

NUCLEAR BEHAVIOUR IN *POLYPORUS* *GRAMMOCEPHALUS* BERK.

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(With 3 Text-figures)

The study of nuclear phenomena in *Polyporus grammacephalus* Berk. has been the object of present investigation.

The nuclear fusion within the binucleate basidium produces a synkaryon which directly passes into the meiotic cycle. Chromatin reticulum appears within the large interphase nucleus at prophase I and resolves into minute chromosomes. These chromosomes form bivalents with their respective homologues ($n=6$). At the beginning of Metaphase I the prominent nucleolus disappears. The intranuclear spindles are frequently associated with polar centrosomes. Their orientation within the basidia follow no definite order. The meiotic cycle is followed by a third homotypic division giving rise to a eight nucleate basidium. Of them four nuclei forms four basidiospores, and the remaining four nuclei gradually degenerate within the basidium.

The primary mycelium originates from a single basidiospore and the secondary one is formed as a result of pairing between two primary mycelia of opposite sex. Both the mycelial types are composed of cells containing nuclei of 'compact and homogenous' type. The interphase nuclei are frequently associated with centrosomes. The nuclear division is typically mitotic.

INTRODUCTION

The present paper is another addition to our knowledge on the karyological behaviour in higher fungi like those that have so far been investigated by Banerjee *et al* (1955, 1956, 1957, 1960, 1961, 1962, 1966a, 1966b, 1967, 1969) on species occurring in India. The fungus in question is *Polyporus grammacephalus* Berk. Though sporadic in occurrence yet it is a common species in Calcutta and its adjoining districts as a saprophyte on various timbers of economic importance. It also seems to grow as parasite on *Spondius dulcis* Willd., *Zizyphus mauritiana* Lamk. and other fruit trees but pending further investigation no definite statement can be made at this stage.

The references indicate that no work has yet been done on this species in so far as karyological investigations are concerned. In order to throw some light in this direction the present investigation was started.

MATERIAL AND METHODS

The fresh basidiocarps of *Polyporus grammacephalus* were collected from living trees of *Zizyphus mauritiana* Lamk. growing on a roadside of Calcutta during the months of June to August, 1965.

The methods employed for this purpose have already been described by Ghosh (1970). For the study of basidial nuclei small pieces of fresh basidiocarp have been fixed, dehydrated and embedded in paraffin in the usual way. The agar film technique has been employed for the study of basidiospores, germinating basidiospores and primary and secondary mycelia as before.

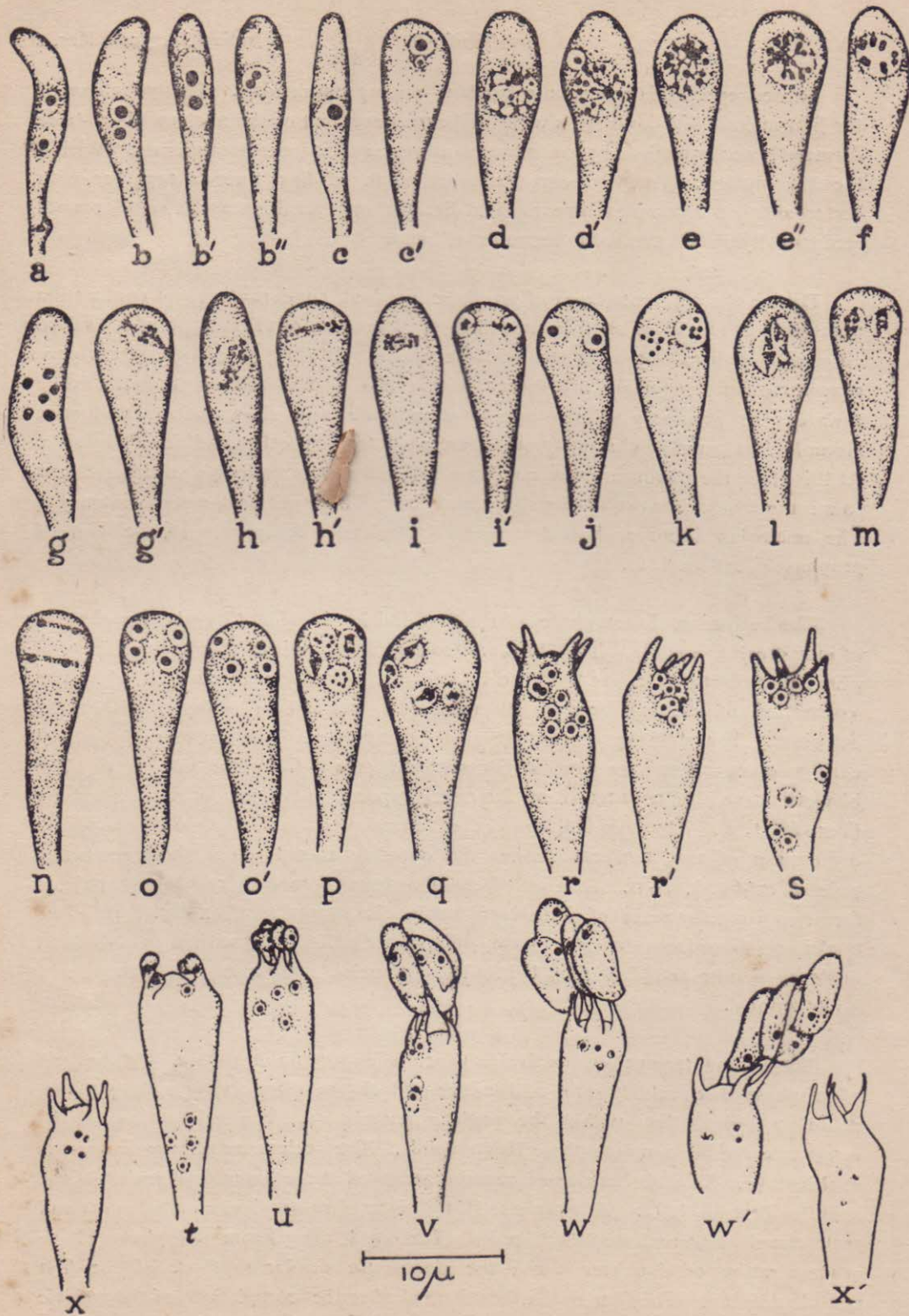
The staining has been done with Heidenhein's Hæmatoxytin, Crystal violet, Feulgen's reagents, and Methyl-green-pyronin for making permanent preparations. Different squash techniques like Aceto-carmine, Aceto-orcein, HCL-Giemsa have also been tried. Most satisfactory permanent preparations have however, been obtained with Iron-Hæmatoxylin following fixation with Sass' and 'Bouin-Allen'.

OBSERVATIONS

The young basidia of the hymenial layer are swollen, thin-walled terminal cells, delimited by basal clamp-connexions. These cells contain dense granular cytoplasm and two small nuclei (1.35μ wide) of 'condensed homogenous' type as described by Pinto-Lopes (1949). They lie along the long axis of the basidium, a little distance apart from each other (Text-fig. 1, a). In the hymenial layer basidia of all the stages of development are present.

The nuclei of young basidia enlarges a little and approach each other before karyogamy (Text-fig. 1 b). The outer walls of the two fuse but their two chromatin bodies remain distinct within the common wall (Text-fig. 1 b'-b"). Afterwards the two chromatin bodies fuse and a synkaryon thus formed is larger in size (2.7μ wide) than those of the fusing nuclei (Text-fig. 1, c). Its structure is similar to the nuclei of young basidium in all respects.

Further nuclear changes are accompanied by the differentiation in shape and size of the basidium. The synkaryon moves upward with the onset of the divisional cycle and enters into 'interphase' (Text-fig. 1, c'). The interphase nucleus enlarges quite considerably (4μ in diameter) and its large chromatin body takes up stain intensely. Sometimes a centriole ($0.3 - 0.5\mu$ in diameter) is seen in association with the nuclear membrane of the 'interphase' nucleus. The centriole contains a stained particle within a diffused area.



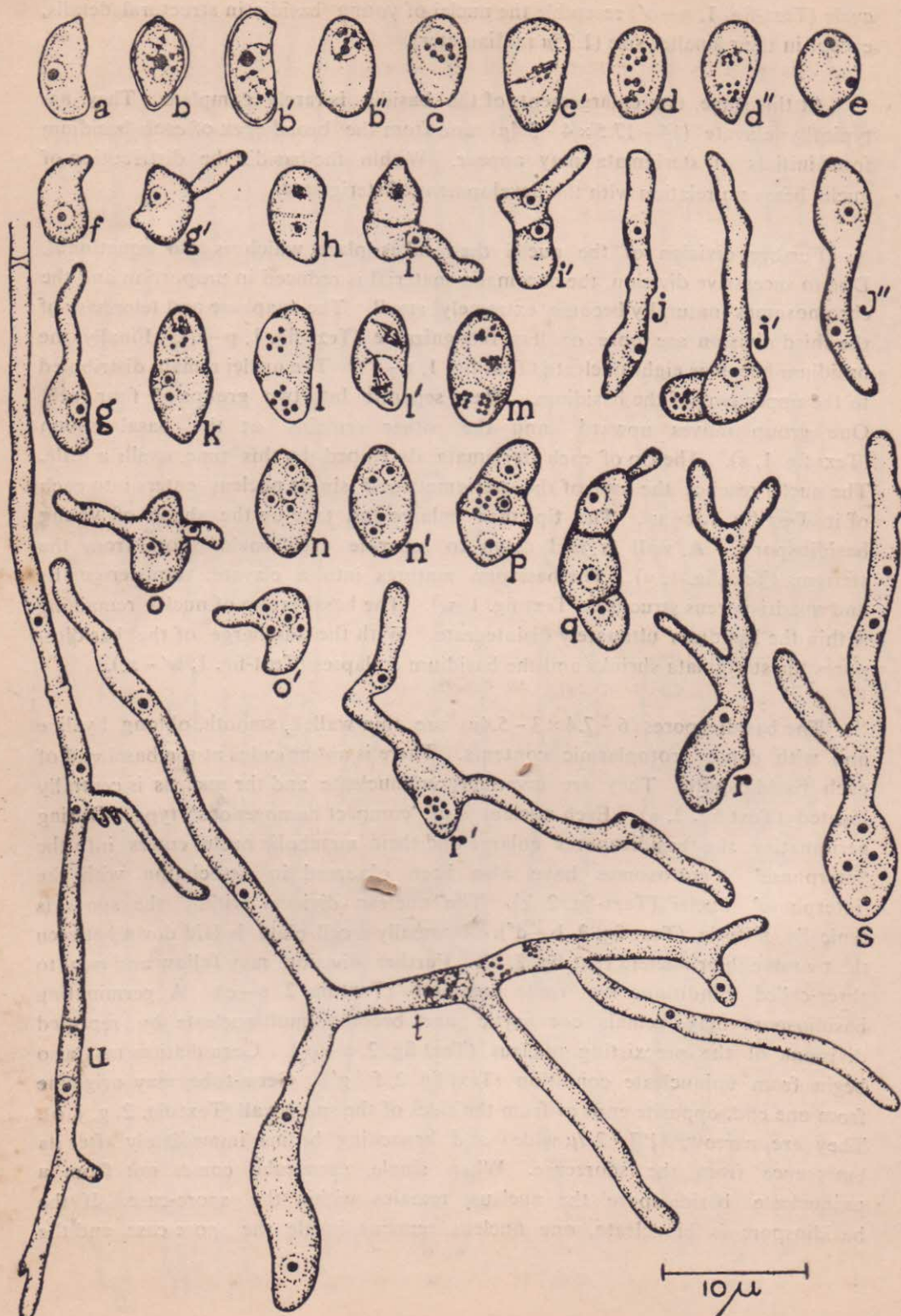
TEXT-FIG. 1. Nuclear phenomena in the basidia of *Polyporus grammacephalus*
Baskin (wide Text).

Subsequently, this interphase nucleus undergoes meiosis. The prophase of the first meiotic division begins with further enlargement of the nucleus ($4-5.4\mu$ in diameter) accompanied by the reduction in size of its chromatin body. Within the karyolymph, generally, chromatin reticulum of fine threads together with heterochromatic granules appears (Text-fig. 1, d-e). At this stage, a small, round, excentrically placed nucleolus becomes visible.

During the different stages of prophase, gradual condensation and contraction processes the reticulum resolves into distinct chromosomes (Text fig. 1, e"). Tailed chromatin bodies appear in one of these stages. In early prophase the chromosomes remain irregularly distributed within the nucleus. They gradually move towards the periphery of the nuclear membrane and show pairing tendency. Homologous pairs of chromosomes come close to each other and lie side by side. At this stage the chromosomes condense and contract extremely and are reduced to minute dots of almost similar shape and size. They take up the stains intensely. The nucleolus becomes less dense and completely disappears at the end of prophase (Text-fig. 1, f).

The beginning of metaphase of the first division is evident by the appearance of intra-nuclear spindle. The fibres are converging at points in opposite direction where stained centrioles are found to be attached with them (Text-fig. 1, g'). The orientation of the spindle may be transverse, vertical or oblique within the basidium. The nuclear membrane then disappears. The equatorially placed chromosomes appear as a closely associated group from the side view. The bivalents have been counted from the clearly spaced polar view of the metaphase (Text-fig. 1, g). The haploid number of chromosomes is 6 ($n=6$). Anaphasic disjunction of the bivalents pushes the chromosomes towards their respective poles (Text-fig. 1, h-i). As a set of chromosomes (genome) reaches the pole, it forms an irregular mass of indistinguishable chromosomes (Text-fig. 1, i). Two nuclei originate from the two masses of opposite poles, each with a well-defined nuclear membrane (Text-fig. 1, j). These nuclei are also 'compact and homogenous'.

These daughter nuclei soon divide which is homotypic in nature. At the end of prophase II, extremely small and condensed chromosomes appear along with a prominent nucleolus within the nuclear membrane. The orientation of the intranuclear spindle in metaphase II is variable. The nuclear membrane disappears at this stage. The chromosomes remain arranged at the equatorial region of the spindle (Text-fig. 1, l). At anaphase II, the sister chromatids are pulled apart and they travel towards opposite poles (Text-fig. 1, m). From telophase II to reorganization of daughter nuclei the stages are similar to those of the first meiotic division. The four nuclei thus formed after the completion of the meiotic



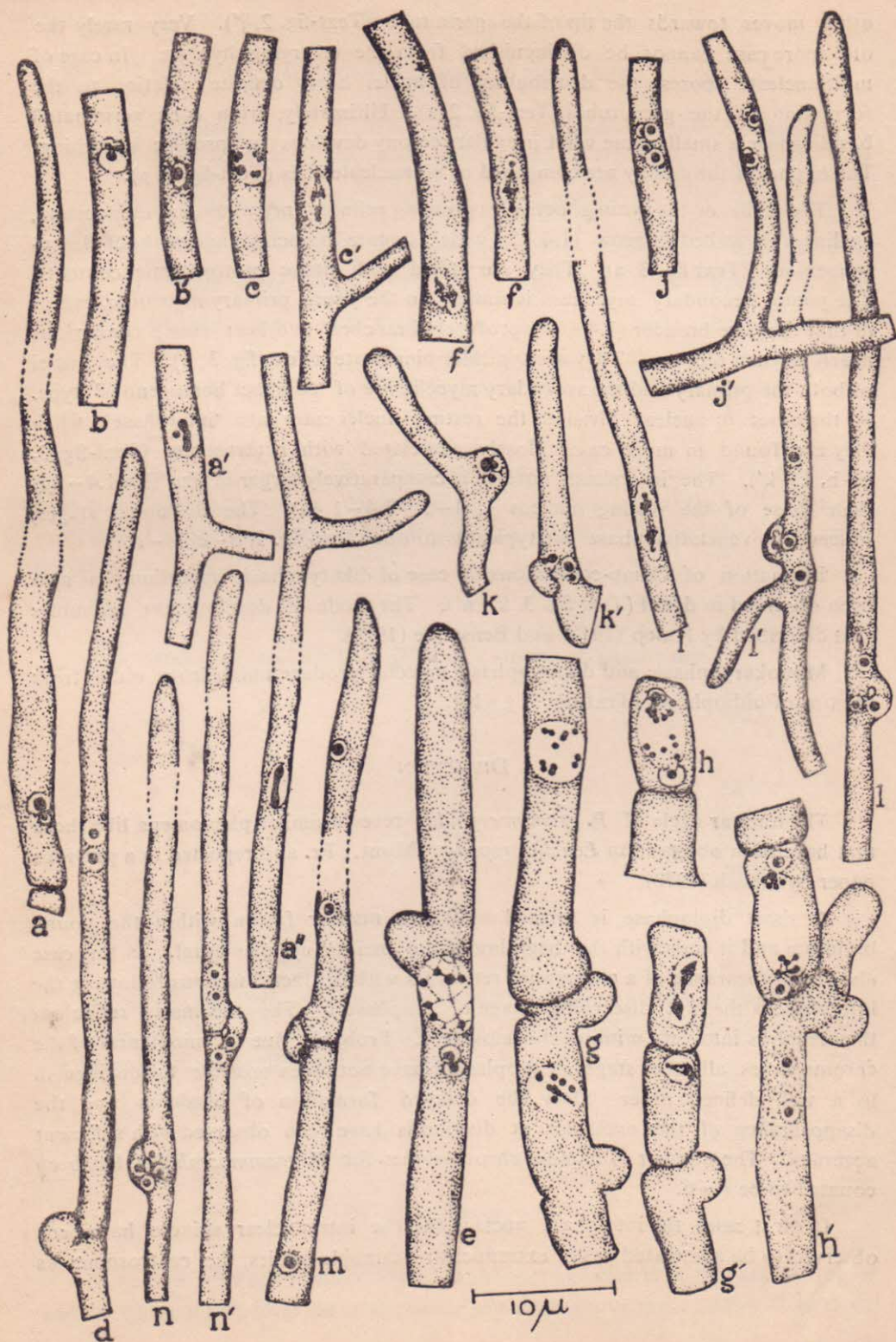
TEXT-FIG. 2. Nuclear phenomena in the basidiospores and germinating

cycle (Text-fig. 1, n - o') resemble the nuclei of young basidia in structural details, except in their smaller size (1.35μ in diameter).

At this stage, the enlargement of the basidia is rarely complete. They are typically clavate ($14 - 17.5 \times 4 - 5.4\mu$) and from the broad apex of each basidium four initials of sterigmata may appear. Within the basidia the distribution of nuclei bears no relation with the development of sterigmata.

Further division of the nuclei then takes place, which is also equational. Due to successive division, the chromatin material is reduced in proportion and the chromosomes naturally become extremely small. The anaphase and telophase of the third division are more or less recognizable (Text-fig. 1, p - q). Finally, the basidium becomes eight nucleate (Text-fig. 1, r - r'). The nuclei remain distributed to the upper part of the basidium. They separate into two groups of four each. One group moves upward and the other remains at the basal region (Text-fig. 1, s). The tip of each sterigmata, developed by this time, swells a little. The nuclei reaches the base of the sterigmata and single nucleus enters into each of it (Text-fig. 1, t - u). The tips then enlarge and take up the shape of young basidiospores. A wall is laid down to separate each basidiospore from the sterigma (Text-fig. 1, v). The basidium matures into a clavate, tetrasterigmatic and quadrisporeus structure (Text-fig. 1, w). The basal group of nuclei remaining within the basidium ultimately disintegrate. With the discharge of the basidiospores the sterigmata shrinks and the basidium collapses (Text-fig. 1, w' - x').

The basidiospores ($6 - 7.4 \times 3 - 5.4\mu$) are thin-walled, smooth, oblong hyaline and with dense protoplasmic contents. There is an apiculus at the basal end of each basidiospore. They are invariably uninucleate and the nucleus is centrally located (Text-fig. 2, a). Each nucleus is of 'compact homogenous' type. During germination, the basidiospores enlarge and their metabolic nuclei enters into the 'interphase'. Centrosomes have also been observed in association with the 'interphase' nuclei (Text-fig. 2, k). The nuclear division within the spore is typically mitotic (Text fig. 2, b - d"). Normally a cell-plate is laid down between the two daughter nuclei (Text-fig. 2, h). Further division may follow and two to three-celled conditions are quite common (Text-fig. 2, p - q). A germinating basidiospore may remain coenocytic and become multinucleate by repeated divisions of the preexisting nucleus (Text fig. 2, n - o'). Germination may also begin from uninucleate condition (Text fig. 2, f - g'). Germ-tube may originate from one end, opposite ends or from the sides of the spore wall (Text-fig. 2, g, j, o). They are narrow, ($1.7 - 2.7\mu$ wide) and branching begins immediately after its emergence from the sporecase. When single germ tube comes out from a uninucleate basidiospore the nucleus remains within the spore-case. If the basidiospore is binucleate, one nucleus remains inside the spore-case and the



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

other moves towards the tip of the germ-tube (Text-fig. 2, j"). Very rarely the old spore case cannot be distinguished from the emerging hyphyæ. In case of multinucleate spores, the distribution of nuclei bears definite relation to the formation of the germ tubes (Text-fig. 2, t). Ultimately, from each germinated basidiospore a small, some what irregular colony develops by profuse branching. The hyphæ of the colony are composed of uninucleate cells (Text-fig. 2, μ).

The cells of the young actively growing primary mycelium are uninucleate, hyaline, thin walled, narrow ($1.4 - 2\mu$ wide), septate, branched and without clamp-connexions (Text fig. 3, a). They are filled with dense protoplasmic contents. The young secondary mycelium is similar to the young primary mycelium, except in that they are broader ($1.4 - 3\mu$), profusely branched and bear clamp-connexions nearly at each septum. They are typically binucleate (Text-fig. 3, d). The nuclei of both the primary and the secondary mycelia are of 'compact homogenous' type. At the onset of nuclear division, the resting nuclei enter into 'interphase', when they are found in most cases closely associated with centrosomes (Text-fig. 3, a - b, k - k'). The 'interphase' nucleus is comparatively larger in size ($3 \times 1.4 - 3\mu$) than those of the resting nucleus ($1.4 - 2 \times 0.4 - 1.4\mu$). The divisional stages, observed in vegetative phase, are typically mitotic (Text-fig. 3, c - c', e - i).

Formation of clamp-connexions in case of dikaryophasic mycelium has also been observed in detail (Text fig. 3, k - n'). The mode of development resembles that described by Kniep (1918) and Bensaude (1918).

Monokaryophasic and dikaryophasic mycelia produce uninucleate oidia from the tips of oidiophores (Text-fig. 3, g - h').

DISCUSSION

The nuclear cycle of *P. grammacephalus* reveals similar phenomena like those that have been observed in *Lenzites repanda* (Mont.) Fr. and reported in a previous paper by Ghosh (1970).

A short diplophase is formed after the nuclear fusion within the young basidium and it ends with the begining of the meiotic cycle as usual. In this case also, the appearance of a chromatin reticulum with distinct nucleous following the interphase is the first discernible stage of prophase I. The chromatin reticulum then resolves into very minute chromosomes. Probably, due to minuteness of the chromosomes, all other stages of prophase I have not been possible to distinguish in a well defined order. Only the delayed formation of bivalents and the disappearance of the nucleolus at diakinesis have been observed with sufficient accuracy. The number of haploid chromosomes for *P. grammacephalus* has been counted to be $n=6$.

In most cases, the interphase nucleus and the intranuclear spindle have been observed to be associated with extranuclear stainable bodies, the centrosomes as

noted before (Ghosh, 1970). After the formation of tetracyte nuclei a third homotypic division follows giving rise to eight nucleate basidium as in case of *L. repanda* (Ghosh, 1970).

The vegetative nuclear division in *P. grammacephalus* is mitotic in nature. Similar observations have been made by Ghosh (1970) in case of *L. repanda* which strongly supports the views of Olive (1953, 1965), McGinnis (1953), Knox Davies and Dickson (1960), Ward and Curysek (1961), Motta (1967) and others.

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