A FUNGUS OF THE FAMILY ENTOMOPHTHORACEAE FOUND ON SUGAR ANTS (*CAMPANOTUS SP.*).

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INTRODUCTION.

During the early months of 1939 when prolonged and soaking rains fell in Pretoria, Transvaal, several " Sugar Ants "—*Campanotus* sp.—were seen at the Division of Botany and Plant Pathology running around with short and white furry growths on their abdomens. Closer examination showed that the growths were of a fungoid nature, and that they apparently protruded from the soft membranes between the abdominal segments. Their nature and position gave the abdomens the distinct ringed or banded appearance characteristic of insects attacked by species of the *Entomophthoraceae.* Search under stones and logs later revealed a fairly large number of the same species of ants, both living and dead, with their entire abdomens coverd with much more conspicuous white fungous growths, which fastened them to the ground and stones, etc. These growths varied from loose, cottony masses of long white strands on some insects (Plate I) to compact and more or less solid creamy masses on others (Plate II). This variation was obviously dependent not only on the age of the growths, but also to a large extent on the dampness of the spots in which the insects were found. Although the growths varied in size, in no case were they ever seen extending beyond the abdomen or growing on any other parts of the insect's body. When the covering stones and logs were removed, many of the live ants on taking fright, were able to extricate their abdomens from the enclosing loose cottony growths, which were then left behind as hollow masses. In the structure of these a suggestion at least, of the bands or rings could still be seen. This characteristic appearance was also present and usually more obvious, in the denser growths from which the abdomens of the dead ants had been extracted. The insects which were able to free themselves were, except for a slight sluggishness, apparently normal in all respects.

When examined under a microscope the growths were seen to consist of masses of large globose fungous spores, and what appeared to be long collapsed and unbranched mycelial threads. (Plate III, Fig 1.) The denser creamy masses consisted almost entirely of large spores, whereas in the loose cottony masses there was a fair amount of the apparently collapsed hyphae. The spores were globose, more or less uniform in size, and averaged about 30 μ in diameter. Those from the loose growths had smooth or echinate walls enclosing coarsely granular contents, while those from the denser masses appeared to be more or less similar to resting spores found in the *Entomophthoraceae* in general (Plate III, Fig. 2). Their outer walls, however, had apparently shrunken giving them a wavy outline. Under the microscope the dissected abdomens of the ants were seen to contain a number of pieces of thick coarsely granular and intertwined hyphae (hyphal bodies). These hyphae differed in length, but were more or less uniform in diameter, although a few of the shortest pieces were somewhat thicker and more irregular than the rest.

THE FUNGUS IN CULTURE.

The fungus grew readily on most of the common artificial media used in the laboratory. It grew and sporulated especially well on potato agar to which five per cent. dextrose had been added, and it was therefore from cultures on this medium that most of the following observations were made.

The cultures increased rapidly in size and soon covered the surface of the medium in a 9 cms. petri dish. The rapid increase was due primarily to the fact that the ripe spores were discharged in all directions several centimetres from the sporophores and that they germinated almost immediately. In the beginning the growing colony was circular, and appeared as a small, sodden, translucent, and colourless disc, in which the growing hyphae could be seen radiating from the point of inoculation. As the disc increased in size the central portion partially lost its sodden appearance, and the radiating hyphae were more distinct and appeared to be more numerous towards the circumference of the colony. After about 15 to 20 hours in an incubator at 28°C., sporophores formed in the centre of the colony and their attached spores appeared as a white powder in a small circular patch, which gradually increased in size and density (Plate III, Fig. 3). The spores were very soon discharged from the sporophores and could be seen scattered around the central white patch inside as well as outside the colony. The spores germinated almost immediately and soon gave rise to small daughter colonies (Plate III, Fig. 4). As growth continued, the distinct circular outline of the mother colony, which was about 2·5 centimetres in diameter. was gradually obliterated by the numerous discharged spores and their resultant colonies, As in the mother colony, formation and discharge of spores took place in the smaller colonies, and after about four days all suggestion of individual colonies was lost, and the entire surface of the medium was covered by an even white powdery layer of spores. The spores that were shot against the lid and sides of the petri dish adhered to the glass, which consequently became covered with a fine powder, in which, after germination had taken place, numerous fine hyphal threads could be seen with the naked eye.

At room temerature ($\pm$ 23°C.) the spores germinated within an hour or two after they had been placed on the medium : the time taken apparently depended, to some extent at least, on the age of the spores. The number of germ-tubes arising from each spore was usually two or three, although one and four were also fairly common (Plate V, Fig. 1). They arose as blunt and hyaline outpushings from the spore-wall and grew very rapidly. (In some cases they had grown eight to ten times the diameter of the spore two and a half hours after inoculation). The hyphae were thick and more or less uniform in diameter ($\pm$ 11·5 μ) ; their contents were coarsely granular except for small portions of the tips, which remained hyaline throughout their growing period. In the beginning the contents of each spore gradually flowed into its growing germ-tubes until eventually the spore was completely emptied. (Plate V, Fig. 2). The protoplasm continued to flow along the growing hyphae towards the tips and so left the proximal portion of each hypha also empty. A septum then formed immediately behind the advancing protoplasm, thus cutting it off from the emptied portion of the hypha and the emptied spore. The distance at which the first septum was formed varied in the different hyphae. In some it was practically in line with the spore-wall, whereas in others it was some distance away. The protoplasm continued flowing with the growing tip and it was again cut off from the emptied portion of the hyphae by another septum. This process was repeated until eventually each hypha consisted of a series of empty segments with only the distal or growing end containing protoplasm. That actual growth took place could readily be seen by the increase in size of the protoplasm-filled segment. A septum did not arise only behind the protoplasm in the growing hyphal tip ; at times several were formed in the hypha that was still completely filled with protoplasm. The protoplasm-filled segments thus formed gradually rounded off at the ends and so became individual short pieces of hyphae, which were capable of independent growth (Plate VII, Fig. 22). Instead of several septa arising in the protoplasm-filled hypha, there were times when only one was formed, in which case a certain amount of protoplasm was cut off from that in the tip, which continued growing in the

normal manner. In this way a segment containing protoplasm was isolated from the growing tip by an ever-increasing number of empty segments. The formation of these isolated and filled segments was commonly repeated a number of times, thus forming a hypha consisting of several protoplasm-filled segments separated by intervening empty segments. The protoplasm did not always flow from the germinating spores and along the hypha as a more or less compact mass. Large irregular spaces were often seen in the contents, giving the hypha the appearance of being only partly filled, and suggesting that all the flowing protoplasm could not keep pace with the growing tip. On the formation of a septum in this partly filled hypha, a segment containing the scattered protoplasm was also isolated from the growing tip by an increasing number of empty segments. The protoplasm in the isolated segment however, usually continued flowing in the direction of the growing tip, and consequently became more compact and accumulated behind the septum. During its flow towards the septum the accumulating protoplasm was successively cut off by septa from the portions of the segment that had become emptied, thus leaving an isolated and protoplasm-filled segment with several very short empty segments immediately behind it (Plate VII, Fig. 20). This process was commonly repeated or more than one septum formed at the same time in the hypha containing the scattered protoplasm, thus eventually giving rise to a hypha consisting of several protoplasm-filled segments separated from one another by a number of very short empty segments. The walls of the emptied spores and the emptied hyphal segments, after remaining in position for some time, usually collapsed gradually and in most cases eventually disappeared altogether.

When the isolated protoplasm-filled hyphal segments were left in the original medium, which had become stale, they did not show any further growth, and remained more or less dormant, but gradually accumulated and assumed various enlarged shapes and often became almost completely round (Plate VI, Fig. 7). After the culture had been growing for several days, i.e. when the surface of the medium had become covered by the large globose spores, the submerged mycelium consisted entirely of these variously shaped pieces of hyphae many of which were still connected by the empty segments. (Although the mycelium grew apparently only below the surface of the medium, a fair amount of collapsed hyphae and a few isolated filled segments could always be seen among the numerous large spores on the surface).

The sporophores, which developed as soon as the mycelium was well established, were more or less similar to the ordinary hyphae, but differed mainly in their aerial habit and their positive photographic reaction (Plate IV, Fig. 2). (Because of this reaction the spores were discharged towards the light and consequently the rate of increase in size of a young culture in the laboratory was not the same in all directions). The tips of the sporophores, unlike those of the growing hyphae, were very blunt and were not hyaline but granular. Although they were usually very short—about two to three times the diameter of the spore—many were seen whose length was eight to ten times the spore diameter.

The protoplasm in the sporophore continued flowing towards the tip, which gradually swelled. After more or less all the protoplasm had entered the swelling tip, the latter was cut off by a septum as a large globose mass—the mother cell in which the large single spore was developed. (The walls of the containing cell and of the spore were in close apposition, but could clearly be seen under high magnification, especially after the spore had been emptied of its contents). The sporophore remained turgid usually with a slight swelling on one side suggesting pressure within, and its tip penetrated a short distance into the spore as a dome-shaped columella. The contents of the spore moved or churned continuously as the protoplasm was entering from the sporophore. This churning movement increased until eventually the protoplasm was in violent commotion. The columella appeared to become slightly flattened suggesting that the pressure within the spore was increasing. It was at this stage that the spore was suddenly discharged from the sporophore as a large spherical body with a prominent hyaline papilla. The maximum distance that the spores

were discharged towards the light was about 35 millimetres. The sporophore remained turgid for a short time after the spore had been discharged, its columella appearing as a slightly swollen tip with or without a minute apiculus at the top. Within a few minutes however, it began to collapse gradually and after about a quarter of an hour appeared as a shrunken and flaccid tube at the end of which the columella was very obvious (Plate V, Fig. 6). After the discharge, traces of a broken membrane could usually be seen around the base of both the columella and the spore papilla. These fragments were obviously derived from the continuous membrane of the sporophore and the mother-cell. Owing to increased pressure in the spore and possibly also in the sporophore, this membrane was eventually ruptured, thus discharging the spore. The force was not always sufficient to free the spore from the sporophore notwithstanding the fact that the surrounding membrane had been ruptured. In this case the spore remained attached by its papilla to the columella as illustrated (Plate V, Fig. 6). At times the surrounding membrane was not ruptured at all in which case the spore remained attached to the shrinking and afterwards flaccid sporophore.

Under favourable conditions the discharged spore germinated almost immediately and a new mycelium was formed. Under conditions which were apparently not so favourable, the spore gave rise directly to a sporophore at the tip of which a secondary spore developed (Plate V, Fig. 4). The latter was commonly discharged in the usual way or else it sometimes gave rise to a tertiary spore, which in its turn was also discharged in the usual way. (Plate V, Fig. 5). At times two secondary spores developed from the same primary spore, and in exceptional cases as many as 16 very small secondary spores were seen still attached to the same primary spore (Plate VI, Fig. 16). In the beginning all the spores were more or less the same size—about 35 μ in diameter—and all possessed the large papilla. In the older cultures, however, the spores varied from 13 μ to 56 μ in diameter and most had lost the papilla. The smaller of these spores were seen developing only as secondary spores and never directly from the mycelium. Besides the big variations in size of the spores in the older cultures, many differed from the rest in having numerous, soft hair-like outgrowths covering the entire surface of the spore (Plate VI, Fig 17). These spores when placed on fresh medium gave rise to one or more germ-tubes in the usual way. (Plate VI, Fig. 18).

In addition to the above, small almond-shaped spores were occasionally found in the older cultures (Plate VI, Fig. 11). These measured approximately 10 $\mu \times 18$ μ and were never seen arising directly from the mycelium. They were in all cases seen to develop from the very small globose spores (Plate VI, Fig. 12), thus it appears evident that they were born only as tertiary spores. On germination they gave rise to germ-tubes somewhat thinner than the normal. (Plate VII, Figs. 13 and 14).

PATHOGENICITY OF THE FUNGUS.

The attempts to infect the same species of ants from which the original cultures were obtained, were unsuccessful, and in no case did any of the live or dead insects develop the typical growths when placed in a petri dish containing the fungus cultures. The larvae of these ants, however, all died within two days after having been placed on the cultures. They were then seen to be full of short pieces of hyphae of various shapes and sizes (hyphal bodies). (Plate VI, Fig. 8). These hyphal bodies where placed on fresh medium, gave rise to normal mycelium (Plate VI, Fig. 9). Within a few hours after death the fungus grew from the larvae and sporulated on the outside. (Plate IV, Fig. 1). Those that were placed on the medium alone, or on cultures together with the adult insects did not die within the same period.

The fungus also attacked termites. In all 198 of these insects were placed on cultures in nine petri dishes and within two days all were dead. Of the 72 placed on the medium alone only eight died during the same time. Those had been attacked by the fungus were full of hyphal bodies (Plate IV, Fig. 3), and were soon covered by the sporulating fungus.

IDENTITY OF THE FUNGUS.

A fungus practically identical with the above was described by Martin,* who found it in 1923 as a contamination on a plate of nutrient agar inoculated from a piece of very rotten wood. The fungus which was obviously one of the *Entomophthoraceae*, was shown to be a species of *Conidiobolus*, and because of the villose appendages of some of the older spores, was named *Conidiobolus villosus* n. sp. The fungus here described however, possesses certain characteristics which make its identity with *C. villosus* somwehat doubtful. Among these are its parasitism, and the stage in its life-history where several minute secondary spores arise from a single spore.

In 1933 a fungus very similar to *C. villosus* was isolated by Kevorkian† from living termites, of the genus *Nasutitermes*, which had been placed in damp chambers for observation. On obtaining sub-cultures of Martin's *C. villosus*, Kevorkian found that it was identical with the one isolated from termites : he demonstrated that it could adapt itself to a parasitic habit, especially on termites, and also observed the additional stage consisting of the production of several minute secondary spores arising from a single spore. After further studies Kevorkian felt justified in making the new combination *Entomphthora coronata* (Cost.) ; in this species he included Martin's *C. villosus*, the fungus which he himself had isolated from termites, an undetermined species of *Conidiobolus* isolated by Derx from an unknown source, and *Delacroixia coronata* (Cost.) Sacc. and Syd.

Notwithstanding the fact that the writer has observed certain additional minor characteristics such as the presence of spores without basal papillae and the small almond-shaped spores, the fungus described in this article must obviously be regarded as a strain of the same species, *Entomophthora coronata* (Cost.) Kev.

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* Martin, G. W.—Morphology af *Conidiobolus villosus*. Bot. Gaz. 83 : 311–318, pl. 16, 3 fig. 1925.

† Kevorkian, Arthur G.—Studies in the Entomophthoraceae I. Observations on the genus *Conidiobolus*. Journ. Agric. Univ. Puerto Rico. Vol. XXI, No. 2, 191–200, 3 pl. 1937.

PLATE I.

A sugar ant—*Campanotus* sp.—with its abdomen covered with, and attached to the ground by the loose cottony fungous growth. N.B.—The head is blurred on the photograph owing to movement as the ant was still alive.

PLATE II.

A collection of sugar ants showing various stages of the fungous growths.

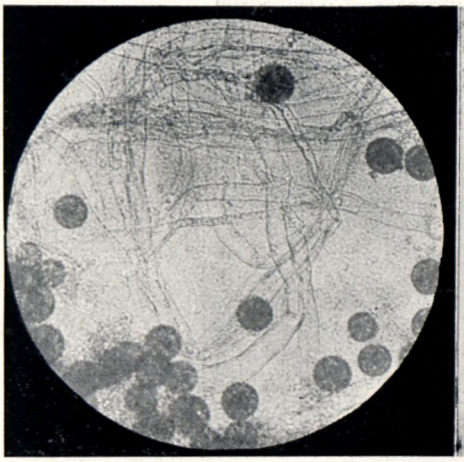

Fig. 1.

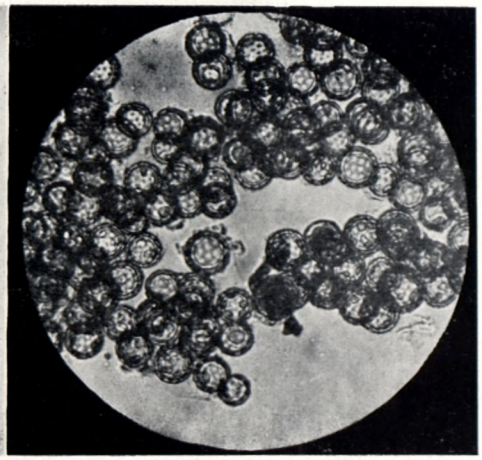

Fig. 2.

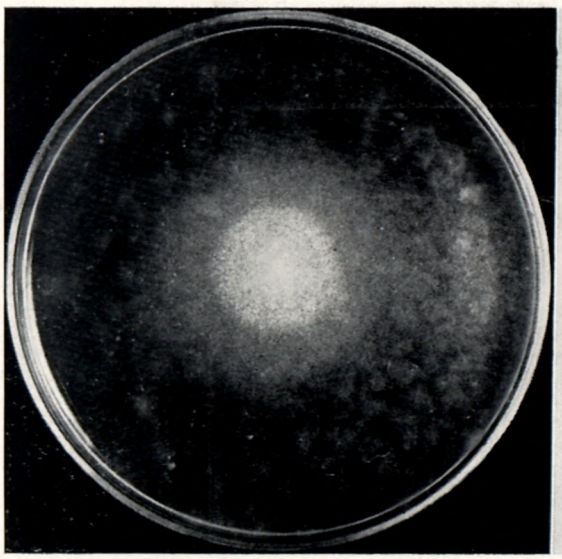

Fig. 3.

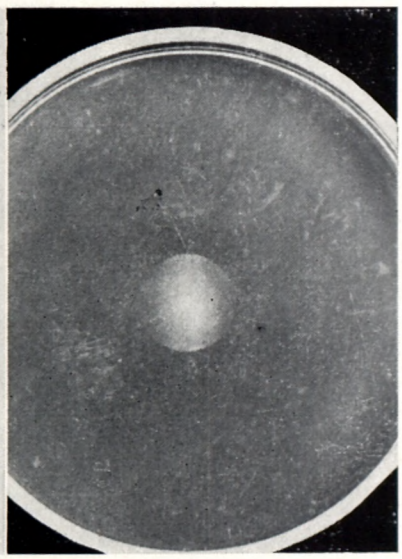

Fig. 4.

PLATE III.

Fig. 1.—The loose cottony growth as seen under the microscope.

Fig. 2.—The compact growth as seen under the microscope.

Figs. 3 and 4.—Development of the fungous colony on potato + 5 per cent. dextrose agar.

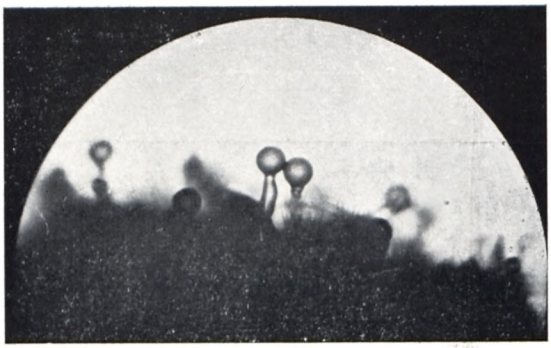

Fig. 1.

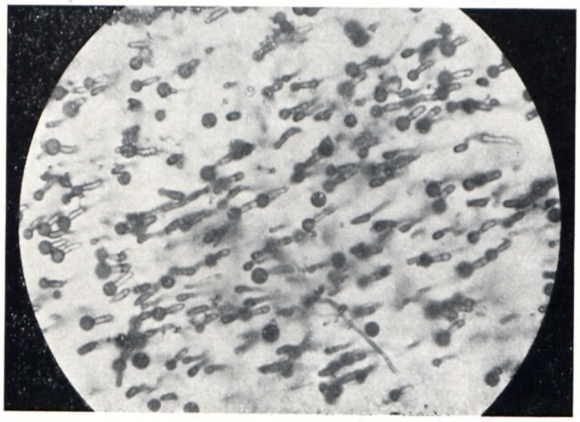

Fig. 2.

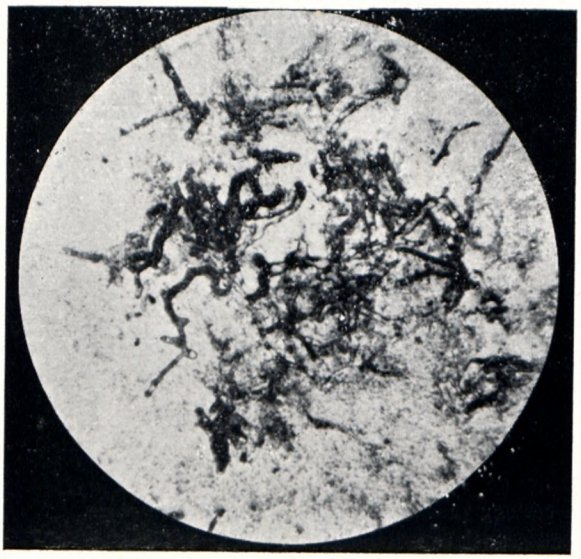

Fig. 3.

PLATE IV.

Fig. 1.—The fungus sporulating on sugar ant larva.
Fig. 2.—Sporulating culture as seen under the microscope.
Fig. 3.—Hyphal bodies from termites.

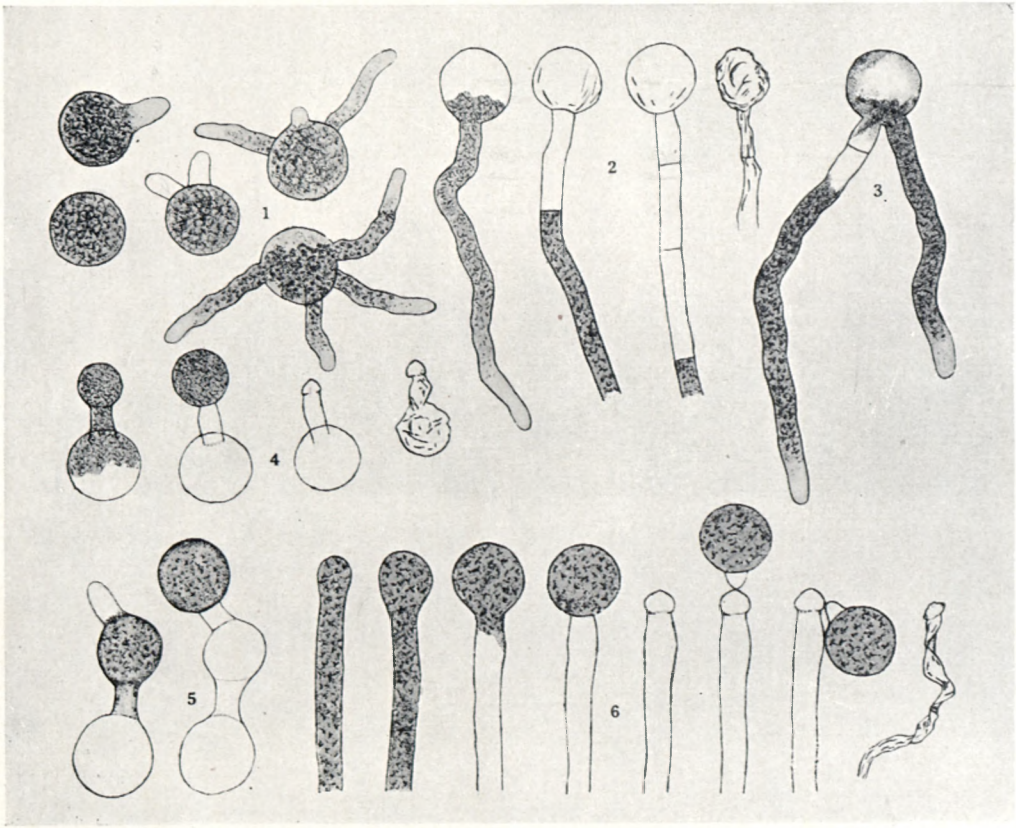

PLATE V (Camera lucida drawings).

Fig. 1.—Spores with 0–4 germ-tubes.

Fig. 2.—Protoplasm flowing out of spore and along germ-tube, emptied spore and hypha collapsed.

Fig. 3.—Spore protoplasm cut off by septum from longer germ-tube and now flowing into new germ-tube.

Fig. 4.—Formation and discharge of secondary spore.

Fig. 5.—Formation of tertiary spore.

Fig. 6.—Formation and discharge of spore: collapsed sporophore.

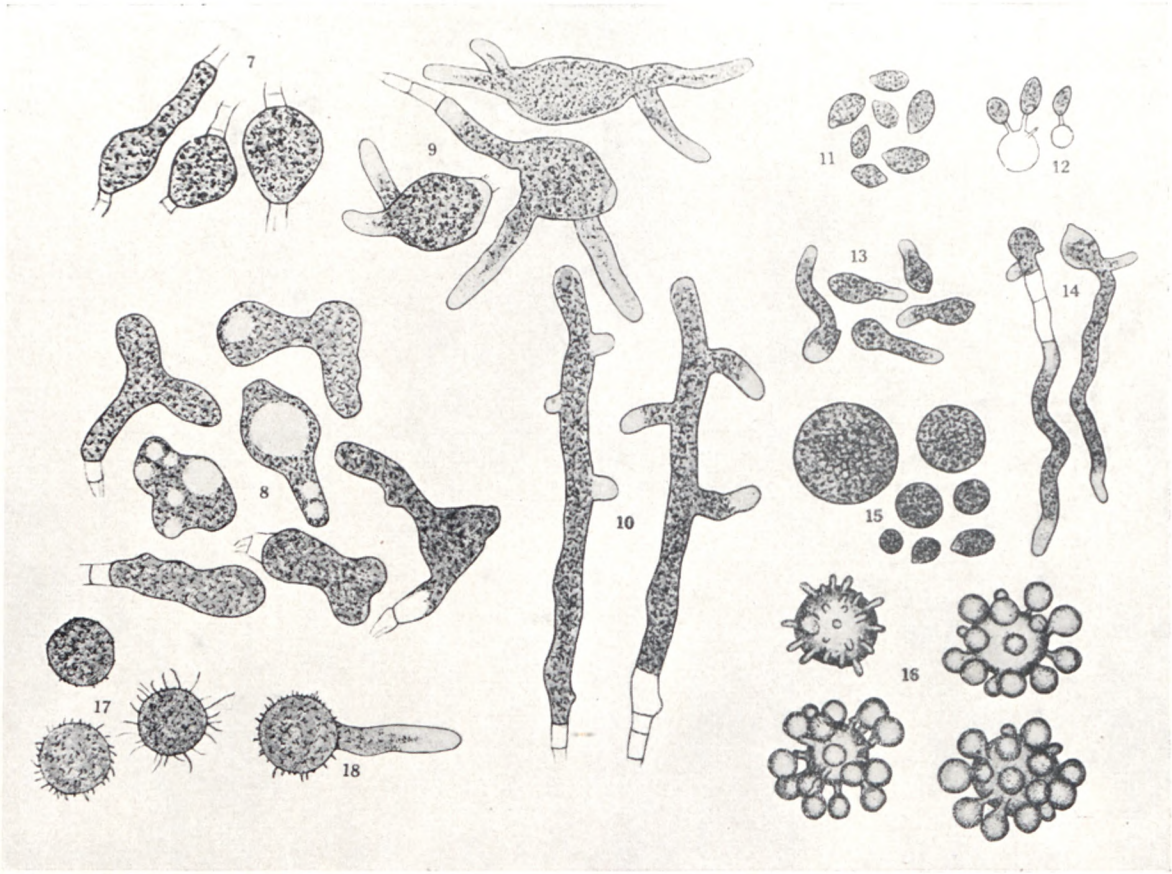

PLATE VI (Camera lucida drawings).

Fig. 7.—Hyphal bodies from culture ± 10 days old.
Fig. 8.—Hyphal bodies from ant larva.
Fig. 9.—Germinating hyphal bodies from ant larva.
Fig. 10.—Isolated segments of hyphae germinating on fresh medium.
Fig. 11.—Almond-shaped spores.
Fig. 12.—Development of almond-shaped spores.
Figs. 13 and 14.—Germinating almond-shaped spores.
Fig. 15.—Spores showing relative shapes and sizes.
Fig. 16.—Numerous small secondary spores borne on single primary spore.
Fig. 17.—Spores with soft hair-like outgrowths.
Fig. 18.—Germination of spores with soft hair-like outgrowths.

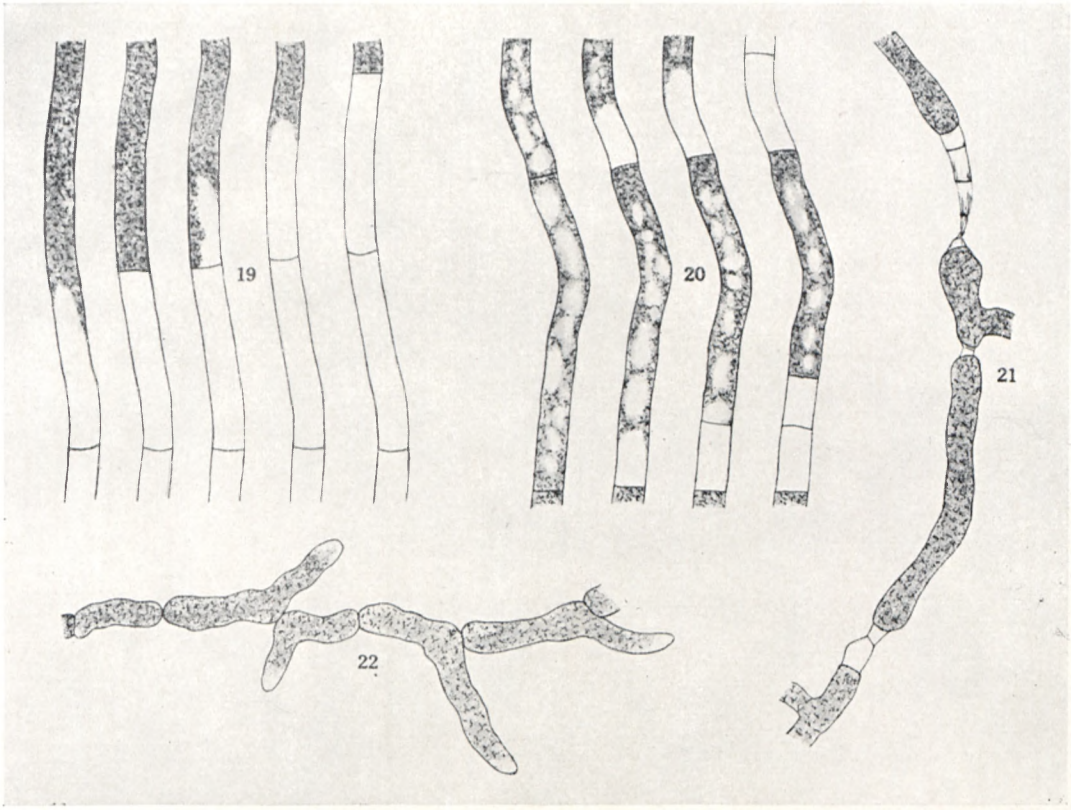

PLATE VII.

Fig. 19.—A hypha with flowing protoplasm successively cut off by septa.
Fig. 20.—Segment formation in partly filled hypha.
Fig. 21.—Isolated hyphal segments commonly found in culture.
Fig. 22.—Protoplasm-filled segments without intervening empty segments.