MORPHOGENESIS OF THE CARPOPHORE OF *COPRINUS CINEREUS*

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SUMMARY

An interpretation of carpophore morphogenesis is presented which is derived both from the literature and from original observations. Cultured material of *Coprinus cinereus* (also called *C. macrorhizus* and *C. lagopus*) has been studied using biochemical assays together with light-, scanning- and transmission electron microscopy to provide details of histology and the levels and distribution of protein, glycogen and NADP-linked glutamate dehydrogenase during the course of development of stipe and cap. Initially, aggregates of hyphae form primordia which subsequently develop into mature carpophores. During stipe development glycogen deposition is at first restricted to cells forming a cup-shaped mass in the stipe base, but very early in primordium development this deposit is depleted and glycogen accumulates in the gills, particularly the subhymenium, where it probably serves as a reserve material for the later stages of cap development. The distribution of cytochemically detectable protein differs from that of glycogen in that the former initially accumulates in the upper regions of the primordial stipe and in the gill hymenium. The stipe showed no conspicuous change in its content of protein during development although in the cap the fraction of the dry wt represented by protein increased substantially. Primordial stipes are composed of overlapping cells of hyphal dimensions with dense cytoplasm and small vacuoles. Stipe development depends on cell enlargement, this seeming to be biphasic; an initial increase in volume being attributable to an increase in cell diameter to give an undifferentiated dikaryotic central region and a differentiated multinucleate cortex. Stipe growth depends on reallocation of cellular components, no one reserve material being identifiable as of prime importance although a few simple sugars do appear to be correlated with the osmoregulatory activity connected with the large scale uptake of water involved in stipe elongation. In the primordium cap the subhymenium is an open tissue of interwoven hyphae, the cells containing large accumulations of glycogen. The hymenium is formed from dikaryotic branches of the subhymenial hyphae to become an organised layer of hyphal tips. Subsequently, although the subhymenial hyphae remain as such, the hymenial cells become inflated. The paraphyses come to form a pavement of appressed and interlocked cells each containing a large central vacuole. Basidia are only slightly enlarged and contain dense cytoplasm with few vacuoles; they contrast with the much extended and inflated cystidia. Despite their inflation, all three cell types still show continuity with the subhymenial hyphae. During cap expansion the major inflationary force is paraphyseal enlargement but as the gill lamellae are removed by autodigestion further cell inflation in the pileal flesh enables it to assume a supportive role. In the earlier stages there is some correlation between declining glycogen content of the cap and increasing activity of NADP-linked glutamate dehydrogenase. It is suggested that cap expansion is driven osmotically and that at least some of the osmotically active materials are synthesised through the urea cycle. At first this activity could be supported by glycogenolysis and amination but later on substrates may be provided by autodigestion of spent gill tissues. It is concluded that cap development is regulated by a metabolism quite distinct from those of either the parental mycelium or the stipe, and that the fundamental metabolic changes occurring throughout carpophore development are orchestrated by light. As carbohydrate nutrients in the medium approach exhaustion suppression of mycelial morphogenesis into hyphal aggregates is lifted, but the subsequent switch from the vegetative

pathway of sclerotium development to carpophore formation is normally dependent on illumination with low intensity blue light. Growth then becomes polarized to form a presumptive pileus and glycogen is mobilized from the surrounding mycelium to accumulate at the base of the stipe. Receipt of a second illumination inhibits further growth of the stipe base and causes glycogen reserves to be translocated to the cap. Glycogen is utilized in the cap to produce glycolytic intermediates, allowing regulation of many aspects of metabolism by catabolite concentration and contributiong to the diverse syntheses required for spore formation and dissemination.

INTRODUCTION

Coprinus cinereus is one of the few basidiomycetes which is amenable to experimental analysis from every aspect. In common with other agarics the undifferentiated mycelium forms a carpophore, the mushroom fruit-body, which contains separate tissues with different functions. In this differentiation the organism evidently achieves the complex morphogenetic integration which distinguishes eukaryotes from prokaryotes; yet the extent of the developmental complexity does not appear to be so great as to be beyond understanding. This organism possesses the less commonly encountered advantage of being readily cultured on any of a range of experimental media as a mycelium which can be arranged to be a monokaryon, heterokaryon, dikaryon, diploid, or of even more esoteric nuclear constitution. The mycelium and its structures are readily used for chemical, biochemical, enzymological and microscopical analysis, and a considerable amount of genetic experimentation has been accomplished. In offering both the complexity of the morphogenetic pathway leading to the carpophore and the vegetative simplicity of the mycelium the organism provides an ideal compromise for the investigation of the control of morphogenesis in eukaryotes; there exists sufficient complexity for the control mechanisms to have the sophistication necessary to make the study relevant to eukaryotes of a higher order of development, yet the undifferentiated mycelium provides the opportunity for the separation and experimental study of events observed to occur in morphogenesis. In this paper we present a collation of recent work on morphogenesis which has previously been difficult to relate because, although the strains used are interfertile, the results have been published under three different specific names (*Coprinus cinereus*, *C. lagopus* and *C. macrorhizus*). This is combined with original research consisting of light- and electron microscopic observations of the structures of developing carpophores and includes determinations of protein and glycogen levels within the carpophore tissues. These together contribute to an overall description of carpophore morphogenesis in *C. cinereus*.

The genus *Coprinus* has been of interest to developmental morphologists and taxonomists for over a century. Two limitations exist which affect the usefulness of the early literature for present day use. First, most of the early studies (reviewed by Reijnders, 1963) made use of sections cut from specimens either collected from the wild or arising on substrates collected from the wild, and in many cases the studies were made for essentially taxonomic purposes. In recent years attention has concentrated on the more functional aspects of carpophore morphology, and most recently attempts have been made to correlate particular metabolic and biochemical events with changes in morphology during carpophore development. These studies make use of carpophores formed by mycelia grown in axenic culture under standardized conditions. Potentially they allow more meaningful comparisons to be made between different reports than was possible before, and they allow the effect of the biochemical

environment on the pattern of differentiation to be identified. The second drawback in the use of the earlier literature is that the nomenclature of the organisms studied over the past century has been applied in a very confusing manner. Unfortunately this is still true today.

Although many *Coprinus* spp. have been studied [e.g. *C. congregatus* (Bret, 1977; Robert, 1977a, b), *C. domesticus* (Chapman and Fergus, 1973), *C. kimurae* (Komagata and Okunishi, 1969), and see Reijnders (1963) for earlier work] by far the most use has been made of '*C. lagopus*' and its allies, yet it is the nomenclature of this group which has become the most confused. In investigating this confusion Pinto-Lopes and Almeida (1970) revealed the wide variety of specific names that have been used to designate particular species, and conversely, the large number of different species which have been described with the same specific epithet. Because of their use in genetical investigations, strains of *Coprinus cinereus* originally isolated from nature by Lewis (1961) and Day (Day and Anderson, 1961) have been widely distributed to laboratories in Europe and North America as '*C. lagopus sensu* Buller' for use in a range of investigations; but nomenclatural confusion still exists and has lead to misleading comparisons. Thus McLaughlin (1977) states that the 'early migration of nuclei into basidiospores reported in *C. lagopus sensu* Buller (Raju and Lu, 1970) does not occur in *C. cinereus*'. However, McLaughlin used strains known to be interfertile with those used by Raju and Lu, so if there is a substantive difference in the timing of nuclear migration it is a population polymorphism and not a species-specific difference. In the hope of avoiding such false comparisons we describe here recent work done on morphogenesis with the strains of *C. cinereus* belonging to the interfertile population originated by the collections of D. Lewis and P. R. Day at the Bayfordbury (Hertfordshire) laboratory of the John Innes Institute. We also demonstrate that strains used in Japan for studies on morphogenesis under the name *C. macrorhizus* are fertile with the 'Lewis-Day' collections.

IDENTITY OF THE MATERIAL

A dikaryon constructed from the monokaryons BC9/6, 6 and H1 of *Coprinus cinereus* (Schaeff. ex Fr.) S. F. Gray *sensu* Konr. has been used extensively in this laboratory for the production of carpophores for experiments on morphogenesis. The H1 monokaryon belongs to the 'Helm' series of wild-types isolated by Day in 1957 (details in Day and Anderson, 1961). Members of this series have been used by Niederpruem and co-workers for morphogenetic studies (e.g. Cox and Neiderpruem, 1975), in the characterization of DNA (Dutta, 1974; Dutta and Ojha, 1972), for analyses of mycelial wall compositions (Schaefer, 1977; Marchant, 1978), and as a comparison for work with *Hygrophorus* (Mehta and Siehr, 1973). The monokaryon BC9/6,6 is one of a set of four laboratory prototrophs prepared by D. H. Morgan by a series of crosses in which H1 was backcrossed for nine generations to it sib, H9. A dikaryon made between BC9/6,6 and BC9/5,5 has been used by McLaughlin (1973, 1974, 1977) for studies of basidiospore formation and hymenium structure. The strains used by Lu and his co-workers (PR2301 and JR52) for studies on recombination and meiotic events (e.g. Lu, 1978; Lu and Chiu, 1976) have a more complex origin. Considerable use was made of PR2301 by Lewis (1961); it is a methionine auxotroph but the original mutation was induced in the wild-type code numbered 1796, and PR2301 was obtained from a cross made between this original

mutant and the wild-type number 68 isolated by Lewis in 1952. The strain known as JR52 is a prototroph of abnormal morphology; its origin is extremely devious but its ancestry includes PR2301 and H1 (Cowan, 1964). Some use has been made of a wild-type (code number ZBw601/40,40) which originated in Czechoslovakia (Moore and Stewart, 1972; Waters, Butler and Moore, 1975a), a strain (number PA1) collected in Poland (Moore and Al-Gharawi, 1976) and another (RF50) collected in France (Senathirajah and Lewis, 1975). These exceptions apart, all of the work done in Europe in recent years on *C. cinereus* has made use of strains whose origin can be traced back to the Hertfordshire population of this organism. The same is true of work done in America and Canada and applies whether the original authors described their material as *C. lagopus* (e.g. Volz and Niederpruem, 1970; Stewart and Moore, 1974) or *C. cinereus* (e.g. Gooday, 1975; Blayney and Marchant, 1977). The genetical work done using these strains has not been reviewed, but some indication of the range of subjects studied can be got from Casselton and Condit (1972), Smythe (1973), Lewis and North, (1974) Rahman and Cowan (1974), Lewis and Casselton (1975), Senathirajah and Lewis (1975), Tilby (1976), Lewis and Vakeria (1977), North (1977a), Sealy-Lewis and Casselton (1977), Casselton (1978), and Moore and Katy (1978).

Coprinus cinereus is a weed in *Volvariella* mushroom beds and a comparative study of these two fungi has recently been initiated in Singapore (Chang-Ho and Yee, 1977). We have found that monokaryons prepared from the dikaryon isolated and used by Chang-Ho and Yee (1977) are fertile with H1, BC9/6,6 and ZBw601/40,40.

For more than 25 years workers in Japan have studied an organism ascribed to the taxon *Coprinus macrorhizus* Rea. f. *microsporus* Hongo (Kimura, 1952). Pinto-Lopes and Almeida (1970) stated that they 'should rather adopt the expression *C. cinereus* (Schaeff. ex. Fr.) S. F. Gray f. *microsporus* Hongo' although they were unable to obtain cultures of this fungus and so could not include it in the mating tests on which the bulk of their conclusions were based. Kemp (1975 and subsequent corrigendum) states that *C. macrorhizus* f. *microsporus* Hongo is interfertile with *C. cinereus* and we have confirmed that the Okayama strains of '*Coprinus macrorhizus*' are fertile with all of the *C. cinereus* strains referred to above. Drs T. Kamada and T. Takemaru of the Department of Biology of Okayama University generously provided us with a spore print from a carpophore formed on the dikaryon (5026 + 5132) which they have adopted as the wild-type for their studies of carpophore morphogenesis. Mono-sporous isolations were made and we have obtained seven strains (coded Okayama-1 to Okayama-7) which are all monokaryons (free of clamp connections but producing oidia) though, as the dikaryon is heterozygous for a morphological mutant, varying in hyphal growth pattern. All seven strains proved to be fertile with H1, BC9/6,6 ZBw601/40,40, PA1, Singapore-7 (a monokaryon derived by us from the dikaryon of Chang-Ho and Yee, 1977) and MC-1,3 (a monokaryon supplied by P. R. Day which was collected at Mount Carmel, Hamden, Connecticut, U.S.A.). By fertile we mean that confrontations between monokaryons on synthetic medium rapidly yielded dikaryotic mycelia and that these produced fertile carpophores when appro-priately cultured. There is no doubt, therefore, that all of these strains are members of the same inter-breeding population and therefore belong to the same species. We accept the recommendation of Pinto-Lopes and Almeida (1970) with regard to the nomenclature of the Lewis-Day material. All of the monokaryons we have isolated from the '*C. macrorhizus*' spore print have produced microsclerotia in culture, a feature unique to *C. cinereus* according to Kemp (1975). However, the basidiospores

originating from the 5026+5132 dikaryon measured 8·4 to 10·3 (av. 9·3) × 5·2 to 6·5 (av. 5·7) μm and were not significantly smaller than those produced by carpophores of the BC9/6,6+H1 dikaryon which measured 8·4 to 11 (av. 9·6) × 5·8 to 7·1 (av. 6·3) μm. Thus although the material from Okayama can be placed in the species *C. cinereus* as suggested by Pinto-Lopes and Almeida (1970), the qualification 'f. *microsporus*' does not seem appropriate. We therefore conclude that the material originating in Okayama from the collection initiated by Kimura (1952) shares membership of the taxon *C. cinereus* (Schaeff. ex. Fr) S. F. Gray *sensu* Konr. with the material whose collection was initiated by D. Lewis in England at about the same time (Lewis and North, 1974). This conclusion is in accord with current taxonomic opinion (R. F. O. Kemp, pers. comm.).

In the discussions which follow all of these strains will be referred to as *C. cinereus* irrespective of the name employed by the original author(s). When other taxons are referred to we follow the recommendations of Pinto-Lopes and Almeida (1970), again irrespective of the name employed in the original publication. When it is necessary to make reference to names judged to be erroneous they will not be italicized.

INITIATION OF CARPOPHORE DEVELOPMENT

The developmental sequence of the carpophore has been categorized into stages by a number of authors. Table 1 summarizes these formalized descriptions as they are used in this paper and indicates how the different categorizations are related. Carpophore formation by a fertile dikaryon of *C. cinereus* growing on a synthetic medium occurs in response to a light stimulus (Morimoto and Oda, 1973; Lu, 1974a) when the carbohydrate content of the medium has been exhausted (Rao and Niederpruem, 1969; Stewart and Moore, 1974). It is not clear how this relates to the situation in the natural environment. *C. cinereus* is a member of the community of composting organisms and is commonly found on plant litter where growth of the mycelium must depend on polysaccharide degradation. The organism is certainly able to produce cellulolytic enzymes (Amarakone, 1976) but very little reducing sugar is accumulated in a freely soluble form in the medium when the mycelium is grown on filter-paper cellulose (Hedger and Hudson, 1974). Comparison with true *C. lagopus* (Iten, 1970) would suggest the ability to secrete proteases too, and the excretion of large amounts of ammonia-nitrogen into the medium by mycelia of *C. cinereus* implies a ready ability to utilize the carbon skeletons of amino acids (Stewart and Moore, 1974). There may well be nutritional differences between fruiting dikaryons grown on synthetic and natural media, but there is no indication that this leads to any significant difference in carpophore structure or development apart from a generally smaller size of carpophore and slightly longer development time in cultures grown on synthetic medium (Ewaze, Moore and Stewart, 1978). It seems justifiable, therefore, to make use of either type of medium as convenient and to consider the data obtained to be fully comparable.

The initial stages in the formation of carpophore primordia by *C. cinereus* have been described by Matthews and Niederpruem (1972). They involve the formation of hyphal lattices in the surface mycelium through interactions between secondary and tertiary branches of intercalary hyphal cells. The lattices produced abundant aerial hyphae to form a rounded aggregation of tightly woven hyphae (Plate 1, No. 1) which subsequently developed into the primordium. Matthews and Niederpruem

Table 1. *Summarized description of carpophore development in*
Coprinus cinereus

Stage 0	*Carpophore initials:* these appear about 88 h after inoculation; differentiation commences in the centre of a hyphal aggregate when it reaches about 0·2 mm. in diameter, histologically distinct zones are evident when it reaches 0·4 mm in diameter, while the inner tissues of 0·8 mm diameter initials are clearly arranged into a distinct 'mushroom' shape; initials 1·5 to 2 mm tall have well developed gill lamellae and a distinct annular cavity; dense polysaccharide deposits can be detected in the cells of the basal bulb of the stipe.
Stage 1	*Primordia:* appear about 110 h after inoculation; 2 to 9 mm tall; gill tissues well developed with basidia about 12 μm long, cystidia 20 to 25 μm; dense polysaccharide deposits can be observed in the subhymenium and cystidia as well as in the base of the stipe; karyogamy occurs.
Stage 2	*Primordia:* about 132 h after inoculation; 6 to 15 mm tall; partial veil intact initially but becoming more or less free; gills beginning to separate, with basidia 15 to 18 μm long and cystidia about 45 μm long; histochemical polysaccharide staining in the cap continues to intensify, that in the stipe base to lessen. Meiosis occurs.
Stage 3	*Immature fruits:* about 144 h after inoculation: 10 to 20 mm tall; partial veil free: stipe begins to elongate slowly; polysaccharide deposits now concentrated in the tissue layers beneath the hymenium, little detectable in stipe base; hymenium fully differentiated with cystidia about 60 μm long, and paraphyses clearly distinct from basidia (about 20 μm long) which begin to form sterigmata and spores
Stage 4	*Immature fruits:* about 150 to 156 h after inoculation; 15 to 45 mm tall, basidiospore pigmentation begins to be laid down; hymenial cells become vacuolated; polysaccharide deposits begin to disperse from the subhymenial cells: stipe continues to elongate slowly
Stage 5	*Mature fruits:* appear about 160 h after inoculation; 20 to 100 mm tall; stipe elongates rapidly; cap expands and opens like an umbrella: spores are discharged; very little polysaccharide is detectable cytochemically.

The descriptions given here are based, with additions, on Stewart and Moore (1974). Carpophore development was originally divided into stages like this by Borriss (1934) but his descriptions are sparse and refer to *C. radiatus*. The various stages of initial development described by Matthews and Niederpruem (1972) are encompassed by Stage O of this scheme. Developmental stages used by Blayney and Marchant (1977) appear to be directly comparable with those described for the same species by Stewart and Moore (1974) which in turn are very similar to the earlier descriptions of the Japanese strains of *C. cinereus* published by Morimoto and Oda (1973).

(1972) make no comment about Brefeld's (1877) claim that *Coprinus* carpophores arise from single cells. However, Waters, Moore and Butler (1975b) did claim that aerial sclerotia of *C. cinereus* developed from an initiation point in which only a single cell was at first involved. Nevertheless, as a result of the extensive hyphal interactions which occur subsequent to inception, clearly shown to include hyphal fusions in development of the carpophore primordium (van der Valk and Marchant, 1978), there is potential for a large population of nuclei to contribute to the developing structure as has been shown to be the case in perithecial development in *Neurospora* (Johnson, 1976). Comparison between sclerotium and carpophore development is relevant because of the strikingly close similarity between the events described for initiation of sclerotia by Waters *et al.* (1975b) and of carpophores by Matthews and Niederpruem (1972). Indeed, it has been suggested that for the dikaryon the two structures are alternative outcomes of a single initiating pathway; the different fates of the hyphal aggregate being decided by environmental features such as light, temperature and carbon and nitrogen supplies (Moore and Jirjis, 1976). The involvement of glycogen in the developmental process is another important point of similarity between sclerotium and carpophore morphogenesis. This is considered in detail in the next section.

ROLES OF GLYCOGEN AND PROTEIN AS RESERVE MATERIALS
IN CARPOPHORE MORPHOGENESIS

Glycogen is involved in various aspects of vegetative morphogenesis (Waters *et al.*, 1975b; Jirjis and Moore, 1976) but has also been implicated repeatedly in carpophore development. The material has been identified as glycogen by enzyme digestion methods (Waters *et al.*, 1975b) and by the spectroscopic properties of the iodine-complex of the extracted polysaccharide (Jirjis and Moore, 1976). In the earliest stages of growth of the dikaryotic mycelium of *C. radiatus* (named as *C. lagopus*) glycogen has been shown to accumulate in bulbous cells of the submerged mycelium (Madelin, 1960); similar accumulations have been detected in *C. cinereus* (Waters *et al.*, 1975a). Madelin (1960) found that the mycelial accumulations were depleted at the time of carpophore formation in *C. radiatus*, and presented convincing evidence for direct transfer of material from the mycelium to the carpophore (Madelin, 1956a). Accumulation of glycogen in carpophore tissue is certainly evident at very early stages of development of the *C. cinereus* carpophore (Matthews and Niederpruem, 1973), and has also been observed in hymenial tissues of late developmental stages in *C. lagopus* and *C. curtus* (Bonner *et al.*, 1957) as well as *C. cinereus* (McLaughlin, 1974).

The periodic acid-Schiff staining procedure readily identifies the endoamylase-sensitive polysaccharide (Waters *et al.*, 1975b) and in this investigation we have applied it to suitable sections of the carpophore to follow the course of glycogen deposition during development in *C. cinereus*. In very young primordium initials deposition of polysaccharide is evident in the stipe base and along the edge of the presumptive pileus (Plate 1, No. 2), As development proceeds deposition in the stipe base is at first heavily accentuated (Plate 1, No. 3); this reaches a maximum in primordia about 3 mm tall (Plate 1, No. 4). At this stage the region in which glycogen has been accumulated seems to be differentiated as a cup-shaped structure in the bulb at the base of the stipe (Plate 2, Nos, 1, 2 ,3). Subsequently glycogen accumulation in the gill tissues becomes emphasized and the relative intensity of staining of stipe base and gill changes between specimens described as Stage 1 primordia (Plate 1, No. 4) and as late Stage 2 primordia (Plate 2, No. 4). It appears that accumulation in the gill coincides with depletion of the stipe base deposit and is probably initiated at about the time that karyogamy starts the meiotic division. The gill accumulations are clearly localized in the subhymenial tissue (Plate 3, No. 1), staining intensity lessens as spore formation is initiated (Plate 3, No. 2), and no polysaccharide deposits can be detected in specimens bearing mature spores (Plate 3, No. 3).

Such cytochemical studies give a strong impression that the glycogen which is initially deposited in the stipe base is subsequently translocated to the subhymenium as the gills develop, and is finally utilized during the phase of spore formation. Blayney and Marchant (1977) claim to have detected the disappearance of glycogen from the elongating stipe by chemical analysis; they compare this with a reduction in the content of insoluble protein and point out that glycogen and protein accumulations have inverse distributions in the stipe. The different distributions of these polymers is readily demonstrable cytochemically. In our material protein was concentrated in the upper regions of the stipe and, particularly, in the presumptive pileus (Plate 3, No. 4); in very sharp contrast with the stipe base localization of glycogen (Plate 1, No. 3). Furthermore, the marked accumulation of protein in gill tissue is

clearly localized in the hymenium at a time when glycogen is concentrated in the subhymenium (Plate 3, No. 5). However, the claim that glycogen is degraded in the stipe to provide material for its elongation (Blayney and Marchant, 1977) is at variance with the statement of Gooday (1975) that this polysaccharide does not act as a food reserve during the rapid phase of stipe elongation. On account of this difference of opinion and because the part played by the carpophore cap is not considered in either of these earlier reports, we have re-examined this point by measuring the quantities of glycogen and protein in the caps and stipes of carpophores of different stages of development (Table 2). The data in Table 2 are quantified in terms of the fresh wt of the sample analysed in order that they should be immediately comparable with data presented by Blayney and Marchant (1977). However, in making developmental comparisons between data expressed in this way it is essential to appreciate that the ratio of dry wt to fresh wt changes significantly as the carpophore develops; data illustrating this point are included in Table 2. Most measurements were done separately on caps and stipes, but protein and dry weight determinations of Stage 1 primordia were done on whole primordia without such dissection. Our data contradict those of Blayney and Marchant (1977) in almost every respect. The protein measurements show no very conspicuous changes in the stipe. Both soluble protein (extracted by grinding in buffer at 0 °C and filtering off the debris) and total protein (estimated after the ethanol-soaked sample had been homogenized and dissolved in strong NaOH at 100 °C) declined in proportion of the fresh wt in parallel with the decline of the dry wt as the tissue developed. Protein estimated in this way was thus maintained at a virtually constant proportion of the stipe dry matter throughout carpophore development. Similar results have been reported by Gooday (1977) who used slightly different methods of analysis, and by Kamada, Miyazaki and Takemaru (1976) who examined the final phase of rapid stipe elongation in *C. cinereus*. We conclude that any changes in the protein content of the stipe resulting from changes in the proteinaceous inclusions identified by electron microscopy (Blayney and Marchant, 1977) must either be so small as not to be detectable by gross chemical analysis or be compensated by reciprocal changes in other proteins. In either case there is no support for the supposition of Blayney and Marchant (1977) that such measurements indicate that protein is degraded to supply the nitrogen for chitin synthesis during stipe elongation. In contrast to the stipe, the total protein content of the cap seems to be maintained at a constant proportion of the fresh wt; so as development proceeds the fraction of cap dry matter represented by protein must be increased quite significantly. This, too, has been observed to be the case in the final phase of development in the Japanese strains of *C. cinereus* (Kamada *et al.*, 1976).

The data recorded for the glycogen contents of stipes in Table 2 are qualitatively and quantitatively more like the results reported by Gooday (1975) than those of Blayney and Marchant (1977). Qualitatively there are profound differences between our results and those of the latter authors. Quantitative comparison cannot be made as data recorded in consecutive tables in Blayney and Marchant (1977) differ by as much as a factor of ten. Thus, we agree with Gooday (1975) that the glycogen reserves of the stipe are massively depleted during the very earliest stages of development. Since in our analyses the stipes were bisected we can make the further point that at all stages up to Stage 5 the stipe base contained more glycogen than did the upper half of the stipe. However, the inclusion of the carpophore cap in our analyses reveals the more important point that as the stipe is depleted of glycogen the cap accumulates it.

Table 2. *Measurements of dry wt and contents of protein and glycogen during carpophore development in* Coprinus cinereus

	Dry wt		Total protein		Soluble protein		Glycogen content		
	Cap	Stipe	Cap	Stipe	Cap	Stipe	Cap	Stipe Top	Stipe Base
Stage 1	107·2±15·7		29·8±7·4		2·5±0·4		2·57±0·69	1·76±1·26	6·27±2·45
Stage 2	113·8±12·5	102·7±5·0	14·8±2·3	16·6±2·5	2·5±0·3	2·4±0·2	2·39±0·25	0·94±0·16	3·10±0·66
Stage 3	95·3±5·1	94·5±7·8	15·1±2·3	16·3±2·6	2·1±0·1	1·7±0·1	5·46±0·83	0·28±0·42	0·49±0·34
Stage 4	78·0±12·2	69·5±12·3	13·5±0·8	13·4±1·9	2·1±0·3	1·4±0·6	4·00±1·94	0·22±0·25	0·43±0·12
Stage 5	74·5±8·8	46·9±9·5	14·6±1·1	9·6±2·6	1·5±0·6	0·9±0·1	0·17±0·10	0·05±0·07	0·04±0·05

All numerical entries are expressed in units of mg per g fresh wt and show the means and standard deviations of from 3 to 11 replicates. Total protein was measured as described by Ewaze *et al.* (1978), soluble protein represents the material which is soluble in ice-cold buffer after grinding and filtration through glass fibre filters (Moore and Ewaze, 1976). Glycogen was extracted, purified and measured using the methods described by Jirjis and Moore (1976). Protein and dry wt measurements of Stage 1 primordia were done without dissection; caps were separated from stipes for all other determinations.

Table 3. *Distributions of fresh wt and glycogen between the parts of the* '*average*' *carpophore of* Coprinus cinereus, *together with the specific activity of NADP-linked glutamate dehydrogenase in the carpophore cap.*

	Developmental stage				
	1	2	3	4	5
	Fresh wt of individual carpophores (mg)				
	70.7 ± 5.8	197.7 ± 56.3	495.2 ± 168.5	423.5 ± 110.5	809.3 ± 136.8
	Percentage distribution of fresh wt within the carpophore				
Cap	44.4	51.0	68.7	71.1	58.6
Stipe top	19.9	15.1	10.7	10.8	15.4
Stipe base	35.7	33.9	20.6	18.1	26.0
	Specific activity of NADP-glutamate dehydrogenase in the cap (units)*				
	15 ± 3	46 ± 9	704 ± 124	598 ± 293	1149 ± 12
	Quantity of glycogen per carpophore (μg)				
	269.1 ± 122.2	531.3 ± 201.3	1856.8 ± 304.0	1361.3 ± 667.5	97.3 ± 64.3
	Percentage distribution of glycogen within the carpophore				
Cap	31.4	50.7	97.3	96.1	85.8
Stipe top	9.0	6.0	0.6	0.7	5.6
Stipe base	59.6	43.3	2.1	3.2	8.6

* 1 unit = 1 nmol substrate used min^{-1} (mg protein)$^{-1}$, the enzyme activity being measured using the amination assay as detailed by Al-Gharawi and Moore (1974).

These results exactly correspond with those obtained by microscopic examination of sections stained by the periodic acid-Schiff procedure, and though neither type of analysis can show definitively that glycogen initially accumulated in the stipe base is subsequently translocated to the cap, any alternative interpretation would need to postulate a most eccentric series of glycogen synthesis-utilization cycles. From Stage 3 onwards more than 90% of the glycogen contained in the carpophore is located in the cap; it declines significantly in quantity between Stages 3 and 4, but the greatest bulk of it is utilized between Stages 4 and 5 (Table 3). This period encompasses the phase of rapid stipe elongation but since elongation is not dependent on the presence of the cap (Gooday, 1974) it is unlikely that significant contribution is made to stipe growth by the glycogen reserves held in the cap. We conclude, therefore, that most of the glycogen contained by the carpophore serves as a reserve material which provides for the later stages of cap development.

The glycogen content of the stipe does decline during stipe elongation between Stages 4 and 5 (Tables 2 and 3) but the small quantity of the material which is involved does not encourage the view that this compound plays a key role in providing for elongation. Thus it seems that neither glycogen nor protein, the two most readily identified reserve materials, can be allocated a centrally important function in stipe elongation. Gooday (1974) showed that elongation can occur in isolated stipes in the absence of added water or nutrients, thus implying that the process can be an autonomous endotrophic one. The scale of the structural changes that take place is so great that if no one substance can be identified as serving as major nutritional reserve material then considerable reallocation of general metabolic resources must occur. Sufficient enzyme activities have already been detected in extracts of stipe tissues to show wide-ranging activity in intermediary metabolism (Moore and Ewaze, 1976),

and the adverse effects of a variety of inhibitors (Gooday, 1974) emphasizes the view that stipe elongation is dependent upon large scale and diverse metabolic activity.

DEVELOPMENT OF THE STIPE

Structural aspects

The importance of very rapid wall synthesis is obvious from the extent of stipe elongation and the time period during which the elongation occurs. During the most rapid phase the stipe may elongate from about 20 to about 100 mm in less than 12 h (Moore and Ewaze, 1976). The kinetics of this elongation have been studied repeatedly since Buller (1924) examined *C. sterquilinus: C. radiatus* has been studied by Borriss (1934, using the name *C. lagopus*), Hafner and Thielke (1970), and Eilers (1974); *C. congregatus* by Bret (1977); and *C. cinereus* by Gooday (1974), Cox and Nieder-pruem (1975, using the name *C. lagopus*) and Kamada and Takemaru (1977a, using the name *C. macrorhizus*). These studies show that the stipe does not elongate equally over its whole length. In all species the upper half, and generally the upper mid-region, is the most active zone of elongation. There do seem to be species differences in respect of the degree of stipe autonomy and the relative parts played by cell division and cell elongation in the overall process. In *C. congregatus* stipe elongation was dependent on both the parental mycelium and the cap during the whole period of development of the carpophore (Bret, 1977). *C. radiatus* seems to occupy an intermediate position; elongation occurs after separation from the parent mycelium, but is dependent on the cap for a growth regulator until the final phase of rapid elongation is reached, so that once the stipe has reached 25% of its final size decapitation does not impair elongation (Borriss, 1934; Eilers, 1974). In contrast, Gooday (1974) found that stipe elongation in *C. cinereus* has no requirement for connection either with the cap or the parental mycelium. Although the tissue which he used were of fairly late stages of development, Cox and Niederpruem (1975) confirmed and extended the observation by showing that primordia above 5 mm in height (which is equivalent to between 5 and 10% of final size) were able to elongate after excision and decapitation.

A contributory factor to this difference in cap dependency could well be a difference in the part played by cell division in stipe elongation. Hafner and Thielke (1970) reported an increase in the number of nuclei in stipe cells prior to its elongation in *C. radiatus*, while Eilers (1974), using the same species, found that the 6- to 8-fold increase in cell length during stipe elongation was accompanied by a doubling of the cell number. Although the DNA content of the stipe of *C. cinereus* has been found to increase abruptly just before the most rapid phase of elongation (Kamada *et al.*, 1976), stipe elongation has been attributed solely to cell elongation (Gooday, 1975; Kamada and Takemaru, 1977a). The increase in size of stipe cells is certainly dramatic. Electron microscopy of Stage 2 primordia reveals that the stipe is composed of overlapping cells of essentially hyphal dimensions with dense cytoplasm, moderately sized vacuoles and regular clamp connections (Plate 4, Nos 1, 2, 3). However, at Stage 4, immediately preceeding the phase of most rapid elongation, stipe cells are already enlarged greatly, being closely packed and highly vacuolated. The cytoplasm is restricted to the peripheral and terminal regions (Plate 5, No. 1) and up to five nuclear profiles can be detected in individual sections (Plate 5, No. 2). Both carpophore stages contain cells with the crystalline inclusions which were identified as protein by Blayney and Marchant (1977) (Plate 5, No. 3).

It is unlikely that the increase in nuclear number evident in developing stipe cells of *C. cinereus* is a preparation for cell division, even though this may be the case in *C. radiatus*. Autoradiographic detection of the incorporation of tritiated *N*-acetyl-glucosamine by elongating stipes of *C. cinereus* revealed a random incorporation along the walls of the cells (Gooday, 1975), no mention being made of the localization in septa which was so clearly evident in a similar study of *Agaricus bisporus* (Craig, Gull and Wood, 1977). Moreover, Lu (1974a) showed that inflated multinucleate cells occurred even in young primordial stipes, the stipe becoming differentiated into a central column of dikaryotic cells surrounded by a cortex of enlarged, multinucleate cells. Thus it appears that the stipe is initially composed of dikaryotic hyphal cells and that these differentiate and inflate centripetally, the increase in cell volume being accompanied by nuclear divisions which presumably maintain the nuclear-cytoplasmic ratio. It is interesting that a developmental mutant of *C. cinereus* in which the stipe fails to elongate nevertheless shows an increase in cell volume during what would be the elongation phase which exactly parallels that which occurs during elongation of the wild-type stipe; the cells of the 'elongationless' mutant increasing in diameter rather than length (Kamada and Takemaru, 1977a). As the stipe does not normally increase in diameter during its final elongation phase this totally abnormal pattern of development strongly suggests that differentiation of normal stipe cells is biphasic. In the initial stage their increase in volume is achieved largely by increase in diameter, and this leads (during intermediate stages) to the recognition of two distinct tissues in the young stipe; the still undifferentiated dikaryotic central region and the differentiated multinucleate cortex. In the second stage (defective in the mutant) resources are channeled so that the cells increase greatly in length without increasing significantly in number.

Metabolic aspects

Elongation of the stipe is clearly dependent on an enormous increase in the volumes of the constituent cells and this is accompanied by very active metabolism of wall polysaccharides which show rapid fluxes during elongation (Kamada and Takemaru, 1977b), the content of chitin increasing particularly during development (Gooday, 1972a, 1975). The importance of rapid wall synthesis is emphasized by the facts that the specific activity of chitin synthase increases during stipe elongation (Gooday, 1973) and that elongation is inhibited by Polyoxin D (Gooday, 1972b), 1975), a nucleoside antibiotic which is known to be a powerful competitive inhibitor of chitin synthase in *C. cinereus* (Gooday, de Rousset-Hall and Hunsley, 1976). However, the wall remains unthickened and most of the cell interior is occupied by vacuoles, implying that the stipe is supported by a hydrostatic skeleton. The ingress of water into the stipe during its development is quite dramatic. In developing from Stage 2 to Stage 5 the stipe of an 'average' carpophore doubles its fresh wt with hardly any change in dry wt (Table 4). For such dung-grown carpophores as were used to obtain the data in Table 4 it is clear that in increasing its length by a factor of five, the average stipe must absorb nearly 200 mg of water. The turgor pressure of stipe cells remains almost constant throughout the period of stipe elongation (Kamada and Takemaru, 1977a) so an appropriate osmotically active solute must be formed and accumulated in the stipe cells in parallel with the absorption of water and synthesis of wall. The identity of this osmoticum has not been established. Although there is

Table 4. *Comparison of fresh wt and dry wt of individual primordia and mature carpophores of* Coprinus cinereus

	Stage 2 primordium ($n = 13$)	Stage 5 carpophore* ($n = 19$)
Cap		
Fresh wt (mg)	135·7 ± 43·7	484·5 ± 236·3
Dry wt (mg)	16·3 ± 5·8	34·1 ± 15·9
Stipe		
Fresh wt (mg)	174·0 ± 81·9	353·5 ± 178·5
Dry wt (mg)	16·0 ± 7·2	16·5 ± 8·6
Length	15·2 ± 1·7	74·7 ± 18·8

Carpophores were grown on horse dung using the standardized conditions described by Moore and Ewaze (1976). Entries are the means and standard deviations of n observations.

* The mature carpophores were harvested either at the time of, or as far as could be judged just prior to, the onset of spore discharge to ensure that the carpophores dealt with had an essentially undisturbed crop of basidiospores.

reason to believe that accumulation of amino-compounds and urea has osmoregulatory significance in the cap of *C. cinereus*, the low and steadily declining levels of amino-nitrogen reported for developing stipes indicates that a different mechanism operates there (Ewaze *et al.*, 1978). In fact, simple carbohydrates appear to be the best candidates as the stipe osmoticum. Rao and Niederpruem (1969) fractionated the alcohol-soluble carbohydrate pools and were able to show a 3-fold increase in the quantity of trehalose as the stipe increased in length from 40 to 90 mm, this sugar finally accounting for almost 18% of the dry wt. These results have been confirmed by Gooday (1977). Trehalose is a non-reducing sugar, and even though glucose levels decline (Rao and Niederpruem, 1969; Gooday, 1977) reducing sugars have been found to remain constant at 5 to 6 mg per g fresh wt as the primordium develops into the mature carpophore (Ewaze *et al.*, 1978). The change in the ratio of fresh wt to dry wt in the stipe as it elongates is such that while reducing sugars will account for about 6% of the dry weight of the Stage 2 primordial stipe, they will account for at least 12% of the dry wt of the mature Stage 5 stipe. Together, therefore, trehalose and alcohol-soluble reducing sugars represent about 30% of the dry weight of the mature stipe. In *Agaricus* mannitol can amount to as much as 50% of the carpophore dry wt (Hammond and Nichols, 1976) but it does not seem to have any role in *C. cinereus*. Total polyols never exceed 6% of the dry wt and decline in quantity as the carpophore develops (Darbyshire, 1974). Thus, although there is good reason to suppose that simple carbohydrates serve an osmoregulatory function in the developing stipe of *C. cinereus*, no one sugar can be identified as being of prime importance; it seems more likely that a diverse metabolism contributes several components to an osmoregulatory cocktail. It must be emphasized that we are referring here to what might be called the adjustable osmoticum. Because of the low dry wt to fresh wt ratio (Table 3) the bulk of the osmotic pressure of the stipe cell must be contributed by compounds of low mol. wt and it is probable that inorganic ions make a very important contribution, as they do in other plants (Cram, 1976). Although the sugar content accounts for only a small part of the overall osmolarity of the cell, it represents a portion which is readily adjusted by metabolism of cell constituents; a property which is not possessed by the inorganic components of the cell.

ANALOGIES BETWEEN CAP AND STIPE

The expansion and elongation of its constituent cells is an essential aspect of stipe elongation, but cell inflation on a considerable scale is no less important in the development of the carpophore cap. We will show below how the inflation of its component cells can be related to both metabolism and morphogenesis in the cap. At this point we wish to emphasize that although cap and stipe development are each centred on cell inflation, the contrasts in metabolism between the two tissues are so great as to indicate that the common result is achieved in very different ways. The reported differences between cap and stipe include: different patterns of metabolite accumulation, both carbohydrates (Rao and Niederpruem, 1969; Gooday, 1977) and amino acids (Ewaze et al., 1978); different patterns of metabolism of isotopically labelled substrates (Ewaze et al., 1978); and profound differences in the activities of particular enzymes, especially polyol dehydrogenases (Rao and Niederpruem, 1969), chitin synthase (Gooday, 1973), NADP-linked glutamate dehydrogenase (Stewart and Moore, 1974), alkaline phosphatase (North, 1977b), enzymes involved in ornithine metabolism (Ewaze et al., 1978), and glucanase and phenylalanine ammonia-lyase (Ishikawa and Serizawa, 1978). All of these data point to fundamental differences in the ways in which cell enlargement is achieved in the two tissues. This suggestion is reinforced by the work done on the induction of developmental variants which resulted in the isolation of, among others, mutants unable to elongate the stipe but with normal cap expansion, or unable to expand the cap but with normal stipe elongation (Takemaru and Kamada, 1970, 1972). Evidently, although the control systems governing cell morphology are able to make similar responses to stimuli emerging from quite different metabolisms, the integration of regulation is such that the pathways of cap and stipe differentiation can be totally separated.

DEVELOPMENT OF THE CAP

Structural aspects

The prime functions of the carpophore cap are spore formation and disseminatory spore discharge. Although considerable attention has been given to the mechanics of meiosis and spore formation, only very recently has much interest been shown in the mechanisms which, by controlling morphogenesis of the cap, contribute to spore dissemination.

The structure and function of the gill tissue in relation to basidiospore development have been studied in detail by McLaughlin (1974, 1977). The results suggest that carbohydrates accumulate in Golgi vesicles in basidia, cystidia and basidiospores and that the accumulations are related to wall synthesis. It was concluded that Golgi vesicles migrated from the Golgi cisternae to the cell-wall and that during migration the contents of the vesicles underwent progressive change in preparation for their contribution to wall growth. At all times, however, the contents of the vesicles were demonstrably different from glycogen; and whereas glycogen and Golgi vesicles co-existed in cystidia, the vesicles were absent from paraphyses and subhymenial cells although these cells contained large deposits of glycogen (McLaughlin, 1974). A subsequent study of the early ontogeny of basidiospores concentrated on the control of spore shape and the parts played by the spore wall and cytoplasmic components in basidiospore development (McLaughlin, 1977).

The process of meiosis has been studied in detail by Lu and his collaborators (Lu, 1967; Raju and Lu, 1970), and a mutant with a temperature-sensitive meiotic defect has been described (Tani, Kuroiwa and Takemaru, 1977). Particular stress has been placed on the morphology and division cycle of the spindle pole body (Raju and Lu, 1973a; Lu, 1978); on chromosome pairing and synaptinemal complex formation (Lu, 1966a; Lu and Raju, 1970); and on the effects of light and temperature on meiosis and genetic recombination. Illumination is required for carpophore formation (Morimoto and Oda, 1973; Lu, 1974a) and although continuous light is not detrimental at 25 °C, at 35 °C it inhibits meiosis and the premeiotic DNA replication cycle (Lu, 1972, 1974b; Lu and Jeng, 1975). The effects of temperature shocks are manifested in the recombination frequencies between chromosomal genes; both high and low temperature treatments increase the frequency of recombinant progeny (Lu, 1969). The temperature-sensitive period is localized to the pachytene stage of meiosis, it starts with the establishment of synaptinemal complexes and ends with their disappearance (Lu, 1970). The increase in recombination frequency is related to a lengthening of the pachytene stage by the temperature shock (Lu, 1974c) and is apparently caused by an accumulation of damage ('nicks') in the DNA as a result of the prevention of DNA repair-synthesis during the temperature arrest (Lu and Chiu, 1976). These investigations have been complemented by a study of spore structure and germination, in which the Golgi apparatus was again implicated as a source of vesicle-bound materials (Heintz and Niederpruem, 1971). Despite overlaps between the different studies, differences of opinion are limited to the interpretation of Golgi body structure (Lu, 1966b; Heintz and Niederpruem, 1971; McLaughlin, 1974) and possibly the time of migration of nuclei from the basidium to the basidiospores (Raju and Lu, 1970; McLaughlin, 1977), although in this latter case neither report includes illustrative data to support the expressed opinions.

Apart from the early classic researches (reviewed by Reijnders, 1963) there have been few comparative descriptions of the cellular details of cap morphogenesis in *Coprinus*, and certainly none on *C. cinereus sensu stricto*. The most obvious featuie which changes during cap development is the degree of inflation of cells in the hymenium. This is clearly evident from even a cursory comparison of hymenium structure of a Stage 2 primordium with that of a Stage 4 immature fruit (Plate 6, Nos. 1, 2). In the younger (primordium) specimen the hymenium consists of a loosely organized layer of hyphal tips of broadly similar size, though occasional unusually large cells may be identified as presumptive cystidia (Plate 6, No. 1). Cells of the hymenium layer-proper originate as branches from the hyphal tissue of the subhymenium and remain in contact with their subhymenial neighbours through dolipore septa. The presence of clamp connections and paired nuclear profiles shows that dikaryon growth is maintained during formation of the hymenium. The subhymenium is a much more open tissue and the cells of which it is composed contain large accumulations of glycogen granules. Generally throughout the primordial gill only a small proportion of the cell volume is occupied by vacuoles. The more mature specimen contrasts starkly with this description. Even though the developmental process is not completed, by Stage 4 the hymenium is already differentiated into two very distinct cell types: the basidium and the paraphysis. The basidium is only slightly enlarged in comparison with primordial hymenium cells, and contains fairly dense cytoplasm with few, generally small, vacuoles; in the tissue illustrated here spore formation is well advanced (Plate 6, No. 2). The paraphyses are enormously inflated in comparison with similarly positioned

primordial cells, and the bulk of the cell volume is occupied by a single vacuole; the cytoplasm being restricted to the periphery. Inflation of the paraphyses produces a hymenium in the form of a closely appressed and apparently interlocked paraphyseal pavement containing interspersed basidia. This is quite distinct from the uncrowded structure seen in the primordium and also differs greatly from the structure of the mature hymenium of *Agaricus bisporus* (Craig *et al.*, 1977). A particular feature of the cell inflation which generates this pavement formation is that it is entirely restricted to the hymenium. The subhymenium remains essentially hyphal (Plate 7, Nos. 1, 2) and despite their considerable enlargement, continuity with the hyphae of the subhymenium can be demonstrated for cystidia, paraphyses and basidia (Plate 7, No. 3; Plate 8, Nos. 1, 2, 3).

It is obvious that inflation plays a central role in differentiation of the cells and tissue which make up the hymenium, but it seems likely that the significance extends beyond this level of structure and that cell inflation has a profound bearing on the morphogenesis of the cap as a whole. Except for the descriptions given by Buller (1924, 1931), discussion of cap morphogenesis in *Coprinus* is generally restricted to the role of autodigestion in the removal of spent gill tissue which might otherwise interfere with spore-fall from the carpophore. However, during this autodigestive period the cap passes through a change in shape of such magnitude that the initially vertical orientation of the gills is transformed to a horizontal orientation (Buller, 1924, 1931). In *C. cinereus* this process is completed in about 6 h and is achieved by a gradual revolution of the edge of the cap in concert with radial splitting of pileal and gill tissues so as to allow the cap to open like an umbrella. In describing and accounting for cap expansion in different species of *Coprinus* Buller (1931) stressed the part played by the pileal flesh. He distinguished clearly the smaller species (*C. curtus*, pp. 27 to 28; *C. plicatilis*, pp. 40 to 42) in which the accent is on differential growth in the central disc of pileal flesh which ensheathes the stipe, from the larger species (*C. comatus*, p. 73) in which differential growth of pileal flesh bounding the gills is supposed to account for the changing shape of the gills. Any role played by the paraphyses is minimized in Buller's accounts; they are described as 'the elastic elements of the hymenium' which 'perform an important mechanical function as the pileus opens' though it is clear that this author considers their role in pileus structure to be a secondary one (Buller, 1932, p. 28), their major function being to separate adjacent basidia. In contrast, it has recently been suggested that in *C. cinereus* the paraphyses make a major contribution to cap expansion (Ewaze *et al.*, 1978) and our observations support this interpretation. Measurement of the profile contour length of the cap at successive stages during expansion shows that it remains constant in the final autodigestive phase (Fig. 1); and even though spent gill tissue is removed by autodigestion a considerable portion remains intact until very late in the expansion process. Yet as the paraphyses differentiate their inflation must increase the area of the gill lamella. In the early stages this appears to be accompanied by extension of the pileal flesh (Fig. 1) but in the later stages the expanding gill is bounded by a flexible but evidently inextensible band of pileal flesh, a combination which will inevitably lead to an outward curvature even without the active participation of the pileal flesh (Fig. 2). The pileal flesh will become an active participant after the autodigestive process has removed the bulk of the gill lamella, but initially paraphyseal inflation provides the major expansionary force.
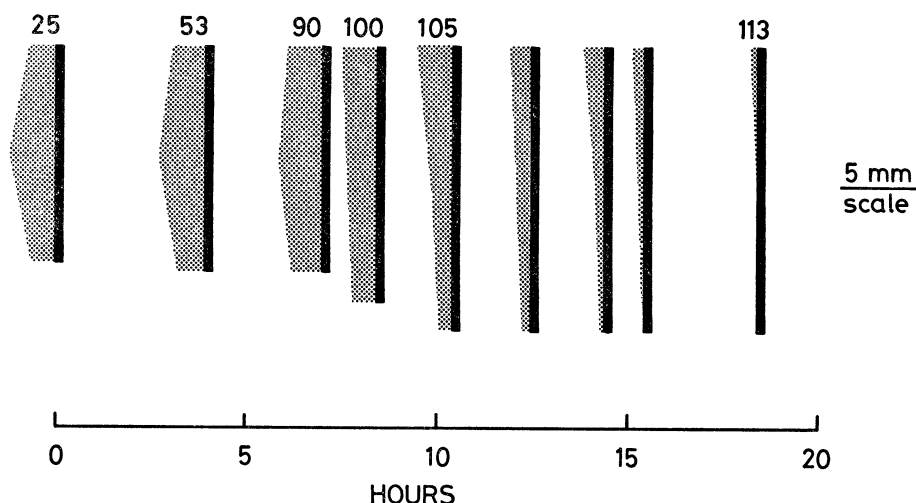
Fig. 1. Gill autolysis in *Coprinus cinereus* and its relation to the overall length of the boundary of pileal flesh. At regular intervals in the final stages of carpophore development a small segment was surgically removed from an otherwise undisturbed carpophore and preserved in formalin. Subsequently the outer contour length (= length of pileal flesh) was measured and the depth of gill tissue measured at the top, centre and bottom of a suitable primary gill in each segment. In the diagrams the shape of the carpophore is ignored (for which see Fig. 2); the vertical bars represent the contour length and the shaded area represents the gill lamella. The numbers above the diagrams record the length (in mm) of the stipe at the time that the sample was taken. In this specimen, spore discharge began at about the 7th hour. These observations show that the pileal flesh remains intact and extended throughout the entire period of gill autolysis, and that for the final period of cap development the length of the pileal flesh is unchanged.

Metabolic aspects

Expansion in the cap is accompanied by a considerable increase in the amount of water contained by the cap (Table 4), and a similarly large increase in the degree of vacuolization of paraphyses (Plate 6, No. 2). These features may indicate that cell inflation is driven osmotically, and it has been suggested that specific amplification of urea cycle activity during the later stages of cap development provides the osmotically active solutes required for this process (Ewaze *et al.*, 1978). Operation of the urea cycle, usually associated with disposal of excess nitrogen, would correlate well with the upsurge in activity of enzymes involved in autodigestion. In *C. lagopus* and *C. comatus* increases in the activities of glucosidase, protease and chitinase have been noted during cap expansion (Iten, 1970; Iten and Matile, 1970; Bush, 1974) and glucanases have been identified in autolysates of *C. cinereus* (Schaefer, Hildreth and Drake, 1977); so the degradation of the substance of the spent gill tissue may well provide the substrates which intermediary metabolism could utilise to support the expansion of the still intact regions. In the earlier stages, prior to the onset of large scale autodigestion, there seems to be some correlation between the activities of enzymes concerned in nitrogen metabolism and the mobilization of glycogen reserves. This is evidenced by the association between declining glycogen content and increasing specific activities of NADP-linked glutamate dehydrogenase in cap tissues as the carpophore develops (Table 3). That this association is not coincidental is suggested by the regulatory properties of the enzymes concerned. Enzymes which, like
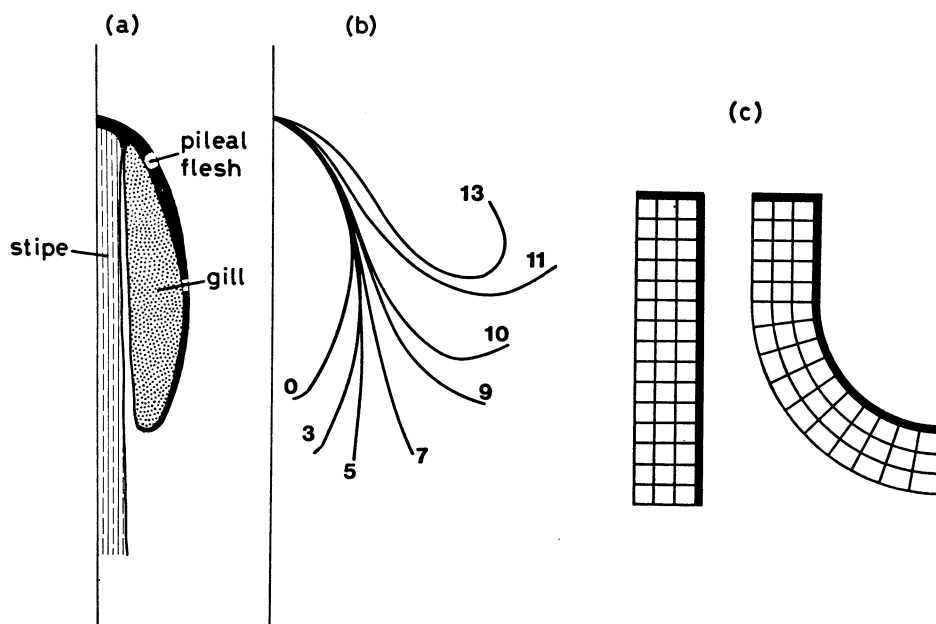
Fig. 2. Carpophore cap development in *Coprinus cinereus*. Diagram (a) is a tracing from a photograph of a vertically sectioned carpophore; identification of the main tissues emphasizes how small is the quantity of pileal flesh in relation to the amount of gill tissue. Diagram (b) records the way in which the profile of the cap changes during its final phase of expansion, the numbers showing the hours elapsed since observation was started at time zero; consecutive photographs were taken of the same carpophore and tracings made of the upper edge of the cap. During the course of the sequence shown the stipe extended from 25 to 95 mm, spore discharge commenced about 5 h from the start of observation. Diagram (c) attempts to show how cell expansion (here depicted only as an increase in length) can account for the recurving of the gill plate if it is assumed that the pileal flesh acts as a flexible but inextensible boundary.

NADP-linked glutamate dehydrogenase, specifically increase in activity in the cap can be shown to be subject to catabolite derepression in vegetative cultures grown in synthetic media, whereas the one enzyme so far known to be specifically absent from the cap (urease) shows catabolite repression (Fawole and Casselton, 1972; Stewart and Moore, 1974; Al-Gharawi and Moore, 1977; Ewaze *et al.*, 1978). Furthermore, there are indications that the glycogen synthesis-utilization cycle evident in the carpophore is encountered in vegetative cultures (Jirjis and Moore, 1976) where it is also associated with the derepression of NADP-linked glutamate dehydrogenase (Al-Gharawi and Moore, 1977). Thus it seems quite clear that these aspects of cap metabolism do not represent a novelty in terms of the reaction sequences involved; much of what occurs in the cap can be shown to occur in mycelia. However, the cap does present a very special regulatory picture, for these metabolic changes which form a part of the normal development of the carpophore can only be induced in vegetative cultures by exposing them to particular (and in some cases most peculiar) synthetic media. What is specific to the cap, then, is that at the outset of its development there is set in train a fundamental change in the emphasis of the metabolism of its constituent cells of such magnitude that the cap cells become metabolically quite distinct from those of either the stipe or the parent mycelium. Since only growth on unusual synthetic media is so far known

to mimic this change in mycelia, it is pertinent to ask how this regulation might be achieved during the normal growth of the carpophore.

PHOTOCONTROL OF CARPOPHORE DEVELOPMENT

It is most probable that in the normal environment exposure to light is responsible for initiating and programming carpophore morphogenesis. In common with *C. congregatus* (Manachere, 1971) and *C. domesticus* (Chapman and Fergus, 1973) light stimuli are required at two stages during carpophore production in *C. cinereus*; the initiation of primordium formation has a requirement for brief exposure to low intensity light, while development of the carpophore, particularly differentiation of the cap, is dependent on further periods of illumination (Morimoto and Oda, 1973; Lu, 1974a). For a number of *Coprinus* species it is known that at both stages the most effective light is in the blue to near-ultraviolet region of the spectrum (Madelin, 1956b; Tsusue, 1969; Lu, 1974a) but detailed action spectra have not been produced. However, it is interesting to note that in the polypore *Favolus arcularius* the two photo-sensitive stages exhibit different action spectra (Kitamoto, Suzuki and Furukawa, 1972, Kitamoto, Horikoshi and Suzuki, 1974).

It is essential to emphasize that the requirement for the first light stimulus can exhibit a very low threshold and has not been recorded by all authors. Lu (1974a) reported that as little as 30 s exposure to blue light with an intensity of 38 ft-c (about 400 lx) was sufficient to induce primordium formation. He recounts that culture dishes not wrapped in aluminium foil received sufficient illumination by leakage through a small hole in the incubator used for supposedly 'dark' incubations. Morimoto and Oda (1973) recognized the need for this first light exposure with the Japanese strains of *C. cinereus* and in the closest approximation to Lu's experiments (4 days prior incubation in the dark as compared with the 7 days dark incubation period employed by Lu, 1974a) they reported a minimum requirement of 6 h exposure to white light of 500 lx for formation of primordia. An earlier study with the same dikaryon had indicated that primordia were formed during incubation in continuous darkness (Tsusue, 1969); this observation was confirmed by Morimoto and Oda (1973) when they grew their cultures on the potato-sucrose agar used by Tsusue (1969). For the bulk of their experiments Morimoto and Oda (1973) used a medium containing glucose (2%), peptone (0·2%) and yeast extract (0·2%). Lu (1974a) also used a glucose-based medium (glucose 0·4%, malt extract 1%, and yeast extract 0·4%). Both of these media are very different from the potato extract +2% sucrose medium (preparation detailed in Takemaru and Kamada, 1972) used by Tsusue (1969), and they support much more vigorous mycelial growth than does the potato-sucrose medium. Indeed, none of the *C. cinereus* strains available to us make good growth on any medium in which sucrose is the sole or the major source of carbon. These observations imply that there may be an interaction between the photosensitive apparatus and the nutritional status of the mycelium which is open to experimental manipulation; specifically that the requirement for the initial light exposure is reduced in threshold as the mycelium approaches conditions of starvation. This seems to be an important observation which is worthy of much more attention than it has received.

The statement that primordium formation requires illumination may consequently need to be qualified by recognition that photosensitivity depends on nutrition. Nevertheless, the primordia formed do not mature unless further exposed to illumination.

The pattern of illumination required for normal maturation is complex. Five phases have been recognized (Kamada, Kurita and Takemaru, 1978) and there are requirements for dark periods and an interplay with temperature in a manner similar to that described for *C. congregatus* (Lu, 1972, 1974b; Morimoto and Oda, 1974; Durand and Robert, 1977).

If the primordia are kept in darkness they do not mature. Instead the base of the stipe elongates to produce what might best be called aetiolated fruits but have been variously termed 'dark stipes' (Tsusue, 1969), 'oversized stipes' (Lu, 1974a) and 'long slender stalks' (Morimoto and Oda, 1973). Buller (1931, pp. 112 to 116) used the term 'pseudorhiza' and showed that it is the solid stipe base which is elongated in the dark. He argued that 'light inhibits the elongation of the stipe-base and so permits the growth-energy of the fruit-body to be concentrated earlier than would otherwise be the case upon the enlargement of the pileus and the elongation of the stipe-shaft'. Lu (1974a) has also suggested that the light signals control the disposition of resources, and in view of the changing distribution of glycogen in the developing primordium discussed above, it seems likely that the utilization of glycogen by the cap and its translocation from the stipe base form at least a part of the developmental pattern activated by the light signal which triggers maturation.

At the biochemical level, analogy with the metabolism of glycogen in animals (Soderling and Park, 1974; Roach and Larner, 1976) would prompt the suspicion that cyclic-AMP may be involved in the polysaccharide transformation discussed earlier (p. 701). This compound has been closely implicated in carpophore formation in *C. cinereus* and, indeed, a very close parallel between glycogen metabolism in mammals and that in *C. cinereus* has emerged from the researches of I. Uno and T. Ishikawa. Although much of the work has emphasised the induction of fruiting in monokaryotic mycelia (Uno and Ishikawa, 1971) sufficient comparisons have been made with wild-type dikaryons to reveal the involvement of cyclic-AMP in carpophore formation and maturation in this species (Uno and Ishikawa, 1973a, b; Darbyshire, 1974). A relation between cyclic-AMP levels and the phenomenon of catabolite repression/de-repression has also been demonstrated (Uno and Ishikawa, 1974). Significantly, cyclic-AMP has been shown to activate glycogen phosphorylase and inhibit glycogen synthetase in *C. cinereus* (Uno and Ishikawa, 1976, 1978); while the activities of adenylate cyclase and phosphodiesterase were shown to increase in dikaryotic mycelia exposed to illumination (Uno, Yamaguchi and Ishikawa, 1974). Preliminary attempts to partition the cyclic-AMP content of the carpophore between its component parts indicate that in the primordium all the cyclic-AMP appears to be located in the pileus whereas just prior to the onset of autolysis the bulk of it is found in the upper half of the stipe (Darbyshire, 1974).

In other fungi levels of cyclic-AMP and activities of dehydrogenase enzymes have been found to respond very rapidly to changes in illumination (Chebotarev and Zemlyanukhin, 1973; Cohen, 1974; Daniel, 1977) and coenzyme levels and the the pathway of carbohydrate metabolism can also be drastically affected by light (Graafmans, 1977; Kritsky, 1977). There is little direct evidence in *Coprinus* for any causal relation between light and specific metabolic changes, although a recent report has indicated that development of the activity of chitinase and of NADP-linked glutamate dehydrogenase in the pileus is dependent upon the light signal that triggers maturation of the primordium in *C. cinereus* (Ishikawa and Serizawa, 1978). Taken together with the work already discussed this shows that it is quite reasonable to

believe that the pattern of illumination received by the dikaryon and primordium may in large measure control the metabolic changes so far known to characterise carpophore development.

CONCLUSIONS

Considering the varied data presently available we suggest that the following description outlines the major events in the growth and differentiation of a dikaryotic mycelium of *C. cinereus*. During growth of the vegetative hyphae a large proportion of the carbohydrate absorbed from the medium is stored as glycogen in swollen cells in the submerged parts of the mycelium. While this is proceeding, in the aerial mycelium neighbouring hyphae interact to form hyphal lattices which act as centres of rapid but self-restricting growth and branching so that hyphal aggregates or mycelial tufts about 200 μm in diameter are produced. In axenic culture these aggregates are formed, often in great number, over the whole surface of the colony. As supplies of carbohydrate nutrients in the medium approach exhaustion repression of the morphogenesis of these hyphal aggregates is lifted. In the absence of illumination all of them will develop into sclerotia by the formation of an external rind layer around the central medulla in which previously accumulated glycogen is metabolized into an unusual secondary cell wall material which is thought to serve a nutrient-storage function. Elevated temperatures (i.e. 35 to 38 °C) are not detrimental to this pathway of development but high concentrations of ammonium salts very effectively prevent the final stages of sclerotium maturation. Illumination with low intensity blue light does not prevent sclerotium formation but in a scattered few of the hyphal aggregates the restriction on their further growth is removed, they increase in size and become polarized internally so that a presumptive pileus is very soon recognisable in the upper part of the cell mass. Translocation of glycogen is also polarized; it is mobilized in other parts of the colony for transport to the developing carpophores, and within the carpophore glycogen accumulates first at the base of the presumptive stipe. Differentiation proceeds to produce a primordium with a clearly defined cap and stipe, but the great bulk of the glycogen reserves remain in the stipe base. If no further illumination is received subsequent differentiation is concentrated on formation of the pseudorhizal stipe base which is negatively geotropic and can extend for many centimetres to drive the primordium to the surface of the substratum. Receipt of a second, more extended, illumination inhibits further growth of the stipe base and promotes translocation of glycogen reserves to the cap and activates the utilization of those reserves. Very early in development the cap becomes the major repository of glycogen in the carpophore, and growth of the stipe must depend on sweeping re-allocation of cellular components. The glycogen is utilized in the cap and the consequent great increases in concentration of glycolytic intermediates creates the conditions whereby many metabolic systems can be regulated by catabolite concentration. At least some of the metabolic systems characterizing carpophore development contribute to osmoregulation. This is true for both cap and stipe, but in the former nitrogenous metabolites predominate, while in the latter simple sugars are formed as osmoregulatory materials. In both tissues accumulation of the osmotica is matched by osmotic inflow of water so that considerable cell inflation provides for expansion and hydrostatic support. In the stipe the cells mainly increase in length to provide for the elongation of that organ. In the cap the cells of the gill hymenium increase in girth. This in turn increases the area of the gill lamella, but as the outer

boundary is constrained by an inextensible border of pileal flesh an outward curvature of the gill inevitably results; the cap as a whole becoming first campanulate and finally revolute.

It is this emerging connection between cellular metabolism and tissue morphogenesis which makes work on *C. cinereus* so attractive. The carpophore is a complex organ structured from tissues with particular functions, yet the whole derives from a mycelium of undifferentiated hyphal cells. Some of the metabolic changes which can be observed to occur endogenously in the carpophore can be reproduced in experimental mycelial cultures. Thus the events and signals which in nature are integrated into the developmental sequence are open to investigation, making this an ideal system for the study of eukaryote gene control and cell biology.

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EXPLANATION OF PLATES

Plates 1 to 8 illustrate carpophore structure in specimens categorized into developmental stages according to the descriptions summarized in Table 1. Tissue preparation: small carpophore primordia were fixed whole, when the carpophore was too large for this to be done segments were removed from part of the cap close to its free edge and from the immediately adjacent portion of the stipe.

For light microscopy (Plates 1 to 3) material was fixed in a solution of 3 % glutaraldehyde in 70 mM phosphate buffer (pH 6·9) for 1 h. Wax-embedded sections were cut 10 μm thick and were stained for glycogen with the periodic acid-Schiff reaction (PAS) as described by Waters *et al.* (1975a), or for protein with aniline blue-black (ABB).

For transmission electron microscopy (Plates 4 to 6) specimens were fixed and embedded, and sections stained as described by Waters *et al.* (1975a). Sections were examined in an AEI EM6B or EM802 microscope operated at 60 kV and images recorded on Ilford N50 plates.

For scanning electron microscopy (Plates 7 and 8) specimens were fixed overnight in a solution of 5·75 % glutaraldehyde in 70 mM phosphate buffer (pH 6·9) at room temperature. They were dehydrated through an ethanol series and, using amyl acetate as an intermediary, were dried to air through liquid CO_2 using a critical point drying apparatus. Dried specimens were coated with gold in a sputter coater and examined in a Cambridge 150 microscope operated at 10 kV.

PLATE 1

Median vertical sections through developing carpophores stained with PAS reaction. Copied from Ektachrome transparencies.

No. 1. Carpophore initial, less than 0·2 mm tall, showing hyphal aggregation. × 67. The specimen is borne on a segment of mycelium.

No. 2. Carpophore initial, 0·8 mm tall, showing differentiation into stipe (st) and pileus (pi). The polysaccharide staining is most evident in the stipe base and along the edge of the presumptive pileus. × 67. The specimen is borne on a segment of mycelium.

No. 3. Carpophore initial, 1·2 mm tall. The stipe cells show an acropetal increase in size. The dense deposition of polysaccharide at the stipe base and edges of the pileus is now accentuated. × 80.

No. 4. Carpophore primordium (early Stage 1), 3 mm tall. The cells of the upper region of the stipe are elongated and have little polysaccharide; those at the stipe base are more isodiametric and have a heavy deposit of polysaccharide. × 70.

PLATE 2

Vertical sections of developing carpophore primordia stained with the PAS reaction. Copied from Ektachrome transparencies.

Nos 1 to 3. Serial sections of a 3 mm tall carpophore primordium illustrating the cup-shaped arrangement of the polysaccharide-rich cells in the base of the stipe. The median section in No. 2 shows a distinct group of polysaccharide-free cells (pfc) surrounded by polysaccharide-rich cells (prc). The non-median sections on either side (Nos. 1 and 3) pass through polysaccharide-rich cells only. × 50.

No. 4. Median section of a carpophore primordium (Stage 2) which was 15 mm tall. The illustration is a montage of 31 separate micrographs. The response to the PAS reaction is intense staining in the gills which at this Stage are still free of endogenous pigmentation. This shows that polysaccharide deposits are found mainly in the developing gill tissues rather than in the stipe base. Contrast with Plate 1, No. 4. × 13.

PLATE 3

Copied from Ektachrome transparencies. Nos. 1 to 3 are LS of gill lamellae stained with the PAS reaction and all × 175.

No. 1. Stage 2. Note the accumulation of polysaccharide in the subhymenium (shy).

No. 2. Stage 3. Staining is now concentrated towards the outside of the lamellae.

No. 3. Stage 5. The spores are pigmented. The vacuolated cells of the lamellae show no polysaccharide deposits.

No. 4. Carpophore initial, 1·2 mm tall. Median vertical section stained with ABB to localize protein. Note the accumulation of protein in the upper regions of the stipe and in the edges of the pileus. This is a serial section with that illustrated in Plate 1, No. 3; contrast the disposition of protein with the localization of glycogen. × 80.

No. 5. LS of gill lamellae of a Stage 3 carpophore stained in ABB. protein is localized in the hymenium (hy). This is a serial section with that illustrated in No. 2; contrast the relative distributions of glycogen (No. 2) and protein (No. 5) in the gills of the same immature carpophore. × 180.

PLATE 4

Nos 1 and 2. LS stipe of a Stage 2 primordium. The elongated cells are overlapping and possess clamp connections (cx), moderate-sized vacuoles (va), dense cytoplasm with glycogen rosettes (gr) and dolipore septa (ds). No. 1, × 3250; No. 2, × 9430.

No. 3. TS stipe cell of a Stage 2 primordium. Note the laminate cell wall (cw) (arrowed), crenulated plasma membrane and dense cytoplasm with endoplasmic reticulum, glycogen rosettes and crystalline inclusions (cr). The parenthesome (par) of the dolipore septum is in contact with the endoplasmic reticulum (arrowed). × 15850.

PLATE 5

All illustrations show longitudinal sections of the stipe of a Stage 4 immature carpophore.

No. 1. The cells are highly vacuolated with cytoplasm restricted to the peripheral and terminal regions of the cell. Vesiculate lomasomes (vlo) are present. × 2920.

No. 2. Terminal portions of two cells, one of which contains at least five nuclei (n). Also present are ring-profiled mitochondria (m), vesiculate lomasomes, and membrane complexes (arrowed). × 6100.

No. 3. Cells with highly vesiculate cytoplasm and prominent crystalline inclusions. × 6210.

PLATE 6

No. 1. TS of the pileus of a Stage 2 primordium. The gill hymenium consists of a loose layer of cells of similar size except for the enlarged presumptive cystidia (cy) connected to a swollen cell of the more loosely organized subhymenium. The hymenial cells are largely unvacuolated and connected to the subhymenial cells by dolipore septa. Clamp connections, paired nuclear profiles and glycogen rosettes are also evident. × 3840.

No. 2. TS of the pileus of a Stage 4 immature carpophore. The hymenium is differentiated into closely appressed basidia (ba) and paraphyses (pry). The basidia have dense cytoplasm with few vacuoles; spore (sp) formation is well advanced. The inflated paraphyses are highly vacuolated with peripheral cytoplasm only. Note the membrane-like sheath covering both basidia and paraphyses (arrowed). × 3580.

Plate 7

Gill lamellae of Stage 5 carpophores; scanning electron micrographs of segments cut from the dried cap with commercial razor blades.

No. 1. Portions of adjacent lamellae which have broken to expose opposing hymenial layers together with spores and connecting cystidia. One of the lamellae has fractured so as to strip the hymenium from the gill and expose the subhymenium in surface view; this reveals the 'mycelial' organization of the subhymenial tissue. × 125.

No. 2. Enlarged portion of the subhymenial layer exposed to surface view. This layer is composed of highly branched and closely interconnected hyphae. × 2500.

No. 3. View of the lamella face as it is exposed by a razor cut transverse to the long axis of the carpophore cap. Cell enlargement is restricted to the hymenium. Note the continuity of basidia and paraphyses with the hyphal elements of the subhymenium. × 1150.

Plate 8

Scanning electron micrographs of the gill lamellae of Stage 5 carpophores. All are views of the lamella exposed by a razor cut transverse to the long axis of the cap. All are intended to illustrate the continuity between the differentiated and highly inflated cells of the hymenium and the hypha-like components of the subhymenium.

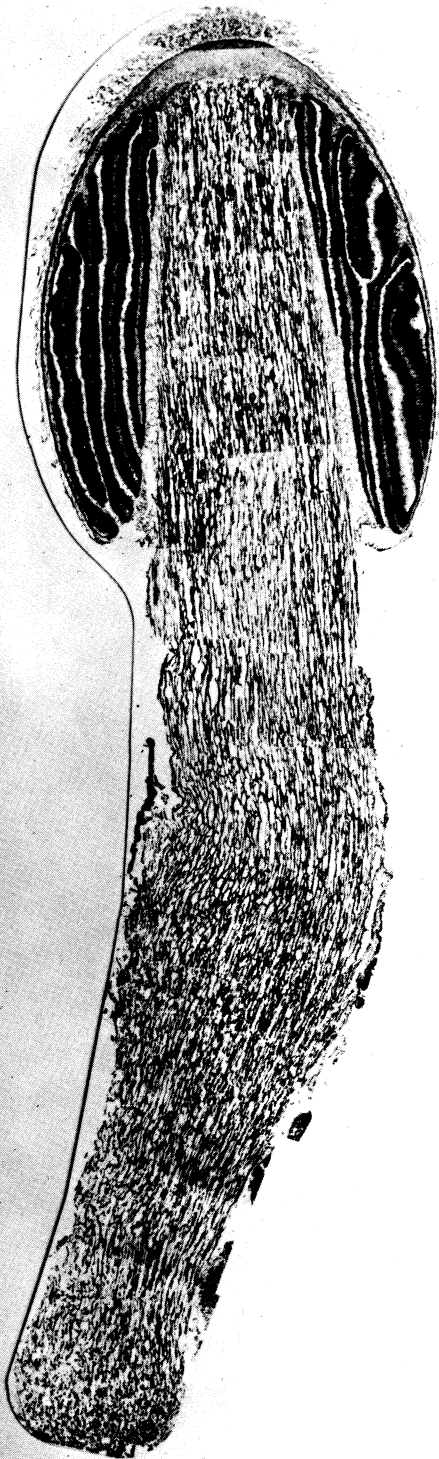No. 1. Enlarged view of the base of one of the basidia shown in Plate 7, No. 3. × 7800.

No. 2. View of a paraphysis. × 4500.

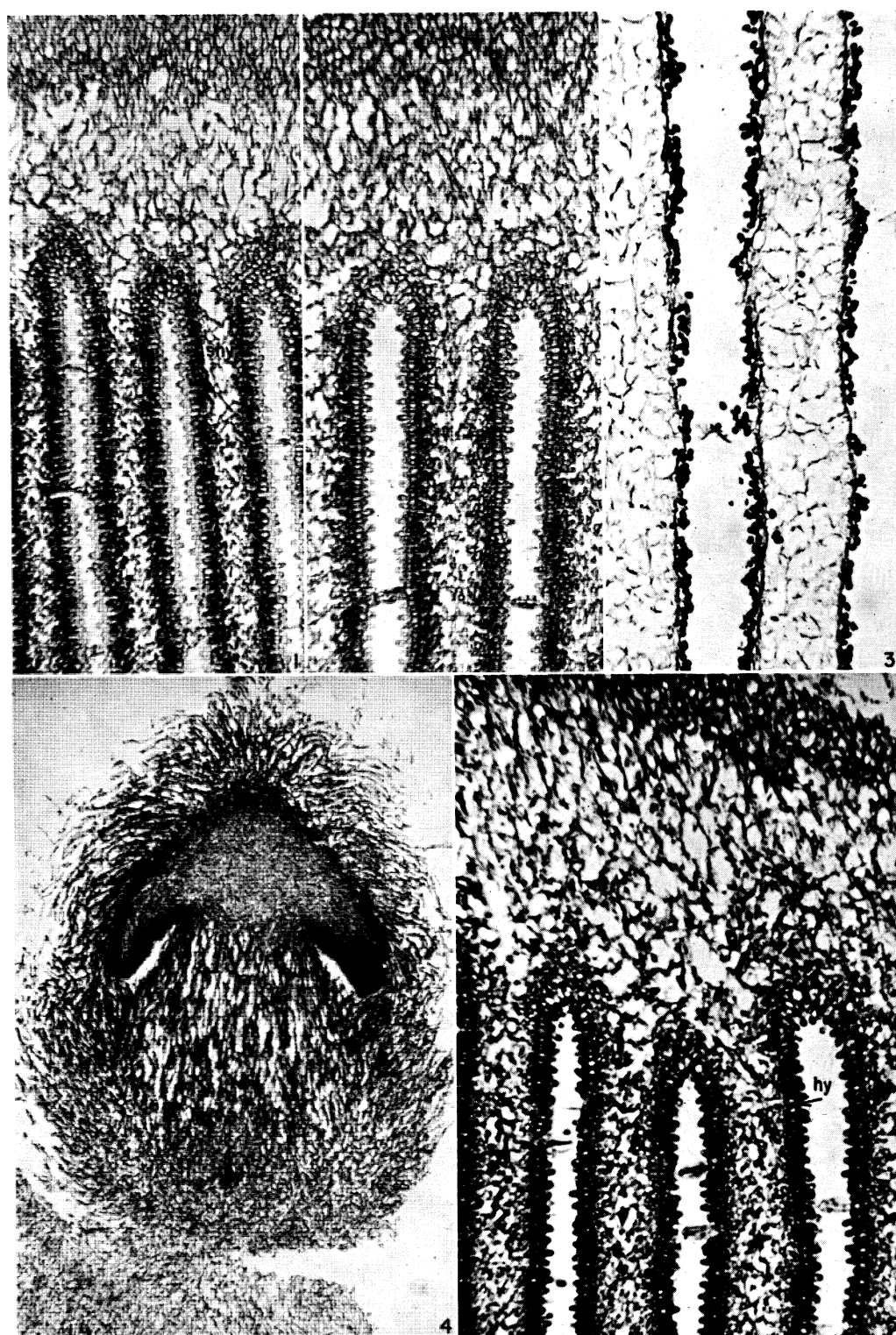No. 3. View of a cystidium. × 2000.

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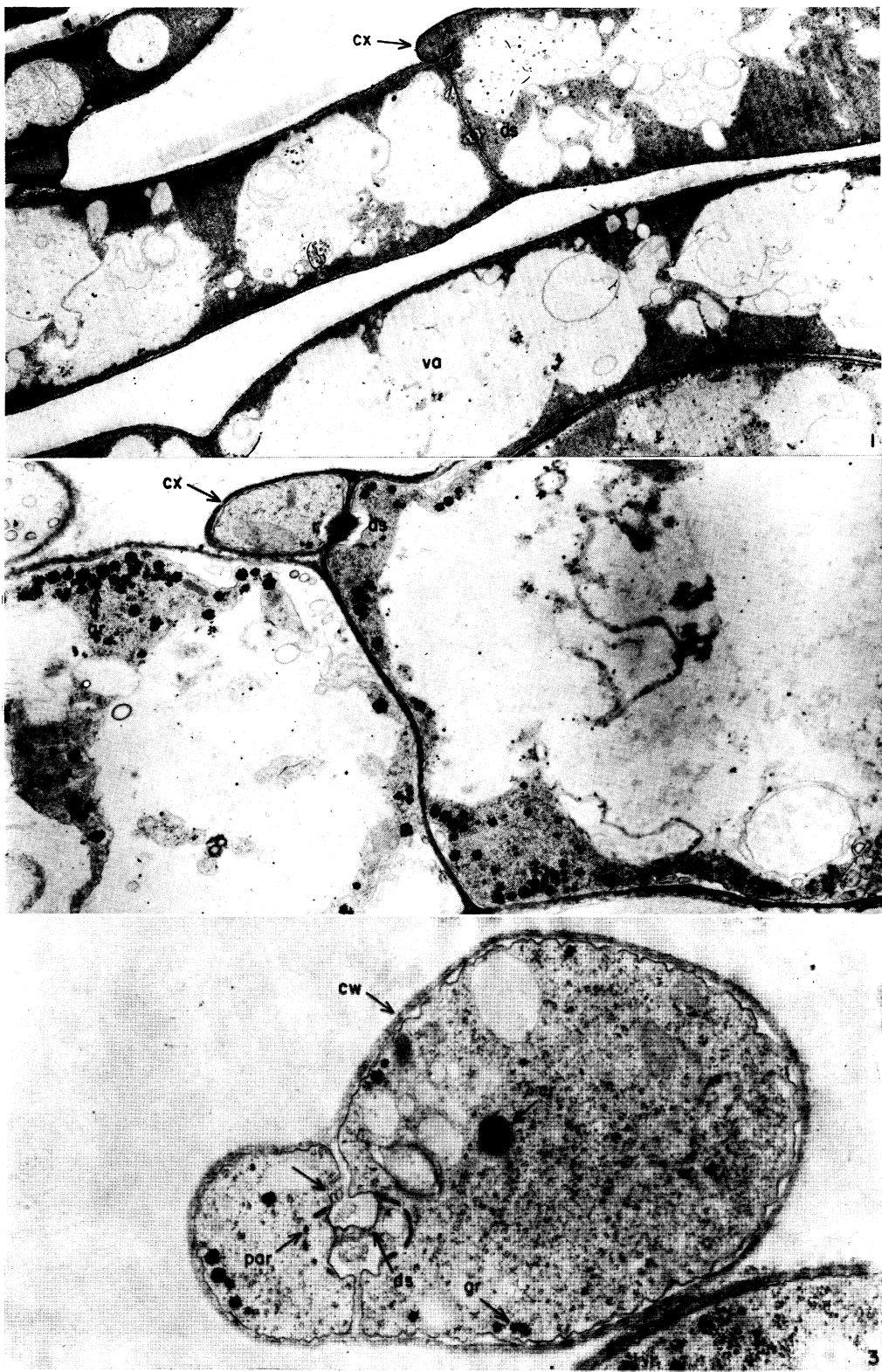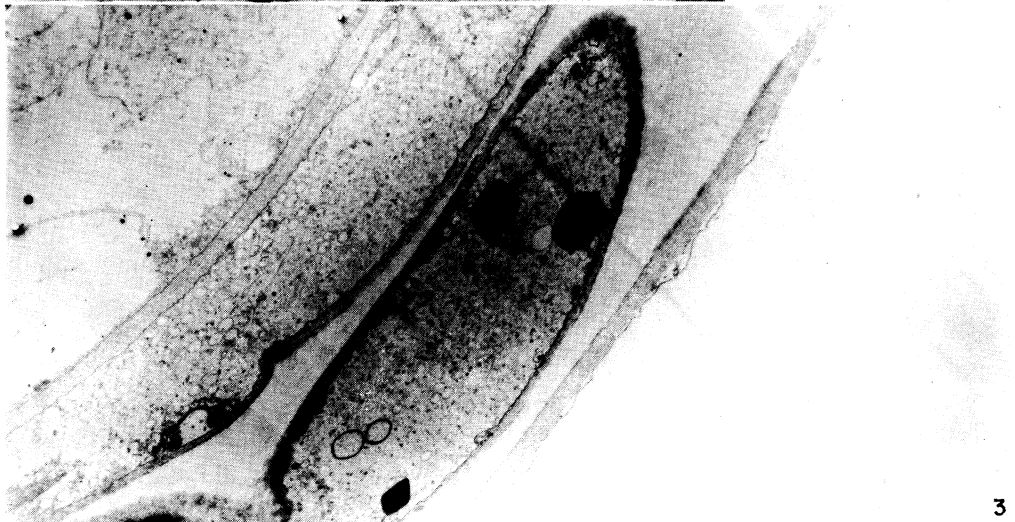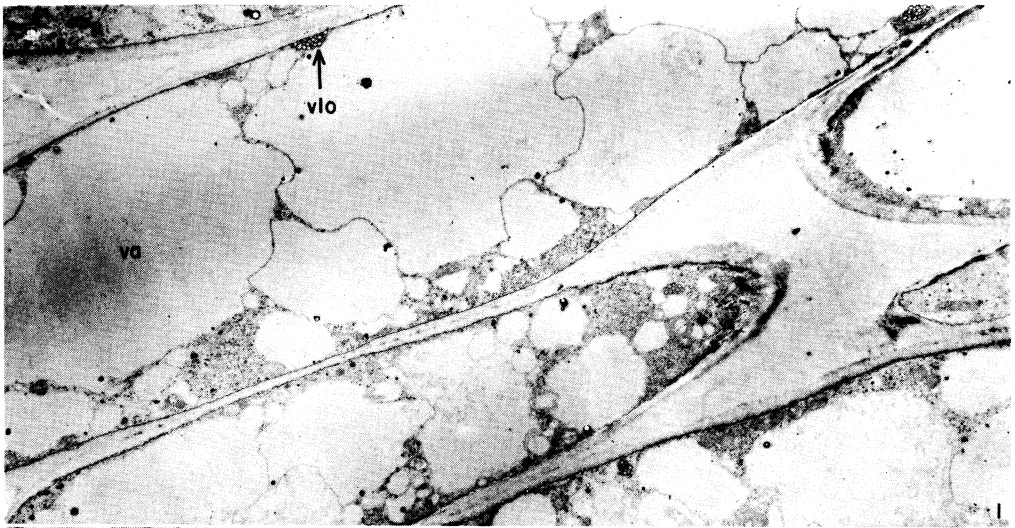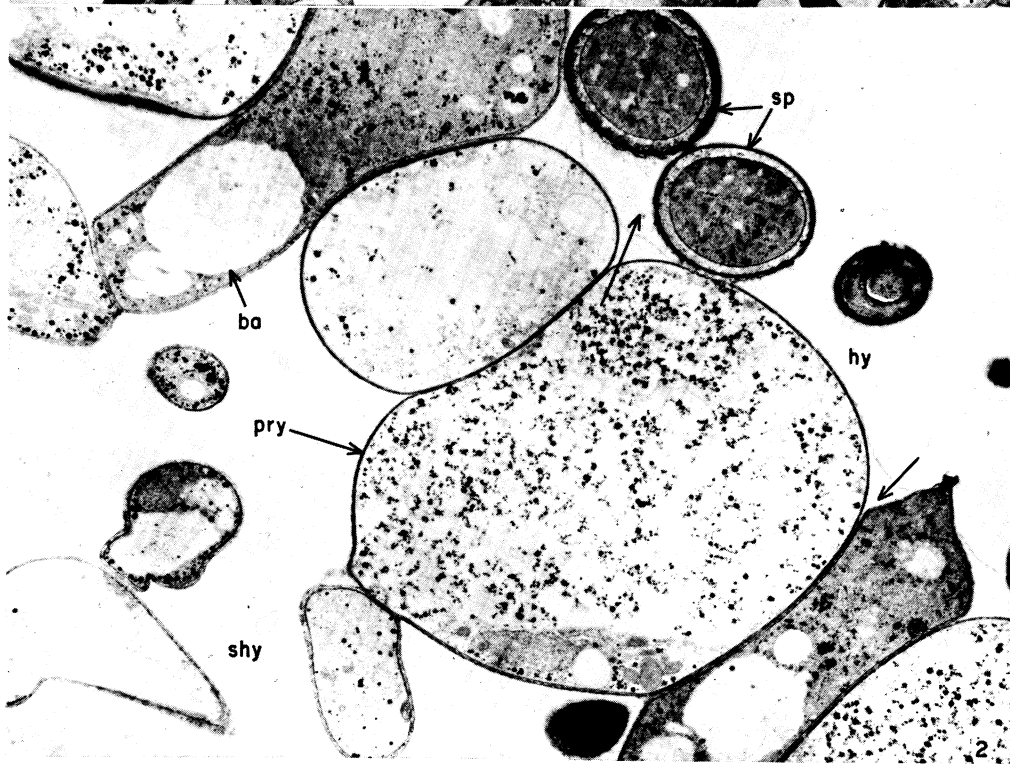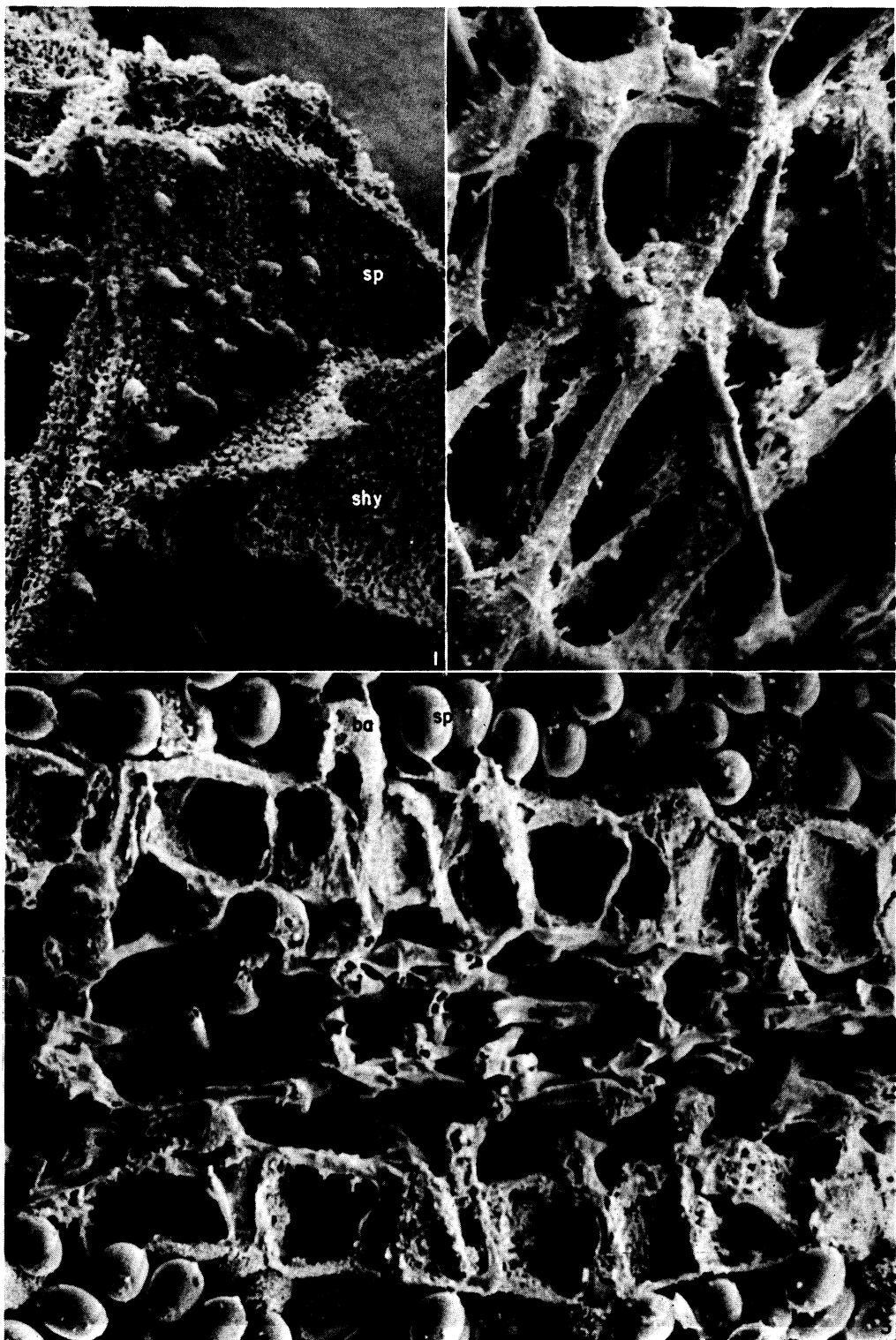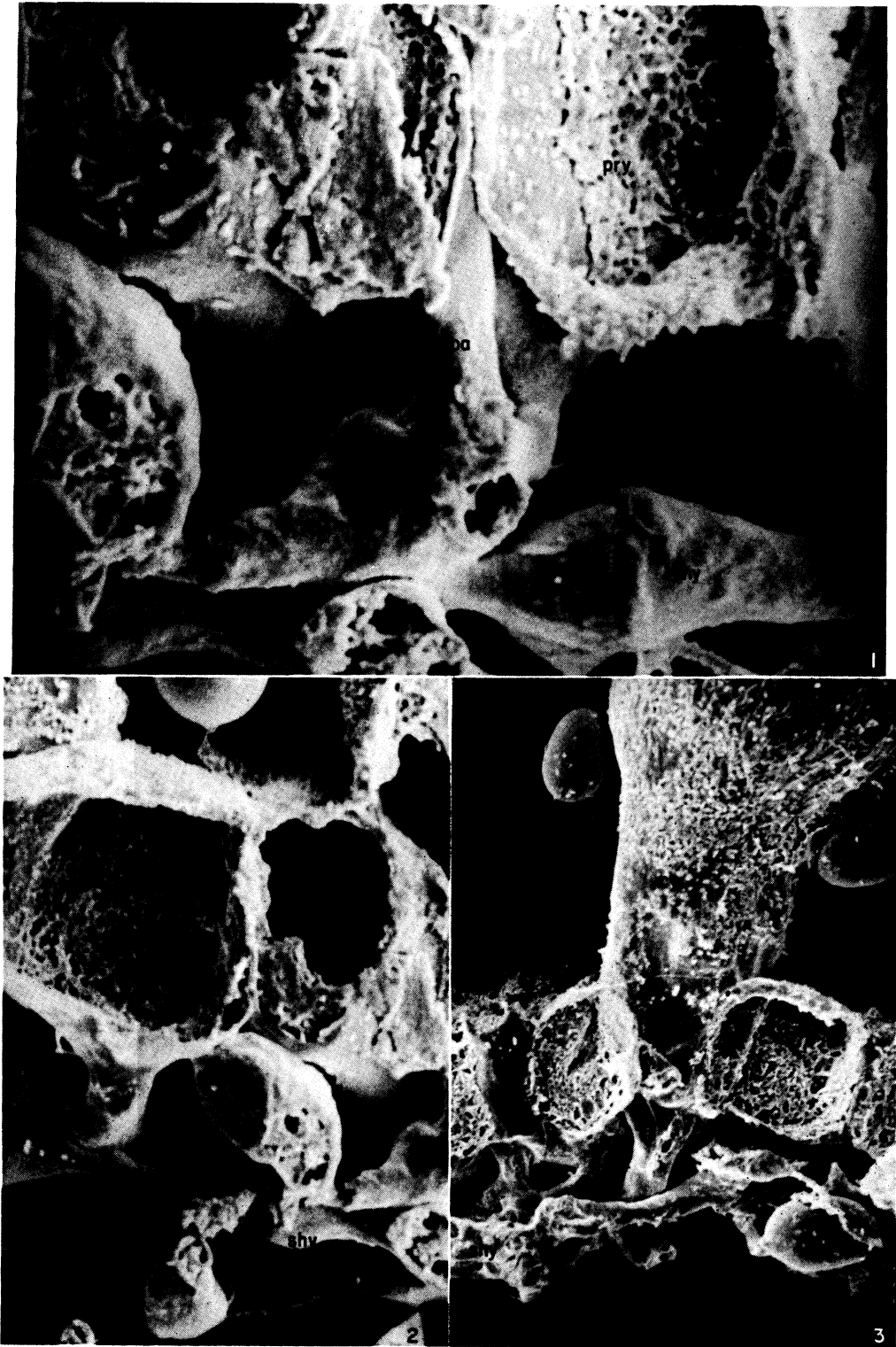
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