binomials which were intended to designate new species rather than to become « nomina nova ».

may apply have been treated as if this provision were part of the rules.

Another particularly difficult problem is the application of the International Rules to sectional names. It is not only necessary to investigate the author's intention as to whether the group-name was actually meant to be a section (which was, in spite of superficial appearance, not the case with Burlingham's groups in *Russula* and *Lactarius*), or its equivalent. A named group beneath a section is usually interpreted as a subsection unless there is evidence to the contrary. Unspecified names (Latin adjectives in plural) below the subsection level, as are often proposed by Bataille, Lange, and others, are not accepted as prior to names with definite rank. The type of the sections and subsections is usually the species after which it is called, and for which it was primarily intended. If there is a discrepancy between the description and the correct interpretation of the type species, or if the sectional or subsectional name is not formed after a typical species the difficulties are often considerable. In such cases a tentative selection of a lectotype has been made with due consideration of the nomenclatorial changes involved with each alternative selection, and with a view of not causing unnecessary innovations in sectional and subsectional names. The range of units between species and order has thus far, from a nomenclatorial point of view, been treated in a very careless way by most authors. Yet, it appears that there should be no exception to the application of the general principles of nomenclature, even in those units that do not immediately influence the binomial nomenclature of the species.

Some of the names of families, initially accepted or even proposed by the author himself, appear to be in disaccord with the pertinent rules governing the naming of families (Art. 23), and had to be changed accordingly. In other cases, the type concept was involved when certain genera were transferred into other families, and the name of the family had to be changed even though the larger number of genera and species belongs to the family whose name must disappear. Family names that were proposed at a time when the rules for the formation of family names were not yet formulated, and were, accordingly, not given the correct ending (in *aceae*), are generally admitted as validly published, and the corresponding change in the ending is made, wherever necessary, in the same way as this appears to be admissible in the case of incorrectly formed specific names (consisting of two words, misspelled, etc.). This goes mainly for the

families that have been proposed by Roze (1876) who gave a description of each of them in a subsequent paper, indicating several genera belonging in each of the families. On the other hand, the family names proposed by van Overeem in his later papers, cannot be considered as validly published since they have no description accompanying the names which are consequently «nomina nuda». All these considerations made it necessary to abandon the family name *Leucocoprinaceae* in favor of *Agaricaceae*, and *Rhodogoniosporaceae* in favor of *Rhodophyllaceae*.

Generally, it is felt that, if ever, nomenclature must be brought into accordance with the rules — now. There are so many changes in the taxonomic field that a few additional changes on the basis of legality will pass almost unnoticed. In the opinion of the majority of those concerned with issues of nomenclatorial order, it is bad policy to keep inconvenient but legal names in the dark, hoping that nobody will discover them. They will eventually be brought to light, and this will be at a time when the consequences will be felt much more severely. It is true that these consequences can then be corrected by conservation. But it is the general consensus that conservations should be kept at a minimum, and, besides, there is in the Rules no provision made for the conservation of specific names — and there should not be.