A case of melano-mycosis of the skin, with remarks, &c; / by Sheridan Delépine.

Reprinted from the 'Transactions of the Pathological Society of London,' 1891.

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By Sheridan Delépine.

[With Plates IX and X.]

GENERAL SUMMARY.

I. Preliminary Remarks.

I am indebted to one of my pupils, Mr. Wild, for bringing this case under my notice, and for helping me in getting the following clinical notes. Had he not been struck by the unusual appearance presented by the ulcers, and removed the few shreds of epidermis which he brought to me for examination, this very interesting case would have entirely been lost to me.

II. History of the Case; Nature of the Lesions.

The patient, a man 27 years of age, was admitted on the 11th of May, 1890, into St. George's Hospital, under the care of Mr. Rouse, for fracture of the right femur. He had eight years previously sustained a compound fracture of the right tibia at the junction of the upper and middle thirds of the leg. As a result of that previous accident there was a scar on the inner and anterior aspect of the leg, which was otherwise apparently sound.

The leg was put up in Desault's and three short splints, and strapped with ordinary soap plaster from the ankle to just below the knee. The foot was bandaged as usual with cotton bandage. At the time of "putting up" *there was no wound below the knee*, and with the exception of the scar remaining from the previous fracture the skin was normal.

On June 13th the strapping was removed from the leg, when it was noticed that both strapping and leg were in two situations covered with a black substance closely resembling soot. In these two places there were also two ulcers. These ulcers were evidently superficial. The skin surrounding them was covered with the brown dust, and wherever this substance was present the cutis was moist, and the epidermis peeled off easily. (A small shred of the cuticle covered with the suspicious substance was removed for microscopical examination.) The lower ulcer was quite near the upper margin of the cotton bandage; notwithstanding this the black stuff did not extend at all beneath it; it, on the contrary, was most abundant where the soap plaster came in contact with the skin, and was absent where this was not the case. There was no trace of it on the outer side of the leg.

The upper ulcer corresponded exactly to the region where the skin had been torn by the broken tibia eight years previously.

These ulcers, although they frightened the patient greatly, were not painful, and healed rapidly after thorough washing and boracic acid dressing.

Microscopical examination of the epidermis.—Under the microscope the cuticle was found to be literally covered with small round bodies, resembling closely red blood-corpuscles both in shape and size, but presenting a dark brown colour. Many of these bodies were crenated, and this increased their resemblance to red blood-corpuscles.

These bodies measured from 3·5 to 6 μ, generally speaking about

5 μ; most of them were spherical, but many also were flattened. I naturally suspected them to be the conidia of some pathogenic mould, and I therefore immediately inoculated some nutrient gelatine tubes; and in less than a week, at a temperature of 20 to 23° C., I obtained an abundant growth of mycelium with conidia, entirely similar to the round bodies found in the skin. The mould thus obtained had all the characters of the *Aspergillus niger*.

III. Description of the Aspergillus niger.

Flügge says (p. 119), in connection with that fungus,—

"We distinguish (after Siebenmann) the so-called *true* Aspergillus (*Asp. clavatus, flavus, fumigatus, niger*, &c.) and the two *Eurotium forms* proper (*Eurotium aspergillus-glaucus* and *Eurotium repens*)."

Further, he describes the *Aspergillus niger* as follows (p. 121):

"Dark brown masses. The knob of the fruit hypha completely spherical. Sterigmata 20—100 μ in length, branched like a hand. Conidia round; when ripe blackish brown; diameter 3·5—5 μ. Sclerotia brownish red, of the size of a rape seed. Best temperature 34° to 35° C."

It will be seen from the drawings accompanying this paper that the organism which I found in the diseased skin brought to me by Mr. Wild corresponds exactly to the above descriptions.

I may add that all the Aspergilli I have examined are extremely variable in their characters, the conidia being about the only parts the appearance and measurements of which can be considered to have any constant value. And even these have to be considered in the light of surrounding circumstances. Thus I found that those which covered the moistened epidermis around the cutaneous ulcer in this case were, generally speaking, vesicular-looking, spherical, and of the size of a small red blood-corpuscle. The pigment seemed to be mostly near the surface, under a double outlined capsule, and at times it projected beyond the surface, having apparently passed through a small opening in that capsule; this gave to the conidia the crenated appearance already alluded to. When, on the contrary, the conidia were obtained from a rather dry cultivation, and before they had come in contact with any watery fluid, then their size was much smaller, barely measuring 3 μ in most cases. They were also more deeply pigmented; the pigment seemed to be uniformly distributed through their contents, &c. &c.

I have observed similar variations in the spores of all the other Ascomycetes I have had an opportunity to study.

These variations are not only the results of conditions of moisture and dryness, but they depend on numerous factors, and, I have no doubt, correspond to various modes of growth and conditions of activity.

The work of Pasteur and his followers strongly points towards the importance which the study of the variability of properties (quite independently of the mutability of species) must have in connection with the origination and modification of diseases.

It seems, therefore, to me that a careful study of micro-organisms which have very characteristic features and stages of growth may help towards the elucidation of some important ætiological problems. I think also that the most suitable objects for such studies are organisms, such as many of the Aspergilli, which are (1st) *facultative parasites*, and which have (2nd) a pretty definite position in the scale of living beings. The first condition gives us organisms which are, so to speak, in a transitional state (that is, admitting that *saprophytes* are capable of becoming gradually *obligatory parasites*). The second condition allows us to compare the various biological relations which determine virulence, either independently of purely physico-chemical factors, or more probably under the direct or indirect influence of external conditions. It is in the hope of helping a little in the investigation of such questions that I have taken advantage of the opportunities offered to me by several cases of mycosis *observed in man* to make a few experiments, some of which I will briefly relate.

Before doing so I must, however, indicate my reasons for believing in the pathogenic properties of the *Aspergillus niger*.

IV. Evidences of the Pathogenic Properties of the Aspergillus niger.

1. *Facts of the case.*

The fact that in the case here related the fungus was found growing abundantly in the skin surrounding and on the plaster covering the ulcers seems to indicate clearly that there was a connection between the Aspergillus and the ulcer. It would be difficult, however, from this to conclude that the fungus was the cause of the ulceration, since it might be urged with equal probability that the fungus had grown only where the skin had become ulcerated for some reason or other.

The removal of the Aspergillus was, however, followed by rapid cure; and this undoubtedly strengthens the view that it was there as a parasite, and not as a saprophyte. Before coming to this conclusion it is, however, necessary to prove that no other organism than the Aspergillus was present in the wound; and that, by inoculation with pure cultivation of the *Aspergillus niger*, it was possible to induce lesions directly connected with the presence of that fungus.

2. *Cases and experiments of various observers.*

Such experiments have been made by a few observers with several Aspergilli, the *Aspergillus niger* included — Lichtheim, Leber, Schütz, &c.; and it has been proved that severe inflammation may be started by these moulds when there is a sufficiently free access of air. It has, however, also been recognised that the *Aspergillus niger* germinates imperfectly in living tissues. Several observers have brought forward cases in which Aspergilli closely allied with the *Aspergillus niger* have apparently caused serious lesions of the integument lining the EXTERNAL AUDITORY meatus—*Pacini, Meyer, Bezold, Bizzozero,* &c.; the CORNEA—*Leber;* the LUNGS —*Rother, Lichtheim, Schütz, Schubert,* &c. There is, therefore, altogether strong evidence in favour of the view that certain Aspergilli may cause lesions; it is also evident that among these pathogenic fungi the *Aspergillus niger* is probably one of the least virulent.

No case of primary affection of the skin by the Aspergillus niger has, so far as I know, *been recorded yet.* I thought, therefore, that although the evidence of the case itself was strongly in favour of the possibility of such an infection, it would be desirable to obtain a new demonstration of the pathogenic properties of that organism.

3. *Inoculation experiments on rabbits.*

I therefore collected conidia from one of the cultivations I had obtained from the spores found on the skin ulcers, and mixed them with sterilised water. Dr. Woodhead had the great kindness to inject this mixture for me into the peritoneal cavity of one rabbit, and into the anterior chamber of the eye of another rabbit. For this I wish to express my best thanks to him here, as, by the interest he took in the question, he saved me the trouble and delays which, in experiments of this kind, are generally experienced

in this country. Thirty-six hours after the injection the inoculated eye was distinctly affected. The conjunctiva was considerably inflamed and swollen; the anterior chamber was partly occupied by a white and opaque material, not looking like pus, and extending from the edge of the cornea towards the centre, leaving the greater part of the space in front of the pupil clear.

There was also distinct photophobia. Forty-eight hours afterwards, the animal having been killed, I found that the whitish matter was almost entirely composed of epithelial-looking cells, among which were a few swollen spores. The greater number of spores had, however, disappeared from the anterior chamber, having probably passed into the lymphatics or been destroyed. Owing to the difficulty of tracing the distribution of brown spores in the midst of the pigmented tissues of the eye, I have so far confined my observations to the rabbit in which the parasite had been injected into the peritoneum.

In this animal there were hardly any evidences of discomfort, except perhaps that thirty-six hours after inoculation it seemed quiet, and kept the abdomen more raised from the ground than is usual in healthy rabbits. Forty-eight hours after inoculation the abdomen was opened immediately after death. There was no evidence of general peritonitis, but at the seat of puncture, and in various other parts, numerous tubercles were found. Their diameter varied from ½ mm. to 3 or 4 mm. Many of them had some blackish material in their centre.

These nodules were most abundant in and about the central tendons of the diaphragm, the suspensory ligament of the liver, the mesentery, and the peritoneal folds about the back and sides of the bladder. There were, however, several small tubercles in the parietal layer of the peritoneum, and on the surface of several viscera, such as the liver and the intestine.

Some of the mesenteric glands were enlarged, congested, and contained numerous spores.

On microscopical section these tubercles were found to contain one or several groups of the brown spores. Even the smallest tubercles, which to the naked eye did not seem to contain any spores, were found under the microscope to follow the general rule.

Around each group of spores there was a zone of small round-cells, more or less altered, but evidently leucocytic in nature. Outside this zone there was in all cases a considerable increase of

fixed connective-tissue corpuscles, which formed round each tubercle a distinct layer of embryonic connective tissue.

In the case of the nodule found on the surface of the liver the subjacent tissues were much altered; thus many of the liver-cells had completely degenerated. In presence of such active changes I was surprised to find that there were only slight indications of germination on the part of the spores, especially where they had accumulated in large numbers. In none of the lesions I examined did I find any typical mycelium, such as has been observed in some of the cases of mycosis of internal organs which have been described. There could not be any doubt, however, as to the activity of the spores, and their appearance corresponded exactly to that which I observed in spores embedded for several days in the midst of a nutrient medium without access of air. Such spores germinate very slowly indeed, even at the temperature of the body.

Notwithstanding this, I suspected that perhaps I had introduced some other pathogenic organisms with the spores of the Aspergillus. I therefore stained several sections by the usual methods used for bringing out various forms of bacteria, but I failed to discover any other organism than the *Aspergillus niger* in them.

It seems, therefore, probable that during the early stages of germination some very irritating product must escape from the spores of that fungus, and that the extension of the mycelial filament causes only a small part of the irritation of the tissues, since, before any serious mechanical disturbance has occurred from that cause, lesions of considerable magnitude have already been produced.

4. *Effects of accidental inhalations in man.*

Involuntarily I obtained on two occasions experimental confirmation of that fact. Whilst cultivating the *Aspergillus niger* on a large scale for the purpose of studying its properties I had twice the misfortune to expose myself to breathe air loaded with conidia. In a few hours I became affected with an intense coryza, and redness of the conjunctivæ accompanied with fever and frontal headache. The day after the bronchi were also slightly affected. Each time I was in much discomfort for about three days, and then the symptoms subsided. Each attack reminded me strongly of hay fever, and to a certain extent of influenza. The first time, either owing to the headache or for some other reason, I felt very ill for twenty-

four hours; this was noticed not only by myself, but by several other persons. (It is quite possible that the spores of some of these moulds may have more to do with the production of hay fever than is suspected at the present time.)

For all these reasons I feel justified in considering that the *Aspergillus niger* is capable of inducing a considerable amount of irritation of animal tissues; and that although it is not under ordinary circumstances a serious source of disease, it may at times induce lesions, of which I give some instances in this paper.

5. *Form of parasitism; influence of heat, moisture, and lowered vitality of affected tissues.*

This fungus is, however, not a true parasite (obligatory parasite of De Bary), but essentially a saprophyte, becoming parasitic at times (*facultative parasite*—De Bary).

The conditions under which the Aspergillus niger *may become parasitic* are well indicated in this case. They are essentially—

1st. High external temperature.

2nd. Moisture due to retained secretions.

3rd. Inactivity or lowered vitality of the parts affected.

The *high external temperature* is necessary to promote the rapid production of the spores by the Aspergilli growing on their ordinary pabulum, which consists chiefly of decayed animal and vegetable matters, as will be shown hereafter. The present case occurred during the hottest months of the year 1890 (June, July), when I found it easy to grow the fungus on almost any material without the aid of artificial heat.

The *moisture* was the result of the retention of the secretions of the skin, the impermeable soap plaster preventing entirely evaporation. It is to be noticed that where the skin was covered with cotton bandage, and therefore comparatively dry, there was no trace of mould.

How the spores got under the strapping can only be surmised. The strapping itself was not mouldy. No other case of mycosis occurred in the hospital, although other portions of the same material had been used. There was no evidence of affection of the skin at the time the limb was strapped. One is therefore driven to conclude that either the strapping or the skin had, just previously to dressing, been contaminated with spores of the *Aspergillus niger*. At that period of the year it is quite possible that the contamina-

tion may have taken place through the air. A single spore, as I have proved by cultivation, is capable of giving rise to several cubic centimetres of spores in the course of a few weeks.

The *effects of lowered vitality* are well shown by the occurrence of ulceration in the cicatrix of an old ulcer which partially seemed to have reopened.

V. Development and Variability of the Aspergillus niger.

1. *Observations.*

The considerations which precede show that the production of parasitic lesions is partly determined by the *respective states of the possible parasite and host;* they therefore invest with interest any study which may help one to grasp the relation between the various stages of the life of any pathogenic organism, or to understand the condition which may influence the greater or less development of any of these stages.

I have summarised the results of some experiments which I have made in this connection. They were carried out by means of ordinary *drop, test-tube, plate* (*capsule*), and *flask cultivations;* in addition I used the *interlamellar method* which I have described elsewhere.[1] Various cultivation media were used. These were either the usual ones—*e. g.* 1, potato; 2, ordinary glycerine nutrient gelatine (Nocard and Roux); 3, agar-agar; 4, glycerine agar-agar; 5, hydrocele fluid,—or simple substances such as—6, distilled water; 7, starch; 8, glucose; 9, cellulose; 10, gum-arabic; 11, egg albumen; 12, peptones; 13, pure gelatine; 14, paraffin,—or, again, various tissues, such as—15, horny epithelium; 16, muscular tissue; 17, adipose tissue. These cultivations were made in sets, and the effects of *temperatures ranging from* 10° *to* 45° *C.* were studied either in parallel specimens or in specimens submitted alternately to high and low temperatures. The effects of *free or limited access of air* were observed in some cases, and the effect of temperature was also noticed with reference to the *vital concurrence* of the *Aspergillus niger* and *Penicillium glaucum.* The action of a few unknown bacilli was also witnessed in some cases quite accidentally. The *importance of moisture* was also ascertained in several cases.

It will be easy to understand the results of these experiments from a study of the following tables.

[1] 'Lancet,' June, 1891.

No. of experiment.	Date of inoculation.	Material inoculated.	Mode of cultivation.	Temperature.	Appearance of mycelium.	Appearance of conidia.	Time when first noticed appearance: Mycelium.
1	1890 June 13	Glycerine, nutrient gelatine	Tube	20°–25° C.	1890 June 14–15	1890 June 15–16	Hours 36
2	„	Glycerine, agar-agar	„	„	June 16	June 18–19	72
3	June 20	Potato	Capsule	„	Rapid	Rapid	—
4	„	Water	Drop cultivation	„	Delayed	—	—
5	„	Nutrient gelatine	„	„	June 20–21 ?	June 21–22 ?	—
6	„	Hard paraffin and distilled water	Inter-lamellar film	„	July	July	—
7	July 2, 8 p.m.	Nutrient gelatine	„	„	July 3, 2 p.m.	July 7	Less than 18
8	July 2	Agar-agar	„	„	? later than 7th	? later than 7th	—
9	Dec. 31	Potato	Capsule	23° C.	1891 Jan. 2	1891 —	48
	1891 Jan. 2	—	—	10°–15° C.	—	Jan. 14	—
	Feb. 24	—	—	23°–30° C.	—	—	—
10	„	Potato	Capsule	23°–25° C.	Feb. 25–26	Before March 7	24 about
11	„	Starch	„	„	Feb. 26–28	„	48 about
12	„	Pure gelatine	„	„	Feb. 26	„	„

the chemical ts formed in fter growth of ungus.	Remarks.	Mother cultivation.	
		Nutrient material.	Date.
faction of ne, crystal- xtractives; te of lime undant	... ...	Ulcer, skin	1890 May 11–June 10.
siderable of oxalate f lime	... ...	„	„
l products tening of otato)	... ...	Nutrient gelatine, No. 1	June 13.
?	... ...	Gelatine, No. 1	„
s crystalline cts soluble ater and ; oxalate of abundant	... ...	„	„
?	Mycelial filaments penetrating between the layer of paraffin and the cover-glass; the ends of the filaments produce conidia without previous formation of fruit-bearing hyphæ or distinct stigmata	Gelatine, No. 5	June 20.
us crystal- e products, le in water alcohol ?); ant produc- f oxalate of lime	Formation of spores delayed owing to experimental drying; abundant formation of oxalate of lime when filaments reach surface; gelatine liquefied and ultimately transformed into crystallisable products	Potato, No. 3	„
—	... ...	„	„
—	... ...	Gelatine, No. 1	June 13.
—	Between Jan. 2 and Jan. 14 a green mould [1] has appeared and grows rapidly, whilst the black fungus has grown slowly	„	„
—	The green stops by March 7; the black encroaches upon the green, and ultimately covers the whole surface	„	„
o softened ightly dis- loured lucose	March 7: green mould[1] appears at side. March 9: the black mould appears to overtake it, and causes it to remain stationary	Potato, No. 9	Dec. 31.
	Mycelium very thin, widely spread; conidiophores discrete	„	„
se or gela- peptone	March 9: gelatine liquefied, mycelium thin, conidiophores separate; no fairy rings	„	„

[1] *Penicillium glaucum.*

No. of experiment.	Date of inoculation.	Material inoculated.	Mode of cultivation.	Temperature.	Appearance of mycelium.	Appearance of conidia.	Time when first noticed appeared: Mycelium.
	1891				1891	1891	Hours
13	Feb. 24	Nutrient gelatine	Capsule	23°–25° C.	Feb. 25–26	Feb. 28–29	24
14	„	Agar-agar (rather dry)	„	„	March 7	0	260
15	„	Glycerine, agar-agar	„	„	Feb. 27	March 9	72
16	„	Glucose (saturated nearly)	„	„	0	0	∞
17	„	Egg albumen (undiluted and possibly too dry)	„	„	0	0	∞
	March 9	—	—	39°	—	—	—
18	Feb. 24	Potato	Capsule	23° C.	Feb. 26	?	48
19	„	Starch (rather dry)	„	„	Feb. 27	Before March 7	72
20	„	Glucose (nearly saturated solution)	„	„	0	0	∞
	March 9	—	—	39°	—	—	—
21	Feb. 15	Peptone, Denaeyer's	Flask	10° C.	0	—	—
	March 5	—	—	39° C.	March 7	March 12	48
22	March 7	Potato	Capsule	39°–40° C.	March 8	Before March 9	12–24
23	„	Potato inoculated with *Asp. niger* on one side and *Penicillium glaucum* on other side	„	39°–40° C.	„	March 8	„
	March 12	—	—	35° C.	—	—	—

the chemical s formed in er growth of ngus.	Remarks.	Mother cultivation.	
		Nutrient material.	Date.
uefied. e of lime	Spores not very abundant, central tuft; fairy rings produced by intermittent growth of mycelium and spores; growth contaminated with *Penicillium glaucum*	Potato, No. 9	1890 Dec. 31.
—	Growth apparently stops after formation of a little mycelium, possibly the medium too dry; placed in 39° C. incubator; hardly any change	„	„
—	Mycelium very yellow; spores hiding nearly the whole mycelium. March 7: contamination	„	„
—	March 9: temperature raised to 39·5° C. No change after many days	„	„
—	... ...	„	„
—	Temperature raised to 39° C., but after many days no change can be observed	—	—
—	... ...	Agar-agar, No .	June 13.
ucose	Rapid extension of a thin layer of mycelium all over the starch; the conidiophores much separated from each other, quite discrete	„	„
—	... ...	„	„
—	Temperature raised to 39°, but with no effect	—	—
—	... ...	—	—
—	Mycelium very scanty at first and distinctly yellow. Conidia also very few, growth evidently difficult. The fluid became contaminated after first opening, and the Aspergillus soon ceased growing	Gelatine, No. 1	June 13.
—	Excessively rapid growth of mycelium, which becomes heaped up in a hemispherical mass, soon becoming folded. Peripheral extension seems to be limited by the abrupt depression of the parts of the potato over which mycelium has grown. Mycelium very yellow; conidia few	Potato, No. 10	1891 Feb. 24.
—	The *Penicillium glaucum* shows no sign of growth till March 12	„	„
—	The *Penicillium glaucum* does not show any sign of growth. The *Asp. niger* grows quicker than at 39° C., especially the mycelium	—	—

No. of experiment.	Date of inoculation.	Material inoculated.	Mode of cultivation.	Temperature.	Appearance of mycelium.	Appearance of conidia.	Time when first noticed appearance: Mycelium.
	1891 March 18	—	—	23° C.	1891 —	1891 —	Hours —
24	March 13	Human epidermis	Capsule	39° C.	March 14	Before March 16	24–36
25	,,	Subcutaneous fat	,,	,,	March 15	,,	48
26	,,	Human muscle	,,	,,	March 14	,,	24
27	March 16	Coagulated hydrocele fluid	,,	39°–40° C.	Before March 18	Mar. 18–19	24–48
28	,,	,,	Flask	23° C.	March 19 0	March 19 0	?
	March 20	—	—	39°–40° C.	March 21 ?	Before March 23	24
29	March 18	Swedish filter-paper	Capsule	39° C.	March 19	March 20	24
30	,,	Pure gum arabic	,,	,,	,,	Before March 23	24
31	April 30	Potato	,,	40°–45° C.	Before May 1	?	12–24
32	,,	Gelatine	—	,,	May 1	?	24
	May 3	—	—	—	—	—	—
33	April 30	Starch	—	40°–45° C.	—	—	—
34	,,	Paper	—	,,	0	—	—
35	,,	Egg albumen	—	,,	0	—	—

f the chemical cts found in fter growth of ungus.	Remarks.	Mother cultivation.	
		Nutrient method.	Date.
			1891
—	After 1 day at 23° C. the mycelium of *Penicillium glaucum* begins to grow. On March 23 green conidia abundant. The *Asp. niger* has grown slowly	—	—
—	In less than 3 days an area measuring 3 inches in diam. is covered with a radiating white mycelium, with a central clump of brown conidia. At the same time a very strong smell of manure is developed, such as is observed in connection with certain animal manure during the hottest days of summer	Nutrient gelatine, No. 13	Feb. 24.
—	Growth of mycelium and conidia very scanty compared with last specimen. Mycelium is denser and whiter. The smell is the same, but after a time a smell of rancid fat is superadded	,,	,,
—	The growth of the mycelium and spores incredibly rapid; in less than 1 week a surface 2½ inches in diameter is entirely covered with conidia; the mycelium is almost invisible. The conidia are of a more reddish colour than in other cases. Smell as above (24)	Gelatine, No. 13	,,
ceoses, &c.	Growth very rapid though not dense. Conidiophores discrete; tendency to arrangement in rings (like fairy rings). Smell of manure	Human muscle, No. 26	Mar. 13.
—	Same as above. On March 23 the cultivation is left at the ordinary temperature (under 20° C.)	,,	,,
Id.	Growth arrested at once.		
Glucose	Mycelium distinctly yellow. Conidiophores discrete, as in starch cultivations. Slight tendency to the production of concentric rings (fairy rings)	Hydrocele, No. 27	Mar. 16.
acid, acetic glucose (?), &c.	Extraordinary mycelial growth. In 5 days the mucilage has become almost white through it. This mycelium forms a kind of felt, holding in its meshes gigantic crystals of oxalate of lime. Conidia scanty. Smell very sour	,,	,,
—	Very rapid growth of mycelium; very yellow and heaped up; pleasant and somewhat fruity smell. The temperature hardly reached 45° C., and that for at most 1 hour	,,	,,
—	Very rapid growth of mycelium	,,	,,
—	The temperature hardly reached 45° C., and that for at most 1 hour.		
—	The temperature remained at 45° C. for several hours	,,	,,
—	Ditto	,,	,,
—	Ditto	,,	,,

No. of experiment.	Date of inoculation.	Material inoculated.	Mode of cultivation.	Temperature.	Appearance of mycelium.	Appearance of conidia.	Time when w first noticed t appearance o	
							Mycelium.	Con
	1891				1891	1891	Hours	Ho
36	May 1, 3–4 p.m.	Nutrient gelatine	Inter-lamellar film, spores 0·1 mm. from surface	40° C.	May 2, 10 a.m.	—	Less than 18	–
	May 2–3	—	—	25° C.	—	—	—	–
37	May 1, 3–4 p.m.	Nutrient gelatine	Inter-lamellar film, spores on surface and within 0·8mm.	40° C.	Before May 2, 10–11 a.m.	Before May 2, 10–11 a.m.	Less than 12	Le th 1
	May 2	—	—	25° C.	—	—	—	–
38	May 1	Nutrient gelatine	Inter-lamellar film, spores 10 mm. from surface	40° C.	May 2, 10 p.m.	∞ ?	About 30	–
	May 2	—	—	25° C.	—	—	—	–
39	May 1	Nutrient gelatine	Inter-lamellar film, spores 10 mm. from surface	40° C.	?	∞	—	–
	May 2	—	—	25° C.	—	—	—	–
40	May 1	Nutrient gelatine	Inter-lamellar film spores, 15 mm. from surface	40° C.	∞	∞	—	–
41 to 45	—	„	—	—	—	—	—	–
46	April 30, 4 p.m.	Rabbit, black	—	—	May 2, 2 p.m. ?	—	—	–
47	„	Rabbit, grey, peritoneal cavity	—	—	—	—	—	–
	May 4	—	—	—	—	—	—	–
48 [1]	A	Egg-albumen coagulated	Capsule in air saturated with moisture	38°	Aug. 8	Aug. 9,	—	–

[1] This additional observation, made after the reading of the paper, shows that on coagu circumstances of moisture and temperature.

me of the chemical roducts found in dia after growth of fungus.	Remarks.	Mother cultivation.	
		Nutrient material.	Date.
			1891
—	Growth of mycelium very rapid; mycelial filaments thick, branching, and septate. Spores beyond 2 mm. in depth hardly altered	Starch, No. 19	Feb. 24.
—	... ...	—	—
—	The surface spores have formed, mycelial filaments very abundant, and already, after 24 hours, a dense network is produced. Conidiophores appear before the end of the first day; the spores are not yet coloured, and the fruit-bearers do not differ from other mycelial filaments. Up to a depth of 0·8 mm. spores have germinated	Starch, No. 19	Feb. 24.
—	... ...	—	—
—	Mycelial filaments very few, short, slender, and without septa. Only a small proportion of the spores have germinated, the others are either swollen or unaltered	Starch, No. 19	Feb. 24.
—	... ...	—	—
—	A few spores only swollen, the others unaltered	Starch, No. 19	Feb. 24.
—	... ...	—	—
—	Spores show hardly any change even after a week	Starch, No. 19	Feb. 24.
—	All these cultivations became rapidly contaminated with a bacillus; the spores became very irregular. Their progress was not followed afterwards	Hydrocele, No. 27	Mar. 16.
—	The anterior chamber of the eye has become full of desquamated cells, epithelial-looking. No distinct mycelium discovered. Spores swollen and beginning to germinate?	Potato, No. 3	June 20, 1890.
—	... ...	„	„
—	Numerous tubercles in the central tendon of diaphragm, the pelvic peritoneum, the mesentery;	—	—
	few small ones in the parietal and visceral layers of peritoneum, over liver and intestine. ll tubercles contain swollen spores, some of which are beginning to germinate. No mycelium yet		
—	Growth extremely scanty	Starch, No. 19	Feb. 24.

iluted egg albumen the *Aspergillus niger* can grow with difficulty under favorable

2. *Typical developmental stages.*

The results obtained were on the whole what might have been expected from the work of other observers in allied fields. Owing, however, to the very characteristic features of the *Aspergillus niger*, they were unusually clear.

I will, therefore, try to summarise these results so as to make them more available than by the inspection of the foregoing table, which I have not been able to render descriptive enough.

Development of the Aspergillus niger.—When grown on the surface of potatoes, or glycerine nutrient gelatine, at a temperature ranging between 35° C. and 40° C., typical spores of the *Aspergillus niger* pass through the following stages:

1st. Swelling and accumulation of pigment under the membrane; passage of pigment as through pores into surrounding medium; crenated appearance.	During the first 2 or 3 hours.
2nd. Appearance of one or two bright spots, then of one or two conical projections, which rapidly elongate, become cylindrical, and often have as large a diameter as the spore itself, at some distance from it. Some measure over 0·2 mm. in length. Two or three septa may have appeared in the longest; the segments nearest to the spore have a double outline, their protoplasma is granular, and contains also pretty large bright granules or vacuoles. The terminal segments are finely granular, thick, and appear to have no cell wall. Small bud-like projections appear on the sides of the filaments; these have the same character as the terminal segments.	Within the first 6 to 12 hours.
3rd. The filaments branch rapidly, and form a network, from which straight hyphæ, some like the others, some thicker, rise up into the air and at various heights from the surface. They become club-shaped. A number of small bud-like projections appear on these bladders; they grow radially, so that the sphere is surrounded by radiating filaments, all very nearly of the same thickness; some of these filaments branch, their extremities become constricted, so that a small rounded spore becomes partitioned off; a second spore is formed in the same way between the end of the filament and the first spore, and so on. These acrospores are the conidia, the filaments supporting them the sterigmata, the erect hypha with its bladder-	From the 12th to the 18th hour.

× 150

Side view of a cultivation of *Aspergillus niger* (obtained from the skin surrounding the ulcer of the leg).

1. Nutrient gelatine with mycelium (indistinct) and numerous crystals of oxalate of lime.
2. Conidiophores (fruit-bearing hyphæ).
3. Conidia (colour exactly that of soot).

like end is the fruit-bearing hypha of mycologists; it may be also called conidiophore for shortness' sake, but this term has not always the same meaning. As yet there is no evidence of any pigmentation, except a bright lemon-yellow coloration of the young conidiophores and of some of the ordinary mycelial filaments. One or two large oval protoplasmic masses are often seen in the thick fruit-bearing hyphæ. These masses gradually pass towards the club-shaped end; this takes place at the time when the sterigmata appear. Another phenomenon may also be observed at the same or perhaps before this time. Among the vegetative mycelial filaments some come in close contact, and between such filaments a kind of conjugation takes place; buds appear on corresponding points in the two filaments; these buds come in closer contact and become flattened against each other; the intermediate membrane is resorbed, and the contents of one segment of one of the filaments pass into the corresponding segment of the other.

From the 12th to the 18th hour.

It is evident that this conjugation must indicate some essential change in the properties of the hyphæ, for up to that period they have a marked tendency to separate from each other as widely as they can, so that if three spores placed together germinate, the filaments thus produced radiate in three different directions, and even their branches seem at first to avoid each other. This tendency is quite uninfluenced by light, heat, or even access of air. I take this to indicate that in the early stages of their growth the filaments impoverish and contaminate the surrounding medium within a certain radius, and that the growing point of other filaments of the same species grows naturally more luxuriantly on the side not exposed to the poisonous emanations than in the other direction. In favour of this influence of the pabulum I may mention that in large groups of spores only few germinate when there is no free oxygenation and the air is not kept very moist. The few filaments thus produced diverge as usual, but they are much more slender than when only two or three start from the same point.

4th. The mycelial filaments become more and more densely interwoven on the surface of the nutrient material. Many of the older filaments lose their protoplasmic contents, and are represented only by a transparent sheath. Some segments retain their protoplasma longer than others. The most superficial filaments become more and more vacuolated, their diameter increases considerably, especially at places where they become dilated

Within the first week.

and bladder-like; these swellings are sometimes so abundant as to give the filaments a moniliform appearance. When the mycelium has become so altered it seems at places to be replaced by a kind of loose cellular tissue. Meanwhile the conidia are increasing in number, and if the fungus is well protected from any disturbing influence chains of conidia of considerable length are produced. These conidia being all of the same size, or nearly so, the chains thus formed look like giant streptococci; at the same time the spores get more and more deeply pigmented as they get older; the link uniting them becomes more and more thread-like, and the spores somewhat flattened. Ultimately they look like rows of thick discs connected together by a central thread. Under certain circumstances they become regularly crenated, owing to the projection of small pigmented granules all over their surface. The sterigmata become often pigmented as well as the fruit-bearing hyphæ, and some of the most superficial vegetative hyphæ.	Within the first week.

3. *Variations.*

Without going into further details I may now mention briefly the various changes which are brought about by various circumstances.

1st. *Influence of pabulum* (temperature, moisture, &c., being the same or nearly so).

Potato, glycerine nutrient gelatine, muscle, skin, all appear to be excellent nutrient media, and on them both the mycelium and the conidia appear rapidly and abundantly.

On *potato* the mycelium generally assumes a yellow colour before the conidia appear; the growth of the mycelium and conidia is abundant.

On *glycerine nutrient gelatine* the growth is rapid, but both the mycelium and the spores are less abundant than in potato cultivation; the production of spores becomes intermittently arrested whilst the mycelium continues to extend peripherally; this leads to the production of concentric rings of conidia. This process is quite analogous to that observed in meadows, and giving rise to the production of the "*fairy rings*," about which Dr. Wollaston wrote a paper in 1807 ('Philosophical Transactions,' 1807, p. 133).

On *skin* the growth is about as rapid as on nutrient gelatine, but the mycelium tends to grow abundantly in comparison to the conidia.

On *muscle* the growth of the fungus is extremely rapid; the mycelium is entirely hidden by a mass of conidia, which is much in excess of what is found in any other form of cultivation. The conidia have also a tendency to be pale in colour, very large, not well separated from each other, probably owing to very rapid growth.

Adipose tissue was found to be a less suitable medium than either skin or muscle; it favoured the formation of a dense white mycelium.

On *hydrocele fluid* the fungus grew rapidly but not thickly, the mycelium forming a thin stratum, and the conidia seldom forming dense clumps; they showed also a tendency to produce concentric rings (fairy rings).

On *concentrated solution of peptones* the growth was very slow, and spores appeared very late.

On *glycerine agar-agar* the growth was much slower, and the small mycelial masses usually took a deep yellow colour long before the conidia appeared; when this happened the whole mycelium was covered with a dense layer of conidia.

On *pure gelatine* the mycelium formed rapidly a thin layer of radiating filaments, with a clump of conidia in the centre, and discrete conidia bearers all over the surface.

On *ordinary agar-agar* only a very slight mycelial growth was obtained after days, but the material used in my experiments seemed to have got rather dry, and was found to grow badly other organisms also.

On *undiluted coagulated egg albumen* growth could be obtained only with difficulty and in very moist air.

Of the non-nitrogenous substances starch and gum arabic were found to be very good media.

On *starch* the mycelium spread very rapidly, forming a very thin layer, and the conidia-bearing hyphæ sprung at large intervals from each other and remained quite isolated; they sometimes reached a large size. They were very convenient for experimental purposes, since each conidiophore could be easily picked with a needle without any other being touched.

On *cellulose* (best Swedish paper washed with distilled water)

the *Aspergillus niger* grew well and quickly, though not abundantly. There was a central clump of conidia with discrete conidiophores all round.

In *mucilage* the mycelium grew with great rapidity and so abundantly that the whole mucilage was permeated with a dense network in about two days; this mycelium was composed of extremely large hyphæ, with very thick walls. Conidia appeared very late and scantily.

On *ordinary hard paraffin*, such as is used for cutting sections, the spores, when kept moist, germinated, and after many weeks gave rise to chains of conidia, very pale, *without the intermediation of ordinary conidiophores*. This acrogenous formation of conidia at the end of apparently ordinary mycelium filaments was observed in many other instances.

In *a thick solution of glucose*, of the consistency of honey, no mycelial growth could be obtained at any temperature.

In *distilled water* spores germinated after a long time, but probably by feeding on the other spores which had not germinated.

From these observations, most of which have been repeated several times with similar results, it is evident that, by *modifying its pabulum, it is possible to obtain several varieties of* Aspergillus niger.

(1) One with pretty *equal* production of *vegetative* and *reproductive* elements (potato).

(2) One with an *excess of reproductive* elements over the vegetative (muscle, glycerine agar-agar).

(3) One with a great *excess of vegetative* over the reproductive elements (mucilage).

In addition, the mycelium may be made to develop a *bright yellow pigment* (potato, glycerine agar-agar).

The mycelium may be made to cover rapidly large areas with a *sporadic production of conidiophores*.

Mycelium and conidia may be made to grow intermittently in excess of each other, and so as to produce an appearance similar to that known under the name of "*fairy rings*" (glycerine nutrient gelatine).

These varieties are *particular to the nutrient media*, since conidia, obtained from any of these cultivations, will give rise to new colonies having types corresponding to those described above, when grown on each special medium.

2nd. *Influence of temperature.*—The effects of temperature were similar to those observed in other pathogenic micro-organisms. On unfavorable nutrient media a temperature of 10° to 15° C. prevents or arrests the growth of the mycelium or conidia.

On the most favorable media the same temperature causes a very considerable delay in the growth of the mycelium, and still more in the appearance of conidia (thus, in one of the most successful cultivations, conidia appeared only after 288 hours, instead of being evident before the end of the first day, as when the temperature is most favorable). Both mycelium and conidia are extremely spare at this temperature.

At 20° to 25° C. the growth of mycelium and spores is tolerably rapid on the most suitable materials, but very slow on the less suitable media. The mycelium has a tendency to form a thin, evenly distributed layer.

At 35° both mycelium and spores grow with exceedingly great rapidity on most suitable materials, a thick mass of mycelium being formed in a few hours, and before the end of the first day conidia being already abundant.

At 39° and 40° C. the growth is very active, and spores are produced abundantly, even on apparently unsuitable media, such as pure cellulose.

Between 40° and 45° C. the cultivation succeeds only on the best nutrient media. The mycelium is produced so abundantly that it forms thick hemispherical masses which soon become deeply folded. The mycelial filaments form a denser felt than in previous cases. Conidia appear very slowly or not at all, at any rate in their typical form.

3rd. *Vital concurrence between the* Penicillium glaucum *and the* Aspergillus niger *at various temperatures.*—The coloured spores of these two fungi gave the means of obtaining some very clear demonstrations of the influence of temperature in the production of an apparent antagonism.

For the purpose of experiments I used either mixed conidia of the Aspergillus and Penicillium, and inoculated various media; or inoculated slices of potatoes in one place with spores of the one, and in another with spores of the other; then exposed them to various temperatures. The results obtained can be easily tabulated as follows:

DESCRIPTION OF PLATE IX.

To illustrate Dr. Delépine's paper on Melano-mycosis of the Skin.

From drawings by the author.

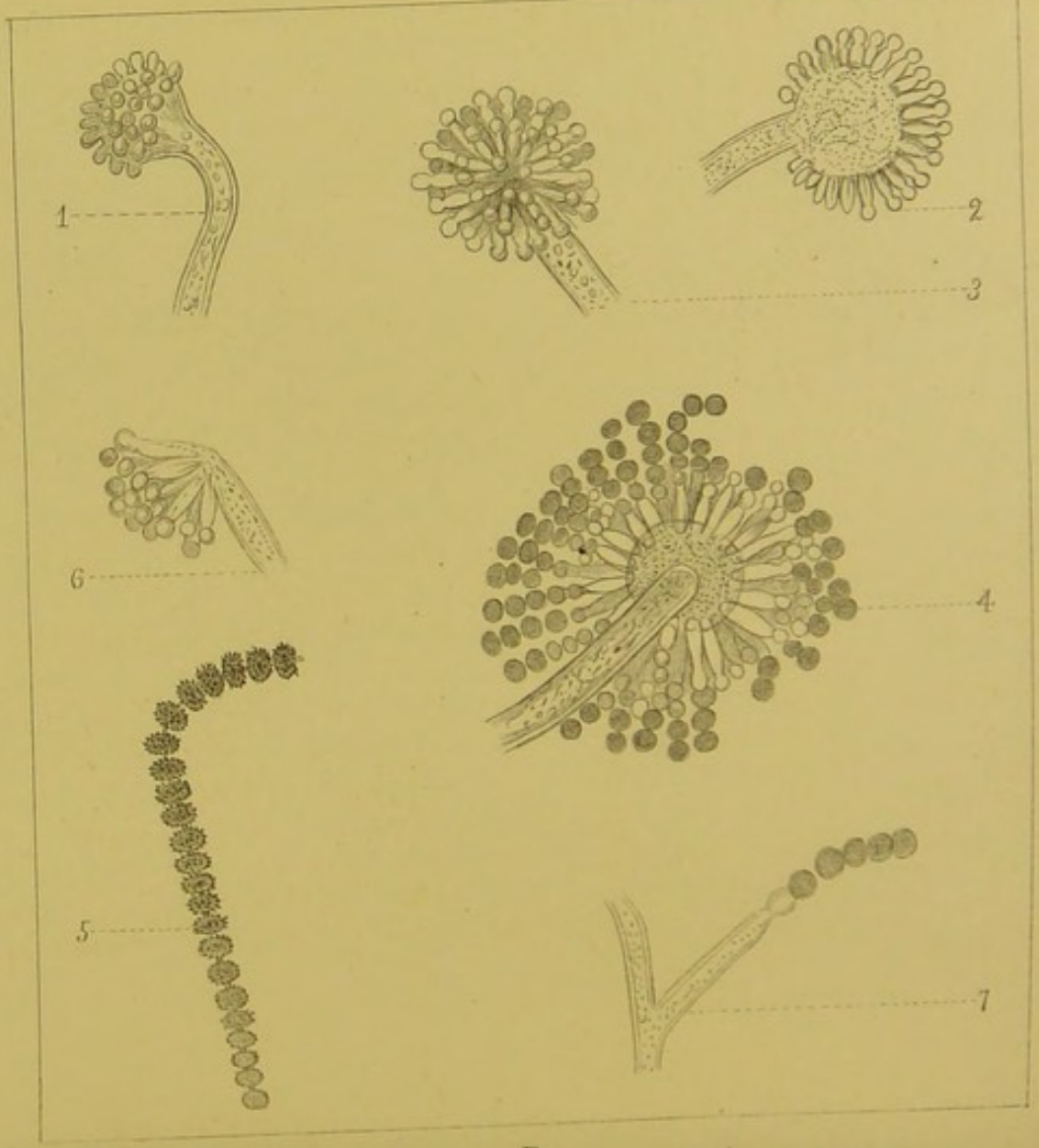
A. A fruit-bearing hypha (conidiophore) grown in a very narrow space between two glass plates (interlamellar method). The fructification is flattened, and the arrangement of the sterigmata is very distinct. The arrangement of the conidia is not typical, as they are not arranged in rows; this was apparently due to very rapid growth. × 450.

1. Hypha.
2. Its club-shaped end.
3. Sterigmata, branching very distinctly.
4. Proliferating end of the sterigmata from which the conidia arise; this region was very pigmented in this case.
5. Young conidia, hardly pigmented yet, very unequal in size.

B. Development of conidia and modifications brought about by nature of pabulum, amount of moisture, &c. (interlamellar method of cultivation being used).

1. First appearance of sterigmata on the club-shaped end of a future conidiophore.
2, 3. Constriction of peripheral end of sterigmata showing the mode of production of conidia.
4. Formation of chains of conidia, their gradual pigmentation.
5. A chain of conidia separated from the conidiophore; the conidia as they grow older become more pigmented, slightly warted, and become gradually separated, remaining connected by what seems to be an axial filament.
6. Sterigmata arising from the end of a hypha which has not become bulbous.
7 Conidia arising from an ordinary mycelial filament without the intervention of conidiophore or sterigmata.

A



B

Sheldon Delaplaine lith.

Danielsen & Co. sculp.

DESCRIPTION OF PLATE X.

To illustrate Dr. Delépine's paper on Melano-mycosis.
From drawings by the author.

FIG. A.—A small group of seven conidia (spores) of the *Aspergillus niger*.

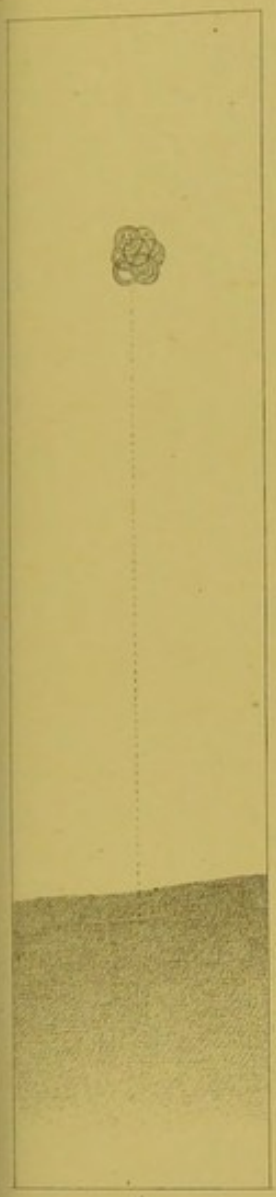
FIG. A.—The spores had not altered, they had been only a few hours in the gelatine.

FIG. B.—The same group eighteen hours later.

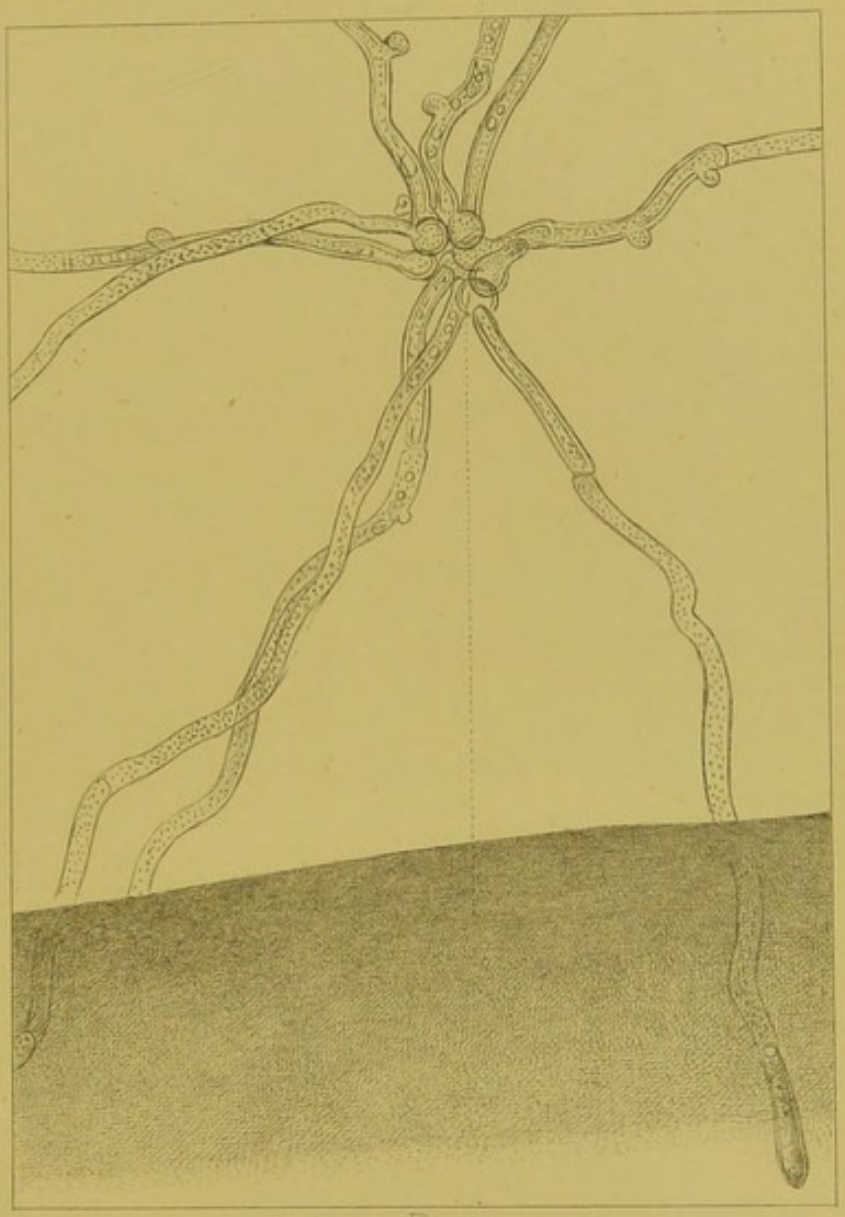
FIG. B.—The spores had swollen and sprouted, some filaments had begun (for about two hours) to protrude beyond the surface. The deposition of oxalate of lime began very soon after this.

FIG. C.—Part of the filaments produced by the same group one month later. × 450. These spores were sown in an enclosed layer of nutrient gelatine (see 'Lancet,' June, 1891), and kept at the temperature of 23° C. in an incubator. The first day they were examined hourly, and after that daily. The dotted line indicates the shortest distance between the spores and the surface of the gelatine (mm. 0.15). The shading indicates the surface of the gelatine.

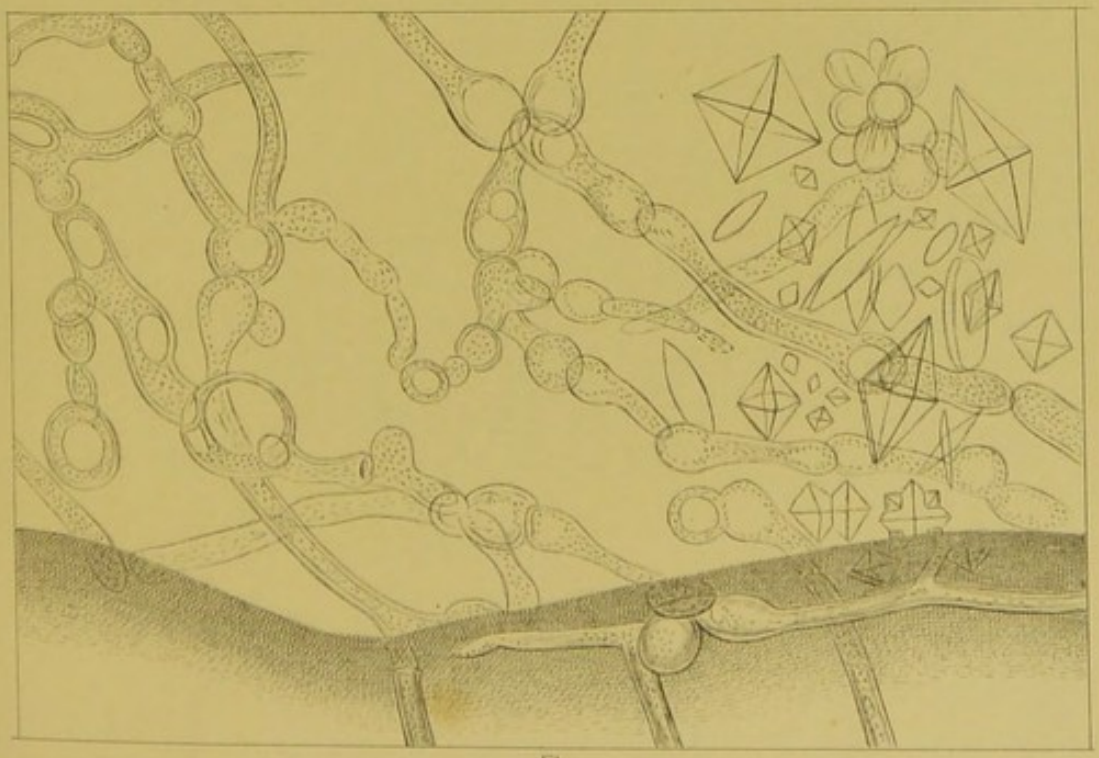
FIG. C.—The swollen and greatly altered mycelium as it appeared just under the surface of the gelatine one month after. Some filaments have conjugated, the protoplasm is much vacuolated.



A.



B.



C.

Sheridan Delépine del. Danielsson & Co. sculp.

Temperature.	*Aspergillus niger* growth.	*Penicillium glaucum* growth.	Result.
10° C.	0	Mycelium and spores abundant	Apparently nothing but *Penicillium glaucum.*
15° C.	Very slight of mycelium	Mycelium and spores abundant	Id.
20° to 25° C.	Moderate growth of mycelium and conidia	Moderate growth of mycelium and conidia	The two fungi grow equally side by side.
25° to 30° C.	More rapid growth of mycelium and conidia	Diminished growth of mycelium and conidia	The *Aspergillus niger* is slowly creeping over the *Penicillium glaucum* and hiding it.
35° to 40° C.	Very rapid growth of mycelium and conidia	0	Apparently nothing but *Aspergillus niger.*
40° to 45° C.	Very rapid growth of mycelium, but not of conidia	0	Apparently nothing but a mycelium, with no distinct features.

4th. *Influence of moisture.*—I was able to stunt the growth and cause swelling of the extremities of mycelial filaments by simply diminishing the amount of moisture in the air surrounding the *Aspergillus niger.* If, after stopping and modifying the growth by drying slightly the preparation, the organism is again exposed to the influence of air saturated with moisture, fresh offshoots are obtained from the clubbed ends of the stunted filaments (these clubbed ends are not to be mistaken for those of conidia-bearing hyphæ). In this way the branching of the filaments can be greatly modified. It is evident that under ordinary circumstances parasites and other fungi may be influenced by similar circumstances, and may thus exhibit alternate forms which are evidently connected with different degrees of growth and physico-chemical activity.

5th. *Influence of air.*—I shall have to refer to this again further on. I may, however, mention that if spores be placed at various depths in a suitable nutrient medium, such as glycerine pepton-gelatine, only the most superficial ones grow.

The fungus is distinctly aerobic, yet within certain limits it can grow in the depth of the nutrient material, and then becomes modified. Spores placed *quite on the surface* grow well and rapidly, yielding within a few hours thick septate filaments, which branch rapidly on the surface of the gelatine, and at 35° C. yield within the first day typical conidiophores. Spores placed at a depth of 0·1 mm. germinate a little more slowly, produce long filaments, which become slightly thicker as they approach the surface, where they become rapidly branched, assuming the same form as in the last case. This is very distinct within twelve hours.

At *a depth of* 0·8 *to* 1 *mm.* they only begin to produce short mycelial filaments at the end of sixteen hours.

At a depth of 1·5 *mm.* the spores are swollen, often crenated, owing to projection of pigment through their wall,. and show a bright nucleus-like body (also at the end of sixteen hours).

Beyond 2 *mm.* from the surface the conidia are only a little swollen or not altered at the end of the first day.

Even the spores placed at *a depth of* 10 *mm.* may occasionally germinate after thirty or more hours; but in such cases the filaments produced are extremely slender, and take weeks to reach the surface. If, however, they succeed in doing so, then, as they approach the surface, they get thicker and thicker until they acquire their normal size. Filaments grown under such circumstances may be as slender as the *Bacillus anthracis* in the leptothrix stage, and, as often the protoplasmic contents of some of the segments of the septate filaments disappear, whilst those of other segments remain, an appearance is produced, by which the few segments still containing protoplasma resemble long or short thick bacilli with square ends.

If mycelial filaments can be made to grow in nutrient matter without reaching its surface, or, after projecting beyond the free surface, are made to re-enter the substratum, they can be made to continue growing without producing conidia. This is probably the way in which mycelial filaments may multiply to a considerable extent in fluid media without producing conidia. As they grow they sink into the fluid and remain surrounded by it until a sufficiently thick layer is produced, the surface of which gradually emerges from the fluid medium.

VI. Effects of the Growth of the Aspergillus niger on Various Media.

In the course of the investigations just related, I observed a number of changes which were distinctly brought about by the growth of the fungus. Without entering into lengthy details, I may summarise these observations.

Nutrient material.	Products found after cultivation of *Aspergillus niger*.
Pure cellulose	Glucose.
Pure starch	Glucose.
Best gum arabic	Glucose, oxalic acid, acetic acid.
Best gelatine of commerce . . .	Gelatose (gelatine peptones ?).
Solidified hydrocele fluid . . .	Proteoses (peptones ?).
Glycerine, peptone gelatine . .	Oxalic acid, abundant.
" " " . .	Crystalline extractives replacing ultimately nearly the whole gelatine.

It is useless to consider the changes resulting from the growth of the fungus in more complicated food-stuffs, since it would be difficult to understand their meaning so long as the changes observed in simple compounds are not quite clear.

It is evident that most of the products mentioned above are the results of some hydrolytic process.

The *formation of glucose* may be graphically represented as follows:

$$\underset{\text{Starch, Cellulose, Arabic acid, or Arabin.}}{C_6H_{10}O_5} + H_2O = \underset{\text{Glucose.}}{C_6H_{12}O_6}.$$

Oxalic acid can be obtained by oxidation of cellulose, starch, and allied products. In the experiments related above the presence of oxalic acid was rendered evident whenever some salt of calcium was present in the nutrient medium. It is well known that lime and other bases are abundantly present in gum acacia when they are supposed to form salts with arabic acid. Lime is also pretty abundant in the ordinary nutrient jelly, as can be proved by the addition of oxalate of ammonia. Whether the oxalic acid is produced by oxidation of the glycerine or of the nitrogenous

compound is difficult to say. I have, however, not been able to make gelatine yield any oxalate of lime under the influence of the Aspergillus; but perhaps there was not enough lime present in the specimen I used.

Gelatoses and gelatine peptones.—The formation of these products was indicated only by their solubility and the biuret test (the quantities experimented upon not allowing the precipitation methods to be used in order to determine further the variety of compound obtained). As *Hofmeister* has shown, these products must be the result of the hydration of gelatine. *Chittenden* and *Solley* have lately supported the same view, although they have not been able to prove it by analysis.

Proteoses or *peptones* were also demonstrated after the growth of the fungus, although the quantities obtained were too small to allow of separation being attempted; these substances must also have been produced by hydrolysis of the albumin and globulins used as cultivation media (hydrocele fluid).

Although these experiments are few, yet they all point so clearly to the same conclusion that, even on their basis, it seems justifiable to assert that whatever the material used (provided it allowed the fungus to grow), the changes brought about were either those associated with digestive processes (glucose, gelatoses, proteoses, peptones) or with regressive metabolism (oxalic acid, &c.).

The production of oxalic acid was, perhaps, the most interesting of these phenomena connected with the growth of the fungus. It was much more abundant in some media than in others; thus the production of oxalate of lime out of gum acacia was extraordinarily abundant.

This production of oxalate of lime was distinctly connected with the exposure of the mycelial filaments to air.

So long as the filaments were embedded in nutrient gelatine, for instance, no crystal of oxalate of lime was observed. No sooner did they project for some distance beyond the surface than crystals began to appear in the superficial layers of the gelatine and in deeper layers along the filaments to which air had access. This last fact is of considerable interest in connection with the probable influence which protoplasmic respiration has on the metabolic products which have just been enumerated.

The production of oxalate of lime takes place by steps, and before typical crystals are formed other crystalline forms are evident,

some of which undoubtedly indicate the presence of some other compounds, for they are soluble in water. Crystals of oxalate of lime after a time accumulate to such an extent as to form with the mycelium a kind of white crust, and it is at this stage that the large cellular-looking swellings of the mycelium occur. Conidia are abundantly produced at the same time. The changes observed in nutrient gelatine do not stop at simple peptonisation; further important modifications must occur, for after a week or two a drop of the liquefied gelatine, when allowed to evaporate, yields a large mass of fine, prismatic, lozenge-shaped, acicular crystals, some of which form very elegant dendritic masses. These crystals are entirely soluble in alcohol and water, and, as I have not ascertained their composition, I will call them for the present extractives.

To the facts which I have just enumerated, and which indicate the fermentative action of the *Aspergillus niger*, I should add also that *Th. van Tieghem* had already, in 1868, connected the *gallic fermentation* with the growth of the *Aspergillus niger* in this fermentation. *Tannin* by hydration becomes converted into *gallic* and *acid glucose* (Van Tieghem, 'Ann. de Sc. Nat.,' 5^{e} Série, viii, 1868).

On the other hand, *Fernbach*, working in Pasteur's laboratory, has shown that *saccharose* could be *interverted* by the *Aspergillus niger*, which he considers must contain a diastatic ferment (sucrase) ('Annales de l'Institut Pasteur,' 1889, pp. 473—531, and 1891, p. 1).

VII. General Considerations on the Hydrolytic Action of Living Protoplasm.

Thus through all these observations it seems proved that *hydrolysis* of almost any organic product can be produced by the *growth of the Aspergillus niger*. It would, according to our present notions of enzymes, be capable of producing a *proteolytic ferment (a gelatolytic ferment), an amylolytic ferment, an inverting ferment, an oxalic acid ferment, an acetic acid ferment, and probably a host of other ferments.*

When we deal with a compound organism like a mammal, it is easy to imagine that the cells of various organs have the power to manufacture special ferments, all specific, and having limited chemical properties. When, however, an organism which, like the

Aspergillus niger, is composed of cells which, independently of any marked morphological alterations, are capable to produce in turn all the hydrolytic actions which are attributed to the presence of special ferments, then one is almost driven to the conclusion that the production of so many different ferments by the same cell is problematical. The fact that active bodies can be extracted from the products of the secretions of certain cells, and may be made to bring about definite changes under definite chemical and physical conditions, seems equally unanswerable. There are, however, marked differences of activity between the artificial digestive fluids and the natural processes of digestion. One is almost tempted to believe that the action of these products is more or less related to their *not distant connection* with a living cell, without which their mode of action becomes more and more allied to that of chemical reagents in an *ordinary dynamical state.*

It is perfectly clear that protoplasm can hardly live without hydrolysing its surroundings, and that *hydrolysis seems therefore to be one of the concomitants of life.* This is not given here as a new idea. When an organism has, like the *Aspergillus niger*, the power to adapt itself to a great variety of circumstances, it becomes possible to demonstrate that this hydrolytic action of living protoplasma gives rise to *products which depend less on the cell itself than on its surrounding media. The adaptability of an organism to various circumstances renders it therefore capable to acquire properties which may become extraordinary when the circumstances themselves are not the ordinary ones under which it is generally known to us.* On these grounds there can be no serious theoretical objection to the supposition that a non-pathogenic organism may at times become pathogenic.

VIII. Bibliographical Appendix (Mycoses).

Author.	Reference.	Year.	Nature of contribution.
Bezold	Ueber Otomycosis zur Ætiol. d. Infect. (Munich)	1881	*Aspergillus fumigatus, nigricans,* and *flavescens* found in the external meatus, as well as other organisms, such as the *Trichothecium roseum*. They excite inflammation. Oil favours their development.

Author.	Reference.	Year.	Nature of contribution.
Bizzozero and Firket	Manuel de Microscopie Clinique (Bruxelles)	1888	The presence of *Aspergilli* in the external meatus was first observed by *Pacini* and *Meyer*, and is of more importance than was suspected at first. Other fungi are also found in the same situation, *e.g. Ascophora elegans, Trichothecium, Mucor mucedo, Peziza.*
Chittenden and Solley	Journal of Physiology, vol. xii, p. 23 (Cambridge)	1891	The primary cleavage products formed in the digestion of gelatine.
De Bary	Morphologie und Physiologie der Pilze, ii, Bd. i (Leipzig)	1866	Morphology and classification. Forms of parasitism.
Fernbach	Annales de l'Institut Pasteur, pp. 473—531	1889	Production of a diastatic ferment (sucrase) by the *Aspergillus niger* (see note at the end of the Appendix).
	Ibid., p. 1	1891	
Flügge	Fermente und Mikroparasiten (English translation by Watson Cheyne, London)	1886 1890	Description of mould fungi. Classification. Pathogenic properties. Reference to various writers, some of which are not mentioned here. Literature.
Fränkel	Deutsche med. Wochensch., No. 31	1885	Cultivation of *Aspergillus fumigatus* at a temperature of 51° C. for half a year prevents formation of spores, but when temperature is brought down to 37° C. conidia are produced again, which are as virulent as if the fungus had been grown in the usual way.
Leber	Grafe's Arch., xxv	—	Growth of *Aspergilli* in the human cornea and in the anterior chamber of the eye of rabbits. Suppurative inflammation.
	Berl. klin. Woch., Nos. 9 and 10	1882	
Lichtheim	Berl. klin. Woch., No. 9	1882	*Aspergillus fumigatus* found in the lungs.
Rother	Charité Annalen, iv Jahrgang, 1877, p. 272 (Berlin)	1879	Woman with extensive bronchitis, and hardly recovered from pneumonia, expectorated *débris* of pulmonary tissue containing mycelium and sterigmata of some *Aspergillus*. The patient recovered.
Schubert[1]	Deutsches Archiv für klin. Med., T. xxxvi	1885	Woman, æt. 75, in a state of marasmus. Naso-pharyngeal cavity covered with mycelium of *Aspergillus fumigatus*. Small ulcerations. Growth arrested by boracic acid after several relapses.

[1] Schubert remarks that the lungs were free, although conidia must have been carried into the air-passages by the air. As he describes the mycelium as greyish white there were either no spores at all, or else he mistook some other fungus for the *Aspergillus fumigatus*.—S. D.

Author.	Reference.	Year.	Nature of contribution.
Schütz	Mitth. a. d. Kais. Ges. Amt., Bd. ii	—	*Pneumono-mycosis Aspergillana.* — Severe pneumonic inflammation occurring in pigeons and geese, and generally caused by the *Aspergillus fumigatus*, and more seldom by the *Aspergillus niger*. Mammals may be affected in the same way.
Siebenman	Die Fadenpilze (Wiesbaden)	1883	Description of the characters and growth of the *Aspergilli*. Literature.
Van Tieghem	Annales des Sciences Naturelles, 5e série, viii	1868	Gallic fermentation (of tannin) under the influence of *Aspergillus niger* and *Penicillium glaucum*.
„	Bulletin de la Société Botanique de France	1877	Development of some *Ascomycetes* (*Aspergillus*).
Wollaston	Philosophical Transactions	1807	Production of "*fairy rings*." Mycelium exhausts the ground in the central parts and persists. At the periphery the visible receptacles of the Agarici (then called fungi) grow and then decay, producing a rich manure, which causes the grass to grow luxuriantly. From the spores produced by these receptacles fresh mycelium is produced, which extends further, a wider circle of receptacles is produced, and so on.
Ziegler	A Text-book of Pathological Anatomy, i, p. 316 (Translated by MacAlister, London)	— 1883	Inflammatory and necrotic changes produced by *Aspergillus fumigatus* and *flavescens*. References to other observers.

Fernbach's experiments with the *Aspergillus niger* are extremely interesting, and bear so much on the mode of production of substances, which may give to the so-called toxalbumins or albumoses some of their prophylactic properties, that I need no excuse for alluding to them here.

When the *Aspergillus niger* is grown in *Raulin's fluid* (saccharose 17·6 gr., free tartaric acid 0·72 to 1·8 per 1000) at a temperature of 35° C., this fungus grows rapidly. Whilst it is growing the saccharose is at first rapidly transformed into interverted sugar. When all the sugar has thus been transformed and used up, the plant, instead of increasing in weight, begins to lose weight. During the first day, when the saccharose is being interverted, the acidity of the fluid is rapidly increasing, but after this it dimin-

ishes. By careful analyses Fernbach estimated the amount of the ferment (sucrase), to the action of which he attributes the inversion of the sugar. (He estimated this quantity by means of the *sucrase unit*, which is the quantity of it necessary to invert 20 centigrammes of saccharose in one hour at the temperature of 56° C. in presence of $\frac{1}{100}$ of acetic acid.)

The results which he obtained are given in the following table:

Duration in twenty-four hours.	Saccharose remaining in fluid.	Quantity of interverted sugar in fluid.	Saccharose used up.	Acidity.	Sucrase in fluid.	Sucrase in fungus cells	Weight of fungus.
	Grammes.	Grammes.	Grammes.	Grammes.	Units	Units	Grammes.
0	4·44	—	—	0·170	0	0	A few spores only
1	1·36	2·36	0·92	0·293	2	58	0·65
2	0·22	1·65	2·57	0·368	3	47	1·265
3	0	0·7	3·74	0·267	5	45	1·78
4	0	0	4·44	0·143	10	44	1·65
5	0	0	„	0·135	13	35	1·61

These experiments show well that the agent sucrase, to which the chemical activity (considered here) of the fungus is due, is closely connected with the cells themselves. It is only when their source of activity seems exhausted that any marked fraction of this activity seems to be transferred to the surrounding media. At the same time, however, the total weight of the plant and the total amount of the "ferment" diminish. These facts, considered in the light of the almost universal fermentative activity of the *Aspergillus niger*, which I have tried to make evident, seem to indicate that perhaps *too much has been attributed to the action of enzymes, independently of their connection with living cells.*

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