STUDIES IN THE SEPTUM FORMATION OF NEUROSPORA CRASSA

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Intrest in how septum formation takes place was aroused by the observation that new septa were formed between existing septa and the total number of septa was greatly increased by flooding the colony with sucrose solutions of 0.75m or above. Shatkin[1] has suggested that septum is formed by the invagination of the inner layers of the cell wall and by the eventual fusion of the infolded parallel surfaces. The present study has been undertaken to give a better understanding about the septum formation in *Neurospora crassa.*

Material and Methods

An albino mutant of *Neurospora crassa* shear and Dodge (IMI-101718) isolated as a contaminant at the Cambridge Botany School in 1956 and identified by Prof. A. M. Srb, to be possessing certain peculiarities different from albino mutant of *Neurospora crassa*, already in his (Srb) possession has been used in this study.

The medium used were Czapek Dox agar[2] modified by addition of 0.005% Difco yeast extract and solidified with 1.5% Davis agar. Standard inoculations were made by a No. 3 cork borer from the edge of a 24hr colony. The inoculum was placed centrally or at the edge of a plate. The incubation was at 22°C.

Observations

During the examination of growing hyphae under a microscope, it has been noticed that septa are formed in succession in the proximal portion of the apical cells as it reaches a certain size so that the apical cell maintains a more or less uniform length.

These septa have a small central pore through which the cytoplasm move from cell to cell. When a cell is injured, the pore in the septum is immediately blocked by pore plugs which are character [3] and appear as shining spherical object

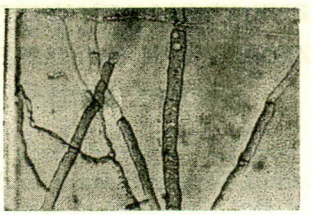

Fig. 1.—*Neurospora crassa.* Pore plugs as refrative bodies in the centre of septa blocking the outflow of cytoplasm from living cells into dead cells.

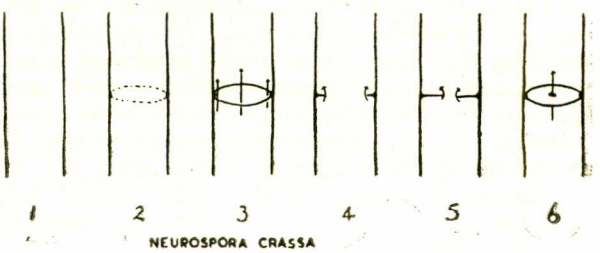

Fig. 2.—*Neurospora crassa.* Diagramatic representation of the different stages of septum formation.

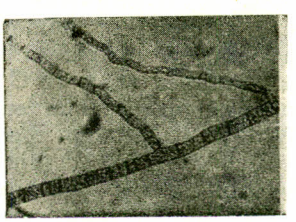

Fig. 3.—*Neurospora crassa.* Long cells have divided into cubical cells after flooding the colony with 1.0M sucrose.

lipoid in (Fig. 1). According to Shatkin[1] the plug is formed by the deposition of spherical particles on the lip of the pore which coalesce and cause the blockage. In the same paper he interprets electron photomicrographs as demonstrating the septum formed by the invagination of the inner layer of the cell wall and by the eventual fusion of the infolded parallel wall surfaces. The diameter of the cytoplasmic connection between the cells is thus gradually narrowed to a diameter of 0.20–0.25μ.

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In the absence of electron microscope detailed observation were made using the light microscope. Observation of the septal distance which is reasonably constant allows the prediction of the approximate location of a normal septum before it is formed. By carefully watching the area where the septum was expected to form, it was possible to see that first sign of septum formation is a faint mark which appears on the wall of the hyphae. It can be seen best in optical section but can be followed round the circumference of the wall by appropriate focusing. Within 30 seconds this faint mark becomes dark and easily seen and a ring of material can be observed lining the inner surface of the hyphal wall. Throughout this time (about 60–75 sec) there is no interference with the flow of cytoplasm. Thereafter the ring grows so that it clearly protrudes into the cytoplasm and interferes with the flow of cytoplasm. After some 150 sec about two thirds the area of the hyphal cross-section is occupied by the septum and the cytoplasm flows through the remaining one third. The septum continues to extend in area and is complete with a small pore after about 5 min (a diagramatic representation of the formation of septa has been presented in Fig. 2). The formation of a second septum commences about 7 mins after the initiation of the first septum.

These observations do not allow any comment on Shatkin's description of septum formation but they do show quite clearly that the process is one of extension of a platform at right angles to the already formed hyphal wall and this platform is pushed out into the actively streaming cytoplasm. There is no suggestion that there is any under-lying cytoplasmic structure or interface for the formation of this wall and the under-lying cause of septum formation at any particular point is difficult to understand.

These speculations are further complicated by the observation of the formation of an extra septum in hypertonic solutions. The production of these (numerous) septa is observed in all fungi examined, which normally produce septa after immersion in sucrose solution of 0.75m or above. The septa appear after equilibration in all the cells of the hyphae including the apical cell more or less simultaneously so that the hypha appears to be divided into a series of more or less cubical cells (Fig. 3).

It is clear that dehydration is not the stimulus for the production of these extra septa, for plasmolysis in 0.5m sucrose does not stimulate septum formation. (In sucrose solutions of 0.5m and 0.4m, no extra septum formation has been observed through these two solutions also produce plasmolysis.) The stimulus must be some property common to solutions of 0.75m and above such as increased dehydration or an increased length of time of equilibration.

Observation of the early stages of the development of these extra septa is difficult because it is not possible to predict with any accuracy the exact point at which a septum is likely to form. Moreover, although the time of their appearance can be predicted, the total time between treatment and appearance is a matter of hours and the normal error will give a variation of several minutes on either side of the predicted time. This period of uncertainty makes concentration for observation of a fleeting morphological aspect difficult. Although the observation of the early stages of the process of extra septum formation may be difficult. However, a general picture can be given.

The hyphae when immersed in the hypertonic solution lose water and show a general collapse of the cytoplasm. This collapsed condition persists for some time depending on the molarity of the solution and then a slow recovery takes place. It is not until the cytoplasm has returned to normal that the extra septa are formed. At this point the cytoplasm can be seen to be moving and the general course of development of the septa appears to be very much as in the case of normal septa, that is, there is a centripetal development of the septum from the inside of the hyphal wall and septal pore in the centre through which cytoplasmic streaming continues.

There appears to be no clue here to the stimulus for septum formation although the method opens up possibilities for further investigation. For example, the *Phycomyces blakesleeanus* which does not normally produce septa, fails to produce them under the stimulus of dehydration.

In the normal production of septa in *Neurospora* there is an interval of about 7 min between the production of septa and of about 5 min for the building of the septa. It is possible that interference with the flow of cytoplasm after 5 min provides the stimulus for the next septum to form and if so this can be related to the stimulus given by plasmolysis? The answer is far from clear but the possiblities of experimentation are many.

Discussion

The phenomenon of the development of septation in arrested hyphae in solutions of higher osmotic pressure is of interest. What facit of this treatment is directly responsible for the stimulus for septation is not yet clear. Dehydration would appear to be an important part because any non-

toxic substance in higher concentration is effective. Dehydration alone is not enough because 0.5m sucrose dehydrates but does not stimulate septum formation. The stimulus appears to work at a threshold, and extension of the stimulus above that point does not lead to an increase in septation. One possible clue is given by Rizvi[3] where the hyphae were treated with sorbose solutions. The sorbose solutions leads to a bursting of the apices in balancing solutions and hypotonic solutions. This leads to a prolonged period of arrestment and increased septation. It therefore looks as though septation can be stimulated by arrestment of the hyphal extension. How this relates to normal septation is also not yet clear. It is possible that the achievement of full size in the septum which takes about 7 min and which is rapid only over the last 1—2 min temporarily restricts the flow of cytoplasm in an anterior direction and signals or promotes the initiation of the next septum. Sufficient evidence is still not available to deal with this matter.

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THE INCIDENCE OF A NEMATODE, PROCAMALLANUS HETEROPNEUSTUS IN THE STOMACH OF HETEROPNEUSTES FOSSILIS

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Introduction

A good number of Nematode species of the genus Procamallanus have been recorded from the Silurid fishes from all over the world. The camallanid nematode from Indian fishes have been studied principally by Ali (1957), Agarwal (1930), Khera (1954), and Kulkarni (1935). In *Heteropneustes fossilis*, Ali (1957) recorded the incidence of *Procamallanus heteropneustus* in the stomach. During a survey of the incidence of Nematode infection in the fishes of East Pakistan,58 specimens of *Heteropneustes fossilis*—a popular East Pakistan fish along with a number of other fishes were examined during July 1965 to December 1965. Infestation by the Camallanid Nematode, *Procamallanus heteropneustus* was found rather common in this fish. In this report an attempt has been made to discuss, the incidence and intensity of *Procamallanus heteropneustus* and their seasonal variation in the stomach of *Heteropneustes fossilis*.

Materials and Methods

'Jeol'fish specimens (*Heteropneustes fossilis*) were collected from ponds, ditches and 'beels' of Chandpur Subdivision. The percentage of incidence and intensity of the nematode (tentatively identified as *Procamallanus heteropneustus* in the stomach were recorded monthwise. The worms were collected after opening the stomach lengthwise and were examined in living condition for morphological and anatomical details. The worms were fixed in hot 70% ethanol and 3% formaldehyde solution.

Results

Of the 58 specimens of *Heteropneustes fossilis*, only 46 specimens were found infected with *Procamallanus heteropneustus*. In heavily infested specimens about 30 to 40 worms were encountered in the stomach. The worms were dull red in colour when fresh, and the colour disappeared when it was isolated and starved. The degree of

TABLE 1.—THE PERCENTAGE ON INCIDENCE AND INTENSITY OF *Procamatanus heteropneustus* IN THE STOMACH OF JOEL FISH.

Month	No. of fish		% of incidence	Intensity
	Examined	Infected		
July 1965	12	12	100	11.7
August 1965	8	7	87.5	10.3
September 1965	10	10	100	10.8
October 1956	14	12	85.7	13.9
November 1965	9	4	44.7	1.7
December, 1965	5	1	20.0	1.0

incidence and intensity of infestation was found to vary in different months.

Morphological features.—The worms are filiform, female (Fig. 1) larger than the male, the female measuring about 7 mm in length and 0.2 mm in diameter, and the male only about 4 mm in length and 0.1 mm in diameter. The buccal capsule is continuous and not separated into paired lateral valves. The oesophagus (Fig. 2) is divided into an anterior muscular and a longer posterior part. The intestine is straight, simple and without

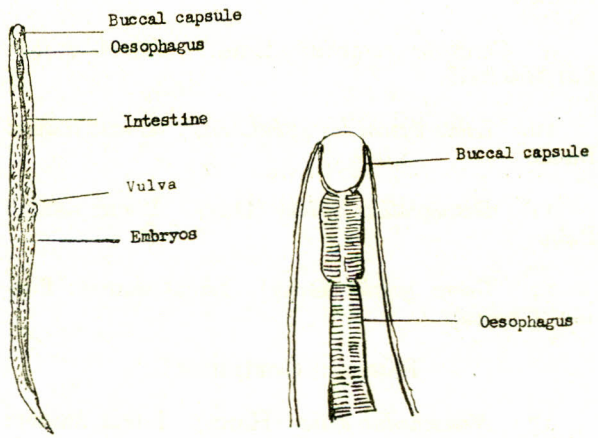

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Fig. 1.—*Procamallanus heteropneustus* entire female;
Fig. 2.—Anterior extremity of *Procamallanus heteropneustus*.

diverticula. The body cavity is continuous from anterior to posterior end and contains the reproductive organs. The worm is viviparous, both the uteri in matured female being filled from end to end with the larvae. The posterior end of the male is curved. The vulva in the female is in the middle of the body.

Discussion

In the course of our investigation with 58 specimens of *Heteropneustes fossilis*, no case of appreciable pathological effect was noticed although about 30 to 40 worms were encountered in a number of individuals. Ali (1957) in India reported the incidence of two different species of the same genus *Procamallanus* namely, *Procamallanus heteropneustus* and *Procamallanus clarius* in *Heteropneustes fossilis* and *Clarias batrachus* respectively, and named them after the host. In *Heteropneustes fossilis* and *Clarias batrachus* we found the same nematode species (*Procamallanus heteropneustus*) in the stomach of both the fish species, apparently identical in morphology and habitat. In none of the *Clarias batrachus* specimens we came across a different species of the fish parasite of the genus *Procamallanus*. The occurrence of a particular species of fish parasite in two different hosts having almost similar structure and ecological habitat is a common phenomenon and as such it would be wrong to name them as two different species. Since the incidence and intensity of this particular species of worm are more pronounced in case of *Heteropneustes fossilis*, this nematode has tentatively been identified and named as *Procamallanus heteropneustus*.

A seasonal variation in the incidence and intensity of *Procamallanus heteropneustus* was observed during the period. The percentage of incidence maintained a high figure of 85 to 100 during July 1965 to October 1965. With the approach of winter in the months of November and December 1965 a sharp decline in the incidence was noticed. Similar was the case with intensity of infestation. The average number of worms per infected fish varied between 10.3 and 13.9 during July to October 1965, while during November and December 1965 the intensity recorded a value as low as 1.0 or 1.7. Observations revealed that most of the fishes of East Pakistan become heavily infested with parasites during winter when the fishes are subject to some adverse environmental conditions. But in case of this particular species, it is interesting to note that the infestation becomes pronounced in hot summer months. The reason of such abnormality is yet to be determined.

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