NOTE.

ON CELL-REGENERATION IN BOTRYTIS CINEREA.—The formation of cell-membranes as a response to traumatic stimuli has been very completely investigated in many groups of plants,¹ but with the exception of certain Mucorineae² little is known of this process in the Fungi. During the course of an investigation of *Botrytis cinerea* it was necessary to examine minutely fragments of mature growths teased out in water. Many of these preparations were maintained for several days, and regenerative changes in the injured mycelium were frequently noted. In these observations, which were merely incidental to the main work, no attempt was made to do more than record the behaviour of the living fungus, occasionally aid being sought in the use of *intra-vitam* stains such as neutral red, methyl violet, and especially Congo red.

The healing process and the rapidity with which it occurs depend to a large extent upon the age and condition of the particular cells affected. Speaking generally, hyphae which have been starved show less regenerative power than mycelium growing upon a physiologically suitable pabulum. No obvious effect of temperature or light was noted, although a more thorough examination devoted to this particular purpose might reveal its presence. Different regions of the same mycelium react unequally to approximately the same type of injury, the regenerative response apparently being related largely to the condition of vacuolation of the protoplast. Young actively-growing vegetative cells are usually filled with non-vacuolate homogeneous protoplasm, whilst of the older cells, those retaining cell-content usually exhibit a multi-vacuolate, honeycomb-like appearance. The conidiophores are highly specialized organs of strictly limited growth. The basal region is tinted and consists of mechanically rigid thick-walled cells in which the protoplast is disposed as a thin parietal layer about one or infrequently two large vacuoles. The upper sporogenous region is hyaline and thin-walled, and the cells are densely filled with homogeneous, finely granular or minutely vacuolate protoplasm in an extremely active condition. The latter cells possess by far the greatest capacity for regeneration: the vegetative hyphae and particularly the older vacuolate cells show this potentiality to a markedly less degree; whilst the cells of the lower region of the conidiophore are almost devoid of it.

In general the types of injury which have been observed to stimulate a healing reaction may be grouped into three categories; (1) acute flexion of cells; (2) punctures and relatively minute wounds; (3) relatively large superficial lesions. In certain injuries two distinct regenerative processes are involved: restitution of the original

¹ See Küster, E.: Pathologische Pflanzenanatomie, Jena, 1903, where the important literature is cited.

² Cf. especially Van Tieghem, P.: Ann. Sc. Nat. Bot., 1875.

cell-wall and the formation of a completely new membrane about the escaped protoplast.

Flexion of the hypha. When a hypha is acutely flexed the bending almost always occurs in the middle region of a cell. If the angle formed be less than forty-five degrees the cell-contents tend to separate into two portions, each of which rounds off its new surface, over which a membrane is formed. The flexion in the hypha tends to become rigid and permanent and the membrane at the point of bending stains more deeply with Congo red.

Punctures and minute wounds. If a cell be punctured or minutely wounded a thin stream of cell-content issues from the lesion and diffuses into the surrounding medium. The force of this jet gradually lessens and finally ceases, presumably with the equilibration of endosmotic and exosmotic forces, and the lesion becomes covered by a new membrane, so that frequently the region of injury can no longer be detected. Wounds of a diameter two-thirds that of the filament have been observed to heal in this way, and in some of the wider hyphae the exact manner of membrane restitution may be observed. Usually the new cell-wall substance is laid down simultaneously over the whole surface of exposed protoplasm, but occasionally regeneration proceeds by a centripetal diaphragm-like growth from the edges of the wound.

Large wounds. Injuries greater in diameter than from one-half to two-thirds that of the cell rarely heal by restitution of the original membrane, and often the entire cell-content diffuses into the surrounding medium. If, however, the wound be situated towards one end of the cell a certain quantity of protoplasm may remain in the other end, and this either dies *in situ* or rounds off its naked surface and forms a transverse septum closing itself in. More rarely this moiety of cell-content has been observed to undergo a process of rejuvenescence and clothe itself in a completely new cell-membrane, forming what may be regarded as an aplanospore. The latter may germinate, producing a hypha which either pierces the original cell-wall, simulating a normal hyphal branch, or grows longitudinally through the parental filament, living apparently in a parasitic manner.

Not infrequently a hypha is sheared across so that a cell is divided cleanly into two portions. The protoplasm usually diffuses from the open ends, but one or both moieties have been observed to round off their naked surfaces and form closing septa.

Severe crushing or considerable laceration of a cell usually results in the death of the protoplast.

Membrane formation by free protoplast. As a rule when a cell is injured part or all of the cell-content is ejected and is lost by diffusion into the surrounding medium. Rarely, however, the entire protoplast is released as a single unit, and of three such cases which were noted, one died, and two assumed a spherical shape and formed about themselves new cell-walls. Subsequently they elongated into slender hyphae giving rise to growths indistinguishable from rather delicate normal mycelium.

In all instances of cell-restitution such as have been described growth proceeds only after the enclosure of the exposed protoplasmic surface by a cell-membrane. In the case about to be described, however, this procedure was reversed and a certain amount of free protoplasmic growth occurred prior to cell-wall formation.

A water preparation was made about noon on Wednesday, retained in a moist condition and re-examined four and a half hours later. It was then noted that a moiety of protoplasm remaining in the lower end of a cell situated at the sporogenous tip of a mature conidiophore had not rounded itself off in the usual manner, but showed marked pseudopodial elongations of its naked surface, one such lobe having penetrated the membrane of the parental cell (Fig. 1).¹ The adjacent cell in the conidiophore was minutely vacuolate and perfectly normal in appearance. The lower portion of the free protoplasm in the injured cell contained a number of minute granules and a few small vacuoles. At the naked growing surface the protoplasm was homogeneous. The lobular prolongations were clear and definite, and, with the exception that no trace of a streaming movement could be detected, bore a striking resemblance to those of an *Amoeba*. The structure was unmistakably a pseudopodial growth of a naked surface of protoplasm. By noon the following day the

FIG. 1.

FIG. 2.

FIG. 3.

protoplast had increased to about twice its original bulk, and the irregularly-lobed pseudopodia were very marked, several having pierced the parental sheath (Fig. 2). Many more minute vacuoles were present in the lower region, whilst along the middle of the lobes and scattered generally through the substance of the protoplasm were large numbers of very fine granules. The edges of the lobes were hyaline and homogeneous. The preparation was again examined at 2.20 p.m. on Friday, when the facies of the specimen was found to have completely changed (Fig. 3). The irregularly-lobed naked pseudopodia had become smoothly digitiform, and were enclosed in thin but quite definite cell-membranes which extended down to the wall of the adjoining cell. In one of the processes a fine transverse septum was visible, and the entire structure merely formed a cluster of minute and delicate radiating hyphae arising in an injured conidiophore. By 10 a.m. the following day the three

¹ The drawings were made with a Zeiss camera lucida; and Swift one-eighth objective NAO. 92 with No. 8 compensating oculars were used. They have been reduced to one-half.

most prominent hyphae had developed into slender filaments bearing lateral branches ; and subsequently all the processes gave rise to normal mycelium.

If water preparations of *Botrytis* conidiophores be maintained for some days, clustered hyphal growths originating in wounded sporogenous tips and identical with that noted above are not rare. The development of such structures by the more usual regenerative processes has not been observed, and it does not appear improbable that they may arise in the same manner as the one described.

The protoplasm of the sporogenous tips of the conidiophores exists in an extremely active and plastic condition, this being exemplified by its immense regenerative capacity ; and, as is evident in the present specimen, this protoplasm is able, under certain conditions and for a period of time, to live and grow when part of its surface is in a free plasmodial state, and only subsequently enclosed in a cell-wall.

If this potentiality be not restricted to the fruiting hyphae of *Botrytis cinerea*, but be distributed widely among the Fungi, and in particular be an attribute of the vegetative mycelium of pathogenic forms, it will have an important bearing upon general ideas of fungal morphology.

That there is no inherent impossibility in the growth and development of naked protoplasm is evident in the existence of the Protozoa and Myxomycetes. Even in the Fungi proper there are forms, such as many Synchytriaceae, in which the organisms live for the greater part of their life as protoplasmic masses free from any enclosing cell-membrane. In the Bacteria, organisms probably of a degenerate fungal nature, evidence has recently been presented¹ of a free plasmodial or 'symplastic' state.

In a research on the behaviour and morphology of the vegetative mycelium of *Botrytis cinerea* in the tissues of *Aesculus Pavia*, which is now being prepared for publication, it was found that certain of the hyphae were in a naked condition, existing as free protoplasmic substance.

Finally it may be noted that for years the Swedish school of phytopathologists have persistently maintained the conception of mycoplasm, and quite recently Eriksson has used his hypothesis in the elucidation of the many problems of the causation of the potato blight by *Phytophthora infestans*.² Mycoplasm involves far more than the mere existence of fungus protoplasm in a naked state, and the merging and subsequent dissociation of the individualities of host and fungus, which is the vital part of this hypothesis, must still be regarded as 'non-proven'. It would seem, however, somewhat injudicious merely to ignore every aspect of this idea or summarily dismiss it as impossible, for in the light of what has been written above it may be that buried beneath much interpretation there is a basis of fact.

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¹ Löhnis, F., and Smith, N. R. : Journ. Agr. Res., vol. vi, 1916.

² Eriksson, J. : Rev. Gen. Bot., tome 30, 1918.

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