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THE ARTIFICIAL PRODUCTION OF SPORES OF MONAS BY A REDUCTION OF TEMPERATURE

A DISSERTATION

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THE ARTIFICIAL PRODUCTION OF SPORES IN MONAS BY A REDUCTION OF THE TEMPERATURE

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THE processes of reproduction among the Protozoa present many interesting problems from the physiological point of view. Aside from the so-called sexual reproduction, which is always preceded by the conjugation of two similar or slightly differentiated individuals, there exist many forms of asexual reproduction, varying from the simple division of the parent organism into two, or in some cases as many as eight, daughter cells, to the more complicated processes of encystment and spore formation. The processes of encystment and spore formation may be preceded by conjugation, but in most cases they are simply a direct transformation of the motile organism into a resting form. Surprisingly little is known about the physical and chemical conditions which determine the transformation of Protozoa into cysts and spores.

It has generally been observed that when a pond in which the organisms live begins to dry out, cysts are formed. This fact caused Cienkowsky[1] to undertake a series of experiments. He kept cultures of Infusoria in small, loosely covered dishes, and allowed the water gradually to evaporate. Before the evaporation had become complete, all the Infusoria had formed cysts. Other investigators attributed this encystment to a lack of oxygen, rather than to the evaporation of the water. Maupas[2] has kept cultures of various Infusoria for long periods of time, and has found that some of the carnivorous forms, notably Oxytrichia, form cysts after they have been deprived of food. Hertwig[3] has observed that the same fact holds good for Actinosphærium. In the same series of experiments he found, however, that the consumption of an excess of food may cause encystment as well as starvation. Other cases are on record also in which various carnivorous Infusoria have been seen to encyst after engulfing a large amount of food. Klebs,[4] in a large number of experiments on fresh-water Algæ, has observed that in Vaucheria zoöspores are formed when the filaments are transferred from the light to the dark. Klebs reared Vaucheria in the following solution, used at concentrations of from 0.1 to 0.4 per cent.: $Ca(NO_3)_2$, four parts; $MgSO_4$, one part; KNO_3, one part; K_2HPO_4, one part; and found that when the filaments were transferred from this solution to distilled water, irrespective of light or dark, zoöspores were formed. He also succeeded in producing parthenogenetic spores in Spirogyra by plasmolyzing the cells with a sugar solution. Klebs makes the general statement that the formation of zoöspores in Vaucheria is aided by lowering the temperature,

[1] CIENKOWSKY, *Archiv für mikroscopische Anatomie*, Vol. I (1866), p. 203.

[2] MAUPAS, *Archives de zoologie expérimentale*, Vol. VI (1888), p. 165.

[3] HERTWIG, *Sitzungsberichte der Gesellschaft für Morphologie und Physiologie in München*, 1899.

[4] KLEBS, *Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen*, Jena, 1896.

but made no experiments to show that a lowering of the temperature itself will actually cause the plant to form spores. He apparently had in mind only the limits of temperature at which spore formation may take place, when the process is initiated by other means. Beyond these observations, which are rather inconclusive so far as the Protozoa are concerned, nothing is known about the conditions which determine encystment or spore formation.

Dr. Loeb suggested that I take up the problem of asexual reproduction from an experimental point of view. Many authors had noticed that the mode of reproduction changes in certain aquatic animals or Protozoa when the pond in which they live begins to dry out. But the question was: How can the lack of water in a pond interfere with the mode of reproduction? Dr. Loeb's idea was that the real physical factor at work in this case was the rapid, or extensive, changes of temperature. As long as

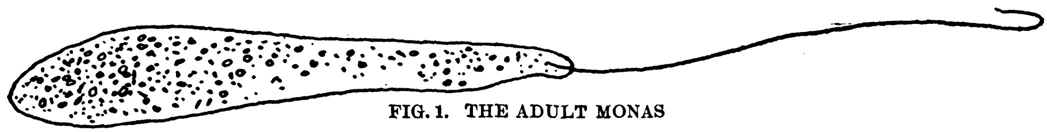

FIG. 1. THE ADULT MONAS

the bulk of water in a pond is large, the daily changes of the temperature of the air will cause only a slow or slight variation in the temperature of the pond. But when the bulk of water is small, the temperature of the latter will follow sudden changes in the temperature of the air more rapidly and completely. In order to test this idea, he suggested that I try whether or not, through sudden changes of temperature, organisms might be caused at any time to reproduce asexually instead of sexually. The experiments were first performed on Stentor, with the results already described in a previous paper.[5] It was found in these experiments that by lowering the temperature the animal would go into a resting stage, which in appearance resembled a cyst; but in no case did I obtain spores. These results could not be obtained by raising the temperature.

During the past year the low-temperature experiments have been continued on several other Protozoa, and in all of them structural changes similar to those already described for Stentor have been obtained. But in one form, Monas, our original purpose has been carried out, namely, the artificial production of spores by means of variations in the temperature.

Monas is a small flagellated Infusorian, of an exceedingly simple structure, and occasionally appears in great numbers in cultures that have been prepared for Paramœcia. It can be easily maintained in the laboratory in great quantities by adding to the culture from time to time a little bread, upon which the Monads thrive surprisingly. In all the experiments the Monads were isolated in small, covered dishes, and the supply of water kept constant by frequent renewal from the aquaria in which the animals had been reared. The temperature was lowered to the desired point by placing

[5] GREELEY, *American Journal of Physiology*, Vol. VI (1901), p. 122.

the dishes in a refrigerator in which constant temperatures, ranging from 1° to 10° C., could be maintained. For each low-temperature experiment a control experiment was performed at the temperature of the room, and great care was taken that all the conditions, with the exception of temperature, should be identical in the two cases.

Monas is more sensitive to changes in the temperature than any of the other Protozoa experimented with. Within a few hours after the temperature has been lowered to 4° C. all the Monads in a dish settle to the bottom and cease their progressive movement. At the same time the cell gradually becomes spherical, the flagellum and mouth-opening disappear, and there is formed a resting cell like those already described in the experiments upon Stentor. These resting cells can be kept at a temperature of 4° to 6° C. indefinitely, and will withstand partial desiccation without losing their power to revert to the normal Monas form when they are removed to the temperature of the room. This reversion to the motile form takes place within twenty-four hours after the room temperature has been reached. The flagellum first makes its appearance and the cells become motile while still in the spherical condition. They soon, however, assume the normal elongated form of the adult Monas.

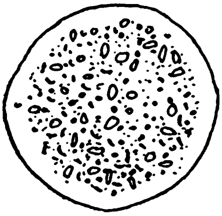

FIG. 2
A resting cell of Monas, formed by an exposure to a temperature of 4° C. during six hours.

If these resting cells of Monas which have been formed at a temperature of 4° to 6° C., instead of being returned to the temperature of the room, be placed on ice at a temperature of 1° C., further structural changes take place as a result of this extreme lowering of the temperature. After remaining at a temperature of 1° C. for five to seven days, the protoplasmic contents of the resting cells break up into small spherical spores, from two or three to twenty-five in each cell. In most cases these spores are discharged from the resting cells as soon as they are formed. They have thick cell walls, and are non-motile. They may be kept indefinitely at any temperature below 8° C., and withstand desiccation perfectly.

When the spores are removed to the temperature of the room and isolated in small, closed cells under the microscope, their development into the motile Monad can be easily followed. The first attempts to demonstrate the development of the spores failed in several instances because of a lack of oxygen in the closed cells in which the spores were isolated, due to the presence of motile Monads which originated from resting cells isolated with the spores. But finally, at Dr. Loeb's suggestion, some fresh-water Algæ were mixed with the spores as soon as they were returned to the temperature of the room. In this way a supply of oxygen was maintained, and the development of the spores began at once. The first change that can be observed is the appearance of a thin layer of protoplasm which grows out of the spore. This protoplasmic layer develops into a small, spherical cell, which gradually becomes separated from the spores. The cell is at first hardly visible,

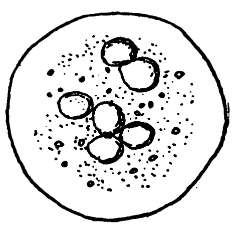

FIG. 3
The formation of spores within the resting cell after an exposure to a temperature of 1° C. for five days.

because of the extreme transparency of its protoplasm, but it eventually becomes granular and develops a flagellum, which appears to originate as a long, delicate pseudopod, extending outward from the protoplasm. The Monad formed in this way finally becomes motile and swims away, leaving behind the empty spore or capsule. In some cases the spores are not discharged from the resting cell as soon as they are formed, but develop within its walls, so that it is possible to find spores in all stages of development, even the young Monads, within the old resting cell. The development of the spores usually occupies from two to four days, although in some cases it may be delayed for a much longer time. The Monads formed from the spores are at first spherical, and swim about with the characteristic revolving movement of this species of Monas. The cells rapidly become elongated, however, and within a short time are indistinguishable in all respects from the adult organism.

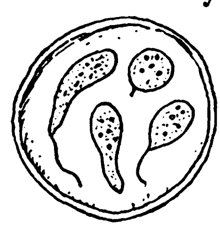

FIG. 5
Young Monads developed from the spores within the old cell wall of the resting cell.

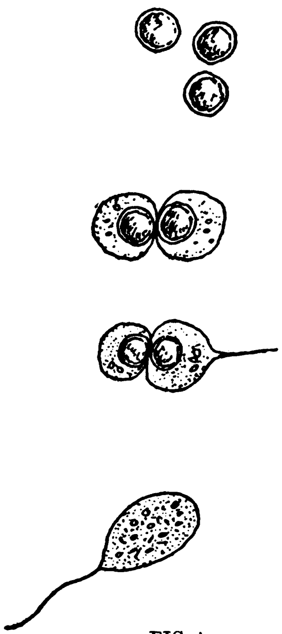

FIG. 4
Four stages in the development of the spore into the motile Monad.

A few typical experiments will be briefly described, as follows:

Experiment I.—On April 11 a culture of Monas was placed in the refrigerator at a temperature of 1° to 4° C. The next day examination showed that all the Monads had formed spherical resting cells. Some of these were removed to the temperature of the room, and they immediately developed into the motile form. On April 24 many of the resting cells had broken up into spores. A small number of these resting cells and spores were isolated under the microscope. The resting cells immediately developed into the motile Monads, as before. On April 26 a few of the spores had formed small Monads, but the development did not go very far because of a lack of oxygen in the closed cells which contained the spores.

Experiment II.—On April 18 a culture of Monas was put on ice at a temperature of 10° C. On May 3 the culture was removed to a temperature of 6° C. A large number of spores had been formed, a large proportion of them remaining within the resting cells. On May 6 many of the spores had developed into young Monads. A large number of the Monads were found swimming about within the walls of the resting cells.

Experiment III.—On May 14 a culture of Monas was placed on ice at a temperature of 1° C. On May 22 a large number of the resting cells and spores were removed to the room temperature and isolated under the microscope in closed cells that contained fresh-water Algæ. Development of the spores commenced at once, and on May 25 they had reached the motile Monad form.

SUMMARY

1. In Monas a reduction of the temperature brings about certain structural changes within the cell that result in the formation of many small spores, each of which has the power of reproducing the organism.

2. It is thus possible by means of variation in the temperature to control the methods of reproduction in Monas. At a temperature of 20° C., Monas multiplies sexually and by simple fission. At a temperature of 1° to 4° C., reproduction by asexual spores takes place.