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CONTRIBUTION TO THE CYTOLOGY OF HYMENOMYCETES: VIII. KARYOLOGICAL STUDIES IN *POLYPORUS AGARICEUS* BERK.

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(With 3 TEXT-FIGURES)

The karyological phenomena in the life-cycle of *Polyporus agariceus* Berk., a centrally stipitate member of the family Polyporaceae has been studied in detail.

The young basidium is distinctly binucleate. The two nuclei fuse to form a synkaryon. With formation of the synkaryon, the dikaryophasic condition ends. The synkaryon undergoes an interphasic enlargement followed by the appearance of chromatic reticulum. Subsequently, from the reticulum typical chromosomes ($2n = 6$) are formed. Each daughter nucleus is formed from a haploid chromosome set which constitute a genome ($n = 3$). The orientation of the spindle of the first division of meiosis (heterotypic) is transverse or obliquely-transverse to the long axis of the basidium while in the second division (homotypic) it is always transverse. A third nuclear division occurs forming the eight nucleate condition of the basidium. Four of these eight nuclei migrate into four developing basidiospores while the other four degenerate in the collapsing basidium. The nucleus within the basidiospore often divides before the liberation of the basidiospore from the sterigma.

During germination, the uninucleate basidiospore enlarges and gives rise to one or occasionally two germ-tubes. The germ-tubes are either quite narrow or of the same width as that of the spores. As the germ-tube elongates, the nucleus in the basidiospore repeatedly divides to form a multinucleate condition. Eventually, by the formation of cross-walls, this coenocytic structure becomes divided into terminal uninucleate cells. Finally, nuclear divisions and simultaneous wall-formations result in the formation of a primary monokaryophasic mycelium.

The secondary dikaryophasic mycelium, with characteristic clamp-connexions and binucleate cells, results only when two compatible primary mycelia unite.

INTRODUCTION

The karyological phenomena, which play an important role in the life-cycles of hymenomycetes, have been worked out by various investigators. The critical review made by Olive (1953) and Boidin's work (1954) have strengthened our knowledge on the subject. From these, it will be evident that considerable work has been done in Agaricaceae, Thelephoraceae and Hydnaceae. In India, the work of Bose (1937) encouraged Banerjee and his co-workers (1955, 1956, 1957, 1960, 1961, 1962) to study the karyological phenomena in some species of Polyporaceae, Thelephoraceae and

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Agaricaceae during recent years. A large number of species, however, still remain to be investigated. The present work has, therefore, been undertaken to throw some light on the nuclear phenomena in the life-cycle of *Polyporus agariceus* Berk., a centrally stipitate species of Polyporaceae occurring in India.

Material and Methods

Fructifications of *P. agariceus* growing luxuriantly on longs of *Shorea robusta* Gaertn. f. were collected from the timber-yards of Calcutta in the months of July to October. Small rectangular blocks (5×5 mm.) were cut from these fructifications and fixed for the study of nuclear conditions in the basidia. Presoaking of the fruit-bodies by rain or water before fixation proved better for obtaining divisional stages of the nuclei. Different fixatives, such as "Bouin-Allen", "Sass", "Carnoy", "Nawaschin (A+B)" and "Formal-Acetic-Alcohol" were used for which "Bouin-Allen" and "Sass" yielded satisfactory results. During fixation, the air from the pore-tubes was pumped out to obtain rapid and effective penetration of the fixatives (Sass, 1929). After fixation the materials were washed, where necessary, dehydrated following Ehrlich and McDonough (1949) to avoid difficulties in sectioning and finally embedded in paraffin.

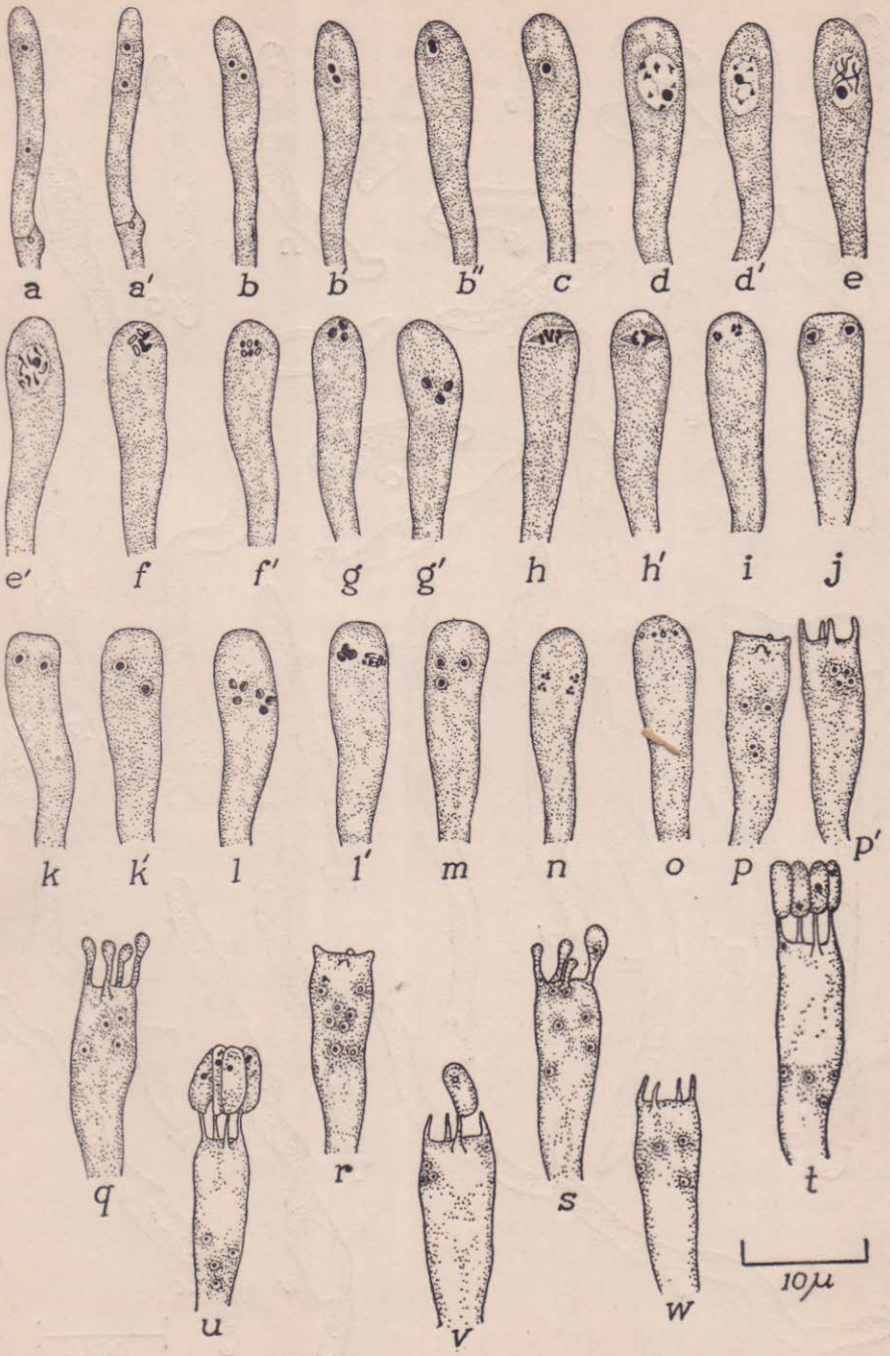
Microtome sections 7μ thick were cut and the sections were stained separately in Crystal violet (1% aqueous solution), Pyronin-Methyl green, Leuco-basic Fuchsine (Feulgen and Rossenbeck, 1924) and Heidenhein's Iron-Alum Haematoxylin. Of these Iron-Alum-Haematoxylin gave best results when used after fixation with "Bouin-Allen" or "Sass". Best preparations were obtained only after mordanting the sections for 1 hour in 4% aqueous Iron-Alum followed by staining in Haematoxylin for 2 hours (Gwynne-Vaughan & Barnes, 1937). Subsequent differentiation was done in saturated aqueous solution of Iron-Alum.

For the study of karyological phenomena in spores, germinating spores, primary and secondary mycelia, agar-film technique (Kniep, 1913) was used. Spore-deposits were directly taken on thin film of cleared 3% malt-agar on sterile slides. A number of slides were immediately fixed while others were incubated at 30°C. for germination of the spores. The germinating spores were fixed at different stages of development. Primary and secondary mycelia of the fungus were allowed to grow separately on agar films and fixed in the same way. Satisfactory preparations of spores, germinating spores and the two types of mycelia were obtained in both "Sass" and "Bouin-Allen" followed by Haematoxylin stain.

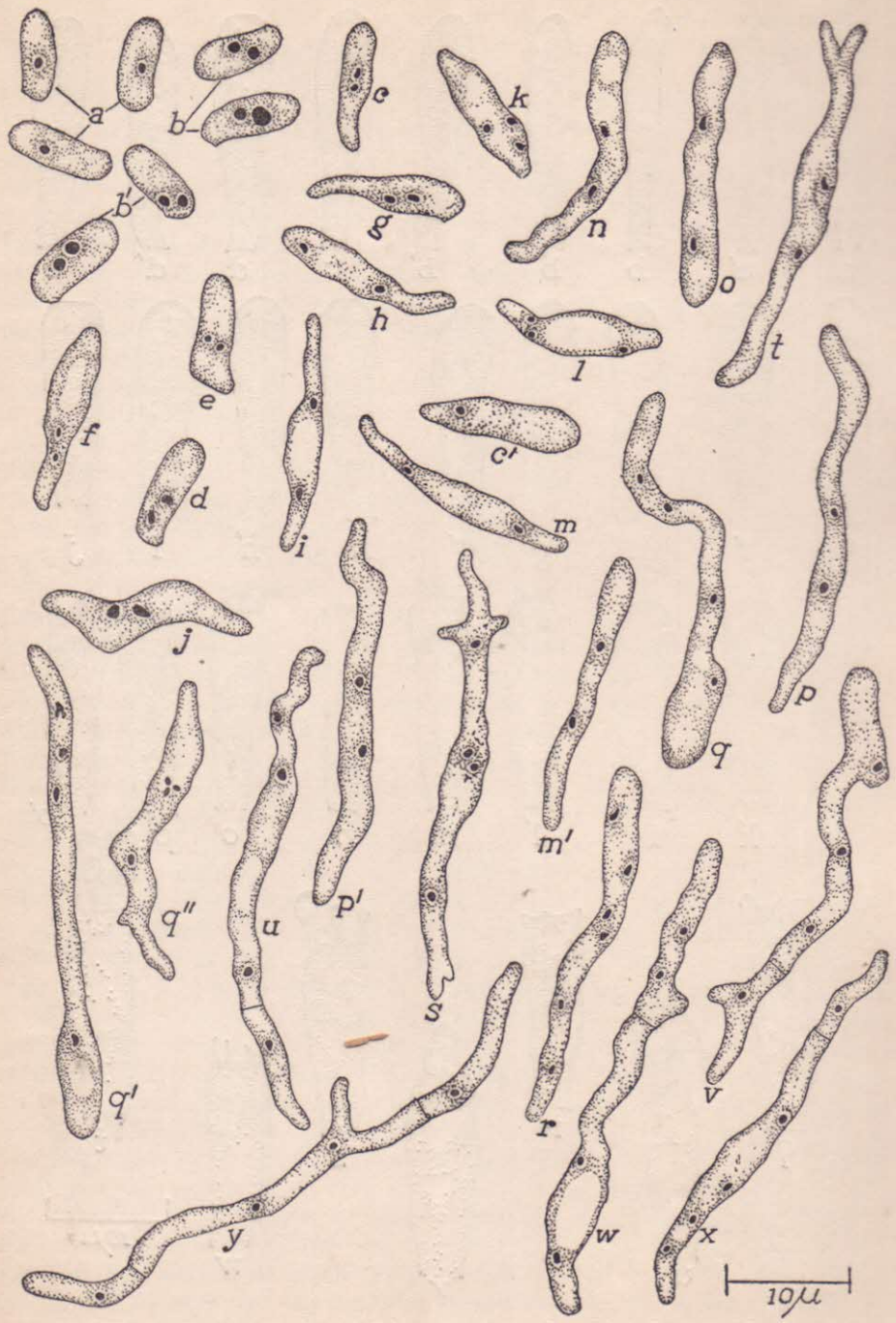
Observations

The young basidium is a slightly swollen terminal hyphal cell with a basal clamp-connexion (Text-fig. 1, a–a'). The protoplasm consists of granulated cytoplasm with two small (about 1μ in diam.), 'homogeneous and compact' nuclei (Pinto-Lopes, 1949). The two nuclei at first remain quite separated from each other along the long axis of the basidium (Text-

FIG. 1, a–a′). Gradually, they approach each other and fuse to form a synkaryon (TEXT-FIG. 1, b–c). The synkaryons are exactly of similar structure and have identical staining behavior with the nuclei of the young basidia but are slightly larger (about 2μ in diam.). The fusion nuclei probably pass through a resting period as is evident from the greater preponderance in the preparations. They then enter the 'interphase' as a preparatory stage of the meiotic division. During this stage, each synkaryon enlarges considerably ($4–5\mu$ in diam.) and within the nuclear membrane 6–7 deeply stained granules and a nucleolus become visible (TEXT-FIG. 1, d). These are the heterochromatic bodies on the chromosomes. This is followed by the appearance of the chromatin reticulum (TEXT-FIG. 1, d′). Eventually, the chromatin appears in the form of six distinct elongated leptotene chromosomes (TEXT-FIG. 1, e). Condensation and shortening of the chromosomes then follow (TEXT-FIG. 1, e′). These six chromosomes ($2n = 6$) then come close together and pair (TEXT-FIG. 1, f–f′). This late occurrence of synapsis confirms Wakayama (1930, 1932). It has not, however, been possible to demonstrate quite convincingly the exact point at which synapsis begins. This is obviously due to the small size of the chromosomes. Then the nucleolus and the nuclear membrane gradually disappear and three bivalents become distinctly visible during metaphase (TEXT-FIG. 1, g–g′). An intranuclear spindle with polar centrosomes appears and the bivalents become arranged near about the equator of the spindle before metaphase (TEXT-FIG. 1, h). With the onset of anaphase I, the bivalents separate and three chromosomes constituting a genome ($n = 3$) move towards each pole (TEXT-FIG. 1, h′). The individuality of the chromosomes are not very clear at this stage owing to their close association. The orientation of the spindle during the first division is transverse or obliquely-transverse to the longitudinal axis of the basidium (TEXT-FIG. 1,h–h′). Three distinct chromosomes constituting a genome are, however, evident during late anaphase (TEXT-FIG. 1, i). These chromosomes gradually coalesce and form an irregular chromatin mass (TEXT-FIG. 1, j) which ultimately rounds up forming a daughter nucleus (TEXT-FIG. 1, k). The resultant two nuclei are identical with the nuclei of the young basidium and remain in one plane either obliquely or at right angles to the long axis of the basidium (TEXT-FIG. 1, k–k′). Both the nuclei then divide simultaneously (TEXT-FIG. 1, l–l′) or in succession (TEXT-FIG. 1, m). Paired chromatides ($n = 3$) are evident at metaphase II (TEXT-FIG. 1, l). In anaphase the separating chromatids are visible. The spindles during the second division are at right angles to each other and to the long axis of the basidium (TEXT-FIG. 1, l′). The four daughter nuclei are either arranged in the upper part or near the middle of the basidium (TEXT-FIG. 1, o–p). A third homotypic division then follows and eight nuclei are formed (TEXT-FIG. 1, r). The divisional stages and orientation of the spindles during third division have not been observed. Rudiments of four sterigmata begin to develop at the four or eight nucleate stage (TEXT-FIG. 1, p, r). The sterigmata increase in size and their tips swell forming spore-vesicles (TEXT-FIG. 1, p′–q). Four of the eight nuclei migrate into the developing basidiospores at random (TEXT-FIG. 1, s–t). Migration of the nuclei may be early while the spore-vesicles are still very small (TEXT-FIG. 1, s) or it may be delayed (Text-FIG. 1, t). After migration the vesicles are cut off from the sterigmata to form four basidio-



TEXT-FIG. 1. Nuclear phenomena in the basidia of *Polyporus agariceus* Berk. (Vide Text).

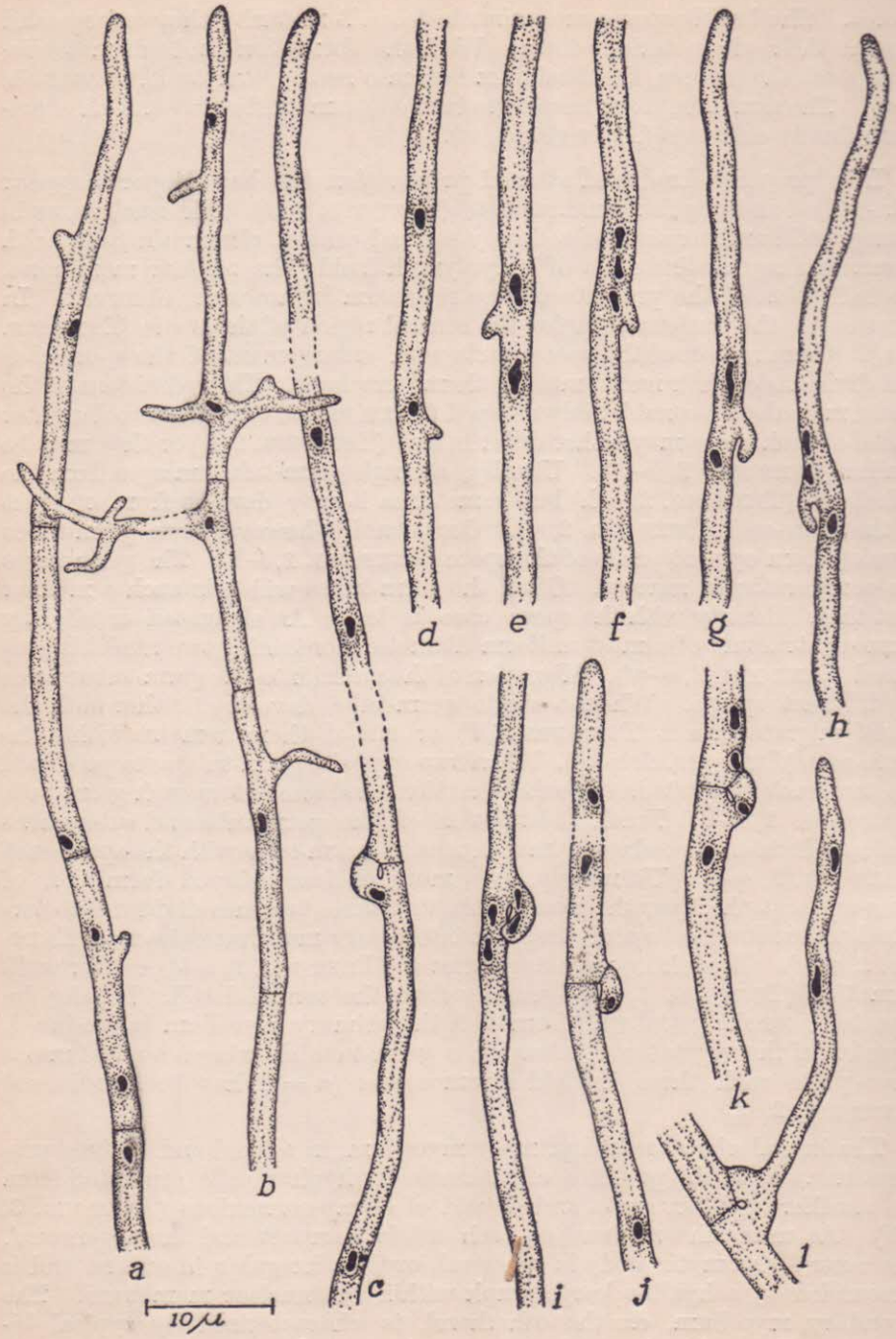


TEXT-FIG. 2. Nuclear phenomena in the basidiospores and germinating basidiospores of *Polyporus agariceus* Berk. (vide Text).

spores. The basidiospores are uninucleate. At maturity they enlarge and assume their characteristic form. With the formation and ultimate discharge of the spores, the basidium becomes nearly empty (TEXT-FIG. 1, u–v). The remaining four nuclei gradually become indistinct and the basidium finally collapses (TEXT-FIG. 1, w).

In a properly fixed and stained preparation the basidiospores appear thin-walled and regularly uninucleate (TEXT-FIG. 2, a). The nucleus, as in young basidium, consists of a deeply stained central chromatin body with a surrounding hyaline zone of karyolymph inside the nuclear membrane. No indication of the presence of any reticulum is, however, observed. In most cases, the nucleus occupies the central region of the spore (TEXT-FIF. 2, a). Germination of the spore starts with enlargement of the spore-case and divisions of their nuclei making them binucleate. These divisions of the nuclei may also be considerably delayed (TEXT-FIG. 2, c′). The two daughter nuclei of each spore may either differ in size (TEXT-FIG. 2, b) or they may be similar (TEXT-FIG. 2, b–b′). Usually, a single germ-tube emerges from the apical end (TEXT-FIG. 2, c–h) but sometimes it may develop from any part of the spore-wall (TEXT-FIG. 2, v). Occasionally, however, two germ-tubes develop from opposite ends of the spore (TEXT-FIG. 2, i–k). The germ-tubes are comparatively narrow. Often the germ-tubes widen in such a manner that their identity with the spore case is lost. An elongated or slightly flexuous structure of almost uniform diameter containing two nuclei is produced (TEXT-FIG. 2, o–p′). Migration of the nuclei into the germ-tube varies in different spores. When a single germ-tube develops, either both the nuclei migrate into it (TEXT-FIG. 2, f) or one of them remains within the spore-case (TEXT-FIG. 2, q–q′). When two opposite germ-tubes are produced the two nuclei migrate in opposite directions and one enters each germ-tube (TEXT-FIG. 2, h–i). Further elongation of the germ-tube and subsequent nuclear divisions produce a multinucleate germ-tube with the spore-case (TEXT-FIG. 2, q–r). Ultimately, by a more or less delayed formation of cross-walls at the tips, this coenocytic structure becomes divided into terminal uninucleate cells and basal or intercalary multinucleate ones (TEXT-FIG. 2, u–y). Branching may start prior to (TEXT-FIG. 2, s–t) or after wall-formation (TEXT-FIG. 2, v–y) usually from the terminal cell. Finally, by repeated branching and wall-formation the primary mycelium is produced. Division of the vegetative nucleus in a germ-tube showing a typical metaphase plate with three haploid chromosomes ($n = 3$) has been observed (TEXT-FIG. 2, q″).

The irregularly branched primary mycelium, in a fixed and stained preparation reveals the presence of uninucleate hyphal cells separated from one another by plain septa and devoid of clamp-connexions (TEXT-FIG. 3, a–b). As usual the nucleus of each cell is 'compact and homogeneous'. The central chromatin body is spherical, oval or irregular in outline and is surrounded by a hyaline karyolymph within the nuclear membrane. The secondary mycelium, on the otherhand, is characterized by typical binucleate cells bearing simple clamp-connexion at each septum (TEXT-FIG. 3, c). The details of conjugate mitosis have not been observed but stages are present showing the essential features of clamp-connexion (TEXT-FIG. 3, d–k). The process in this form agrees in salient points with the observations



TEXT-FIG. 3. Nuclear phenomena in the primary and secondary mycelia of *Polyporus agariceus* Berk. (vide Text.).

made by Bensaude (1918) and Kniep (1917). The essential features in the formation of a clamp-connexion is the development of a slender lateral pocket from the middle of the terminal hyphal cell (TEXT-FIG. 3, d). The pocket gradually curves downwards, touches the main hypha and ultimately fuses with it (TEXT-FIG. 3, e–k). Meanwhile, the two nuclei divide conjugately near the pocket in such a manner that one of the daughter nuclei of the upper pair remains within the pocket (TEXT-FIO. 3, i). One cross wall appears across the spindle of the dividing nuclei near the base of the pocket and the other across the spindle of the main hypha and touching each other at an angle (TEXT-FIG. 3, j). Thus the terminal cell receives a pair of conjugate nuclei as soon as the division is complete while the subterminal cell and the lateral pocket remains temporarily uninucleate (TEXT-FIG. 3, j). Finally, when the lateral pocket fuses with the subterminal cell, the latter becomes binucleate (TEXT-FIG. 3, k). Branches mostly develop due to proliferation of the clamp-connexion and subsequent migration of two nuclei (TEXT-FIG. 3, l) into the developing hypha.

DISCUSSION

In this investigation, evidence has been presented to consider that the karyological phenomena in the life-cycle of *Polyporus agariceus* is in general agreement with the previous observations made by Banerjee and his coworkers (1955, 1956, 1957, 1960, 1961, 1962). The nuclei of the young basidia are 'compact and homogeneous', each containing a central chromatin mass, a hyaline zone of karyolymph and a nuclear membrane. The two nuclei, in each basidium, fuse to form a synkaryon which then enters the 'interphase' and enlarges considerably. According to Swanson (1961), this enlargement is due to synthesis of large molecules of nucleic acids and proteins. With the initiation of meiosis, one or two nucleoli and a number of deeply stained chromatin bodies become visible within the nucleus. As the division, proceeds, chromosomes appear and the diploid and haploid chromosome numbers have been found to be 6 and 3 respectively. Following meiosis, a third nuclear division takes place and eight nucleate basidia are produced at maturity. This additional division of the tetracyte nuclei has also been observed by Goto (1936), Biggs (1938) and Banerjee *et al.* (1956, 1960, 1961, 1962) but its significance is yet to be determined. Of the resultant eight nuclei, four migrate at random into four developing basidiospores, one in each, and finally cut off at the tips of four sterigmata. The uninucleate basidiospores are morphologically alike and germinate to produce primary mycelia of uninucleate cells. The secondary mycelium consists of binucleate hyphal cells with clamp-connexion at each septum. The binucleate condition terminates in the young basidium when the two nuclei fuse.

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