Fig. 1.

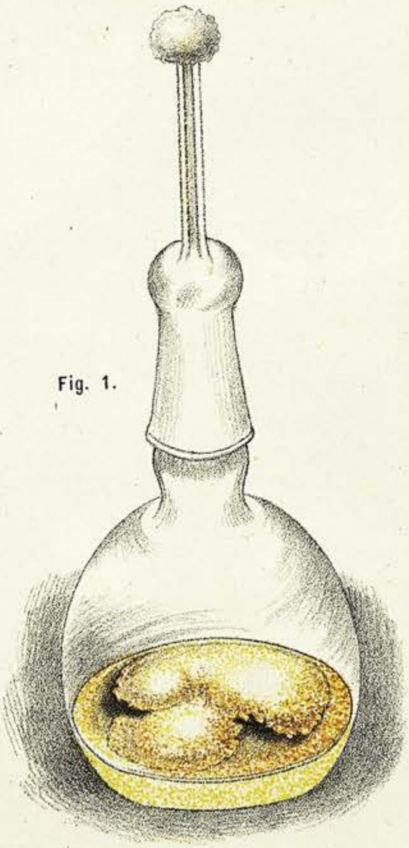


Fig. 2.

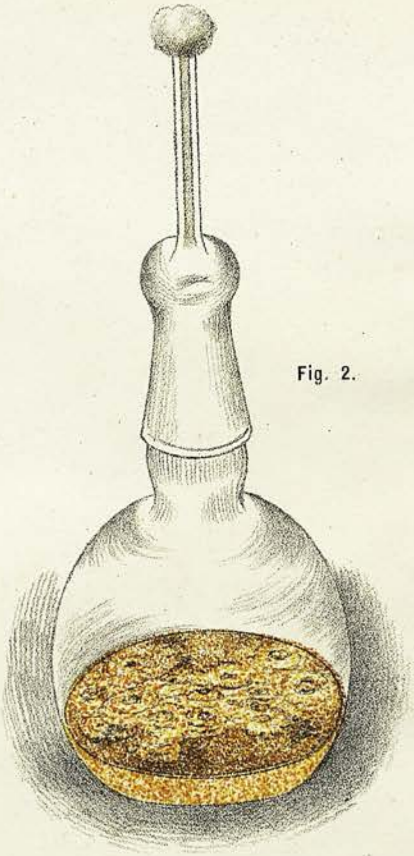


Fig. 1.—Trichophyton Vulgaris (FRENCH).

Fig. 2.—Trichophyton Vulgaris (ENGLISH).
(Modified by the addition of acid tartrate of potash).

Fig. 3.—Trichophyton Vulgaris (ENGLISH).

Fig. 4.—Trichophyton Vulgaris (AUSTRIAN).

Fig. 3.

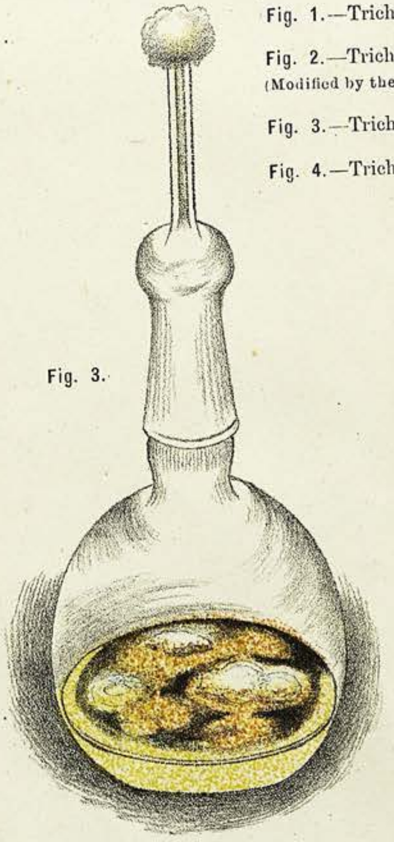
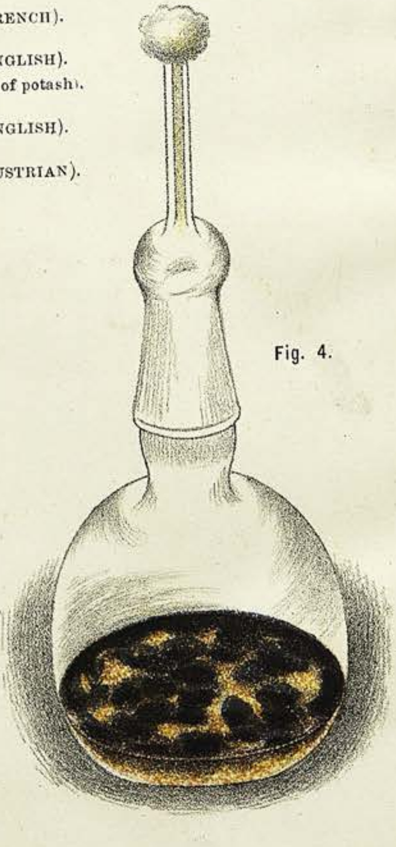


Fig. 4.



T. Dobb & Co., Printers, L'pool.

+

(*Private Copy.*)

INTRODUCTION

TO THE

STUDY OF THE MOULD-FUNGI PARASITIC ON MAN.

Being a Thesis presented to the Senatus Academicus of the University of Edinburgh for the Degree of Doctor of Medicine.

BY

H. LESLIE ROBERTS, M.B., C.M., Edin.

LIVERPOOL:

T. DOBB & Co., PRINTERS, 229, BROWNLOW HILL.

1893.

Note.

This Thesis is the fruit of an investigation undertaken by me during the last five successive years. It enters on subjects which are associated, in the closest way, with the external conditions of climate, race, and country; and, therefore, it was found necessary to investigate them in different countries. For this reason I have availed myself of the advantages which continental laboratories afford the Bacteriologist. I may specially mention the laboratories of Dr. Unna in Hamburg, of Dr. Hebra in Vienna, of the Pasteur Institute in Paris, and I am sensible of the aid which was generously given by the Directors of these Institutions. But during the last three years the work has been pursued in my own laboratory, in the intervals between professional duties, on those fundamental lines of bacteriological research which were laid down for our safe direction, by Pasteur and Koch. The necessity, therefore, of experimental inoculation of living animals, was unavoidable, and I am indebted to Prof. Gotch, F.R.S., and to Mr. Bryant, the President of the Royal College of Surgeons, of England, for their kindly aid, which enabled me to obtain the legal right to perform these experiments.

I have endeavoured to separate the subject of the Thesis from the narrow groove in which the Physician is too apt to consider it, for my aim has been to give the parasitic moulds their right place among the Cryptogams; and I have, therefore, made use of the terms employed by modern Botanists in the description of the higher Fungi.

Of the incompleteness of this work, in respect of the far-reaching subjects which it enters on, no one is more conscious than its Author, and this I have intended to show in the title; for much time and research are yet needed before the Natural History of these Fungi can be written with certainty and satisfaction.

Liverpool, 20th April, 1893.

CONTENTS.

CHAPTER I.

Introduction.

During recent years the attention of Bacteriologists has been directed by the discovery of certain facts to the question of racial variations in the lower and higher pathogenic fungi. It was steadfastly held by the founders of Bacteriology that the individuals of a species uniformly presented, under all circumstances, the type characters of the species; and when these individuals were associated etiologically with certain diseases, they were described as fixed forms. When deviations in form were met with in the same, or closely allied diseases they were categorically put down as new species. Tulasne, for the first time, pointed out the possibility of pleomorphism and he demonstrated that many forms which were currently described as distinct species were in reality merely different stages of development of the same individual. But the value of his discovery and its bearings on the problems of pathology were not recognised for many years, till the recent, greatly enlarged, cultivation of the pathogenic fungi, brought into prominence the fact that the individuals of the several pathogenic species presented astonishing differences under cultivation. When reared side by side in a common soil there are often marked physical differences, sometimes in the matter of colour, or growth-energy; and when the inquiry is pushed still further, aided by the microscope, minuter, but still characteristic, variations may be discovered, such as alterations in the shape of the cell-elements, or fruit-bearing organs. Sometimes marked differences of another sort are discovered, such as the kind or degree of modification impressed on the soil by the growth of the fungus. If the soil is gelatinous, one individual may liquify the gelatine, while another may not, although both specimens may be, undoubtedly, members of the same species. Many instances might be drawn from the recent study of the lower fungi to illustrate this principle, and in an excellent paper by Adami,* the bacteriologist who doubt its truth may find for his consideration a large collection of well-arranged examples. No one who has cultivated the moulds, which are pathogenic on man, on a sufficiently large and extensive scale, collecting his specimens from different countries, can fail to be impressed with the same truth. Fungi-culture has taught us that these Cryptogams are no exception to that general principle that Plants are specially liable to variation under cultivation, a principle which was illustrated by Darwin, in his own masterly style, in his work on "Animals and Plants under Domestication," but, in spite of which, many observers and bacteriologists have worked as if it had never been written at all. It is part of the object of this Thesis to show how wide is the range of these variations, and how differences, perhaps worthy of being considered racial, may be originated by changes in the conditions of cultivation. Extreme caution is needed when we come to deduce inferences; for every step we take into this new field of inquiry may discover facts which may cause us to modify, or even abandon, a previous conclusion. Now we are only at the verge of the unknown and this Thesis is offered as a contribution to aid us forward more by suggestions of facts than by demonstrated conclusions.

* "Medical Chronicle," vol. XVI., No. 6, p. 366.

CHAPTER II.

COMPARATIVE MORPHOLOGY AND BIOLOGY.

In respect of the evolution of forms, or species, the moulds, parasitic on man and the higher animals, occupy a position intermediate between the unicellular organisms and the higher fungi. In the lower fungi each individual is a single cell, reproducing itself by division. We can say, generally speaking, that no formed structures intervene between the parent cell and its offspring. The protoplasm of the parent divides, and the result is the formation of the new individuals. In the higher fungi a formed structure intervenes between the spore and the new individual; a special portion of the fungus is set apart to form a seminal apparatus, and the offspring is the result of the fusion of the male and female elements. This is the highest form of reproduction although the higher fungi, like higher plants, still retain other methods of reproduction.

On the other hand the fungi parasitic on warm-blooded animals, resemble the higher fungi in the development of a formed structure, but a special seminal mechanism has never been observed in any of them so long as they keep to their parasitic habitat. But the first step toward a reproductive apparatus is seen in the formation of fruit-bearing branches and in the formation of buds. Some of the Aspergilli, however go beyond this in their saphrophytic life and arrive even at the formation of a seminal apparatus or sporocarp. We can distinguish three parts in the parasitic moulds 1, spores, 2, thallus, 3, fruit-bearing branches (gonidiophores) and fruit (gonidia).

1. *Spores.*

True *sexual* spores have never been observed in these fungi in their parasitic habitat, and in the great majority of them it seems probable that this seminal method of reproduction never occurs under any known circumstances. It is true that well-marked sporocarps develop on Eurotium repens and E. Aspergillus glaucus* but these individuals have the least affinities with the group, and their pathogeniety, if any exists, is of the feeblest order. But the homologies between the species of the parasitic moulds and the "*Main series of the Ascomycetes*" suggest that the seminal modes of reproduction may have been, originally, possessed by the trichophytic and epidermophytic moulds. The present state of our knowledge, however, will not permit of any solution of this question. In Plate VII figs. 3, 4, 5, I have figured a very curious *appearance* of reversion to the sexual type which a common specimen of trichophyton exhibibited when cultivated under special circumstances, and which is described in the chapter on Variation. Such an appearance might lead one to favour the view that the power of forming archicarps and antheridia had originally been possessed by the ancestors of the trichophytic fungi, but that in course of time and change of circumstances it had been lost. But the resemblance may be merely accidental, and such conclusions fanciful. Yet there is nothing unreasonable nor indeed altogether lacking the support of evidence, in the supposition that the parsitic moulds are *descended* from a higher type of fungus. Their habits of life seem to indicate this. They readily change their parasitic mode of existence for a saphrophytic one, when the necessary (and perhaps original) conditions of temperature are afforded them, and under the conditions of cultivation they attain a higher and richer form of development.

Again I have noticed that individuals which had been passed through soils for four generations under the favourable circumstances of cultivation showed a very marked sluggishness to re-adopt the parasitic life. I shall have occasion to refer to this again.

Gonidia are bud formations springing from certain parts of the mycelial filaments. They represent the highest form of reproduction which is commonly attained by the group. The purely trichophytic fungi stop in their development on their hosts, short of this mode of reproduction.

* See Fig. 94, p. 203. De Bary, on Comparative Morphology and Biology of the Fungi, translated by Prof. Balfour, 1887.

The filament which bears the gonidia may be called a gonidiophore ; it resembles closely the gonidiophores of the Botrytes group. The description of the gonidiophores will be reverted to again. The form of the gonida varies not only in different species but in the same species, at different stages of their development. Thus the gonidia of trichophyton capitis vulgaris are pear-shaped while attached to the gonidiophores, but assume the spherical form when free. Their size differs with the state of their ripeness. Thus an unripe spore of achorion vulgaris which does not exceed 3μ—4μ, will attain the size of 8μ when germinating Species are sometimes distinguished by the different sizes of their spores as Aspergillus fumigatus with its small spores from Aspergillus glaucus with its large spores, but this method of distinguishing species is not, I believe, worthy of confidence. For while it may be safe for such well defined species as, aspergillus glaucus and fumigatus in which the function of spore-formation is far more highly developed than we find it in the trichophytic moulds, it becomes misleading in dealing with ill-defined moulds, in which the power of gonidia-formation is feeble and extremely variable. Very great differences in the quantity of gonidia may be observed in the different species of the parasitic group. Thus in the Aspergilli where this mode of reproduction is the recognised type of the genus, the quantity is enormous, but among the less clearly defined species of achorion and trichophyton, in which bud formation is not the established type the quantity at all times is scanty and feeble. The gonidia of some species are coloured. The aspergilli display a strong tendency to the formation of colouring matter, but sometimes the gonidia are colourless when the rest of the fungus is coloured.

Vicarious spores, or *sporiferous cells* :—All the purely trichophytic and epidermophytic fungi propogate their species by vicarious methods. As parasites even bud formation is not resorted to. Some (if not all) of the mycelial cells tend to become sporiferous cells. Their description belongs to section on "Thallus" when they will be more fully considered.

2. *Thallus.*

The thallus, or mycelium, of the parasitic moulds consists of a colony of cells formed by acrogenous and laterial growth. The germ spore when ripe and supplied with nourishment throws out one or more buds which elongate into cells whose length exceeds, many times their own breadth. The mycelial cells are tubular in shape, and are invested when young with thin transparent walls composed of a substance closely allied to cellulose and stained brown or yellowish brown by the sulphuric-iodine solution, the protoplasmic contents at first completely fill the cavity of the cell, and, when examined in an inert fluid at the same temperature at which the mycelium has been cultivated, it appears finely granular, of a pale grey colour, and transparent to light. Degenerative changes may occur rapidly, the granules becoming coarser, fat globules (and sometimes air vacuoles ?) forming in the cell-substance while the walls may bulge outwards.

The limitation of the cell is formed by a transverse wall which occupies a position close behind the growing point, while the growing point itself enlarges to form an adjacent cell. After the formation of the septum the cell still maintains its independence, and, so far as the closest scrutiny can discern, has no open communication with the adjacent cells which lie in the axis of its poles. The cell obtains its nourishment independently for itself, which passes directly to it through its limiting membrane, for there is no circulation of food particles or protoplasmic matter in the mycelium of these fungi. But the development of the cell does not necessary cease with it apical limitation, many of them throw out a bud on one side or another, generally immediately *beneath* the transverse wall at the growing end of the cell, less frequently from the opposite pole of the cell. The bud develops into a cell the cavity of which generally maintains open communication with the parent cell. By the continuous formation of apical cells and branch cells, a reticulate filamentous structure is formed which constitutes the body or thallus of the fungus.

The size and, indeed, the shape of the mycelial cell is subject to much variation : speaking generally the length of the cell varies directly with the nourishing qualities of the soil which supports it. For each cell being self-dependent for its nutrition, its limitations are determined by the supply of food in the soil in which it thrives. Thus the mycelium of trichophyton vulgaris, and achorion vulgaris, or indeed any of the epidermophytic fungi when growing in human skin is extremely short, its length barely exceeding its own breadth. It is this shortness which has led observers into the error of regarding these cells as *spores*. They are primary mycelial cells whose form differs widely from the form of mycelial cells of the same identical species under cultivation. This is due, however, to the poverty of the soil. Exactly the same sort of cells may be produced under cultivation if we will only imitate the original conditions. Thus when spores of achorion or trichophyton are put to germinate in ordinary water, or in mineral water, devoid of sugar, or proteids, the form of the mycelial cell exactly imitates the shape and size of the cell in its natural habitat. On the other hand when nourishment is abundant, the limitations of the cell are more liberal, and it then assumes the, comparatively, gigantic proportions delineated in Plate I., d.

During the dynamic or growing period, the formation of the thallus is mostly uniform, but during the static period, when all active growth has ceased, many changes occur in the mycelial cell. The walls become thicker and appear with a double contour, the contents become more coarsely granular, and highly refractile oil globules may form at intervals.

More notorious are the ampulliform swellings which form in two positions namely in the *terminal* cells in the axial line and in intercalary cells. The former I have ventured to call the end-ampullæ, the latter intercalary-ampullæ. The end-ampullæ are regularly oval or spherical in shape varying in size from 8μ to 30μ and even larger; in the intercalary vary similarly in size and are oval in shape. The significance of these swellings has been much disputed. By Duclaux and Unna, and most cultivators of these fungi, they are regarded as pathological. Some mycelial swellings are undoubtedly pathological, but I am inclined to regard these regularly formed ampullæ as serving some end useful to the individual and its race. They are formed generally at the turning-point in the life of the individual, when the climax of growth development has been reached or nearly so, and when in individuals of higher types of structure the formation of seed or fruit would naturally be taking place. They are most abundant in the middle portion of the colony where a dense weft is formed which bears some analogy to the sclerotium of the higher fungi. I am inclined therefore to regard these regular ampullæ as reserve stores of nutriment. In Plate II, Fig. 2, I have figured an end-ampulla, observed in a three-year-old specimen; the contents are seen to be nearly exhausted.

This central weft, where the ampullæ are chiefly situated, is so dense in some varieties of the trichophytic species that it cannot be teased out without breaking up the structure of the weft. It is sharply separated from the deep layer of the thallus, which can be drawn off as a separate membrane, but passes gradually into the upper layer with its erectile and sporiferous hyphæ. It is in this sclerotioid weft that the brown colour of old specimens develops.

The *sporiferous cells* are modifications of the primary mycelial cell formed by a process of partitioning off by the repeated formation of transverse walls. (See Pl. I., i.) They resemble the gemmæ of certain Mucor-formes (De Bary.)

The formation of the sporiferous cell takes place during the static period of the life of the fungus, or during what corresponds to the fruit-bearing period of the higher fungi. One or more mycelial cells may participate in their formation, which is accompanied by a general enlargement of the cell, with a repeated formation of transverse walls. The length of each sporiferous cell exceeds slightly its own breadth, so that the form approahes to the circular, and its breadth is about double that of the mycelial filament which bears it. In cultivated specimens of saccharomyces albicans, trichophyton vulgaris and achorion vulgaris they are sharply defined from the rest of the mycelium. It is possible that these sporiferous cells are analogous to *resting spores*, but exact observations are wanting as to the period of rest required by them, or as to whether their re-germination corresponds with any particular time of the year. But certainly they have not that definite character which marks the resting spores of the higher fungi, such as the teleutospores of Puccinia graminis.

The *reproduction* of the lower parasitic moulds, whether in their natural state or under cultivation, takes place commonly by vicarious methods, instead of by bud formation or by seminal formation; the vegetal mycelial cell takes on the function of a spore. Thus the tendency of *all* the mycelial cells is to become sporiferous, whether or not they pass through the form of gemmæ. But the conditions under which they obtain the fitness of spores are not yet known, and it seems probable that each mycelial cell possesses the sporiferous power in very different degrees.

3. *Gonidia and Gonidiophores.*

The fruit-bearing branches of the lower parasitic fungi are of the botrytis type. They are liable to much variation, and seldom or never attain that typical perfection which characterises the gonidia or gonidiophores of the higher parasitic moulds namely the aspergilli and mucors.

In some varieties of trichophyton the gonidiophores are slender branches arising from a mycelial filament, and possessing parallel walls and numerous septa at short intervals. (See Pl. I. j,, and Pl. III. fig, 3). Sometimes the gonidia or buds spring from a fruit-ampulla after the type of the aspergilli, but sterigmata have never been observed. In other varieties the arrangement of the fruit-bearing organs approaches closely to the botrytis type. (See Pl. I., m.n., also Pls. V., and VI.) Spores form singly or in small groups at the end of a short stalk or basidium. The basidium is broader at its base than at its summit. Sometimes the spores are aggregated into large bunches at the extremity of the gonidiophore, which is not, however, enlarged as in the aspergilli.

The fruit-bearing organs of the aspergilli and mucors are formed on a more precise type. The gonidophore is a branch of a mycelial filament, and its extremity forms a fruit-ampulla, the surface of which bears the stalks, or sterigmata, from which the spores are formed by abjunction. Their description will be reverted to under the special description of the parasitic moulds.

SPECIAL MORPHOLOGY.

I. Species, Trichophyton.

1. *Trichophyton capitis vulgaris**:—a white mould with yellowish brown under-surface, of feebler vegetative power than achorion, more fastidious in choice of food than the common blue mould, also less rapacious and more limited in its growth, but capable of growing at higher temperature than penicillium glaucum. Minimum temperature about 15°c., optimum 30°—31°c. Mycelium 3—4μ broad, length of mycelial cell varies from about 5μ—120μ Ampullæ 20μ—40μ gonidia 2—3μ. Varieties differ in respect of growth-energy, pigment-formation, gregarious or agregarious tendencies of colonies, parasitic virulence, and elective affinities for different parts of the body,

2. *Trichophyton Corporis*:—A well-marked variety, but not well-known. Flourishes in Vienna where its attacks on man are frequent. Its pathogenic virulence far exceeds its vegetative power. The fungus forms filaments and sporiferous cells in the epidermal scales.

3. *Trichophyton Muridarum*—A provisional name proposed by the Author for a group of trichophytic fungi which attack rodents. Very little is known about their life-history. The trichophytic fungi of man and the higher animals, such as the horse, probably descend from them.

4. *Trichophyton? Sporuloides*, Robin. A doubtful or, probably, spurious variety, consisting of spheroidal bodies similar or identical with Torulæ, found in neglected states of the hair, such as plica.

5. *Trichophyton? Ulcerum*, Robin. A doubtful variety described by Lebert, and Robin, found in the crusts of atonic ulcers of the leg, consisting of "nucleated" sporiferous cells 0.005—0.10 mm. in size, developing into moniliform filaments, some of which are branched (cf. Babes' oidium subtile cutes). No exact observations relating to this.

6. *Microsporon furfur*:—Considered by the Author as a variety of the trichophytic species, not as belonging to a distinct species. It is a low form of mould, consisting of threads and sporiferous cells. the latter sometimes abundant and may be detached in groups. Secretes a brown pigment. It has feeble pathogenic properties, but produces a desquamative pigmented condition of the skin of man, named Pityriasis Versicolor.

*See footnote p. 15.

7. *Microsporon Audouini.*—Probably identical with trichopyton capitis vulgaris, or, at most, a variety, mistaken by old observers for the fungus of Alopecia areata, then named Porrigo decalvans, but in reality a bald form of ringworm.

8. *Microsporon mentagrophytes.* An obsolete name of a fungus identical with trichophyton vulgaris, or a simple variety of the same. It is found in the follicles of the chin of man producing the disease known as hyphtogenous sycosis. It was supposed by the old observers not to penetrate into the interior of the hair-shaft, but to form an investment around the intrafollicular part of the shaft, separating it from the wall of the follicle.

II. Species, Achorion.

1. *Achorion vulgaris* closely allied to trichophyton vulg. Mycelium formed of thin wavy filaments 3—4μ broad, septate and richly branched, free extremities of filaments club shaped. Ampullæ of various sizes, 40μ and more, and varying in form and arrangement according to the variety. Between the filaments are often found chains of irregular oval sporiferous cells. Secretes a pale yellow pigment; thallus forms extensive masses on the surface of the skin, of dry chalky consistence and pale yellow colour; produces the disease called Favus. There are several varieties, described under the section on Variation.

III. Genus, Aspergillus.

1. *Aspergillus Fumigatus,* Fresenius. The fungus forms a greenish, often bluish growth so like penicillium glaucum, the common blue mould, that it is very difficult to distinguish one from the other by the naked eye. It is more delicate in structure than any other species of Aspergillus. The gonidiophores, 5μ diam., end in club-shaped ampullæ 8—20μ diam. in its broad part. Sterigmata unbranched, usually 6μ long, but occasionally reach 15μ, awl-shaped, closely packed on the hemispherical cup. Gonidia round smooth, single-contoured, mostly bright and colourless, 2·5—3μ in diam. Selerotia unknown, Siebenmann frequently observed groups of brown translucent spherules 17·5—25μ diam., believed by him to be resting spores. Similar bodies have been

seen by the Author, associated with trichophytic fungi. A. Fumigatus has an optimum cultivation temperature of 37°—40° c. (Siebenmann).*

2. *Aspergillus niger*. High growth-energy, forms dark chocolate brown masses, gonidiphores 18μ long, 10—15μ broad, fruit ampullæ spherical, sometimes 75μ broad. Sterigmata 20—100μ long, branched like a hand. Gonidia round, very numerous, dark brown, or greyish brown when ripe, surface smooth or warty and thickened, diam. 3·5—5μ. Sclerotia have dull brown or reddish colour, of the size of a rapeseed (Siebenmann).

3. *Aspergillus flavus*, forms greenish yellow or yellow growths gonidiophores 0·4 cm. long, 7—10μ broad. Fruit-ampullæ spherical, varying in colour from brown to golden yellow according to the soil and amount of moisture. Sterigmata unbranched. Gonidia round, yellow to brown, with a fine warty surface, diameter 5—7μ. Sclerotia very small and black. (Siebenmann).

4. *Eurotium aspergillus glaucus*. A fungus of feeble pathogenic virulence and found on man only as a epidermophyte. Fruit-ampullæ round, bluish green or yellowish green colour. Gonidia 9—15μ yellowish green, of round shape in some varieties, warty or spinous in others. Forms ascospores. Found growing on leathern articles, bark-liquor, preserved fruits, dried plants, damp wood, dripping. It thrives best between 10°—12° c. (Siebenmann).

5. *Eurotium repens*. At first white, ultimately dark green; fruit-ampullæ, not so compact as in E. asp. gl. Gonidia oval, smooth, colourless greyish green, or 5—58μ in longest diameter. Occurs on preserved fruits, bread, etc., optimum temperature 10—15°c. (Siebenmann).

IV. Saccharomycetes Group.

Saccharomyces albicans, Reess (Oidium Albicans, Robin) belongs to the group of Sprouting-fungi, grows as a parasite on the mucous membrane of the human digestive organs. Vegetates by sprouting, or by the formation of filamentous mycelium, the filaments springing out of the spout-cells. Resembles closely S. Mycoderma (Gravitz). Presents close analogies with Exoascus pruni which is parasitic on the young fruit of

* Siebenmann, Die Fadenpilze, zweite Ausgabe, 1889.

species of Prunus (De Bary). The S. albicans derives special interest from its position in the Exoascus-group, and, hence, its relation to fungi parasitic on living plants.

Saccharomyces Cerevisiæ, Torulæ.

Sprout-cells which closely resemble, or are identical with S. cerevisiæ are occasionally found with other harmless micro-organisms on the human skin. I have specially observed them in epidermal scales taken from human ringworm of the arm, and have noticed the sprouting form of their vegetation contemporaneous with the later stages of development of the trichophyton. A similar fungus has been described by Bizzozero*

V. Mucorini.*

1. *Mucor mucedo.* Found in aphthous patches on oral mucous membrane, probably as a saphrophyte. ~~It was shown by Lichtheim‡ to possess pathogenic properties.~~ Fruit-branches 1.3—4 mm., colourless, simple or branched, generally bearing a sporangium, sporangia yellowish brown to black, smooth, or granular, or spinous. Spores oval. Commonly found in all decomposing organic substances especially when rich in nitrogen (Frank).

2. *Mucor racemosus.*—Resembles M. Mucedo, but more delicate in structure. Fruit-branches at highest 1.5 mm., sometimes simple but generally breaking up into short branches with grape-bunch arrangements. Sporangia yellowish to bright brown, smooth or finely granular. Spores round. Found on organic substances especially when rich in carbohydrates. (Frank). ~~Shown by Lichtheim to be pathogenic or certain rodents~~.

Both these species belong to the higher fungi, as they are capable of propogating their species by seminal methods. Zygospores which result from the conjugation of the two several cells have been described by Bainier.

VI. Penicillium Group.

Penicillium minimum (Siebenmann) :—Observed in the human ear in a single case (Bezold), consists of delicate branched colourless septate

* Virch, Arch, Vol. 98, p. 441.

† De Bary, l.c: p. 150.

~~‡ Lichtheim, Zeitschrift, f. klin. Medicin, Bd., 5. H.,~~ 2.

* See Appendix, Note A.

mycelium 2μ diameter. The fruit-bearing branches end neither in ampullæ or clubs, but exactly like those of penicillium glaucum. Length of pencil of rays 20μ The spherical smooth gonidia $2{\cdot}5$—3μ are simple-contoured, colourless when seen singly, blackish brown in masses resembling Aspergillus niger. (Siebenmann).

mycelium 2μ diameter. The fruit-bearing branches end neither in ampullæ or clubs, but exactly like those of penicillium glaucum. Length of pencil of rays 20μ The spherical smooth gonidia $2{\cdot}5$—3μ are simple-contoured, colourless when seen singly, blackish brown in masses resembling Aspergillus niger. (Siebenmann).

CHAPTER III.

Natural History of the Fungi Parasitic on Man.

The Natural History of the parasitic moulds has for its object the portraying of the life of these fungi in nature apart from any interference on the part of man. It therefore excludes all facts obtained from the observation of these fungi under cultivation, although the practice of fungi-culture cannot be excluded in the study of the subject.

Our knowledge is yet too fragmentary to allow us to trace the species of these fungi through all their natural evolutions; to follow them from host to host, from the animal of low temperature to the animal of high temperature, such as man. But this is the *aim* of natural history in respect of the parasitic fungi. It would be impossible to write this chapter solely from the point of view of a physician, that is, from the clinical aspect of the diseases which the parasites occasion. The outlook must be taken from a biological standpoint. Man must be regarded as a host, and the skin as a soil in which thrives the plant-parasite. The disease is the modification of the soil and this is only an illustration of a general law that every soil is modified by every organism it nourishes and every organism is changed in some way by the soil it lives on. But the observation of the disease affords only one aspect of the natural life of the fungus, and that is a purely relative one. In reality the disease is the behaviour of the tissues towards the fungus, and the facts we glean from these obvervations must be placed under the head of *reaction*, and clearly discerned as a series of events apart from the simple life of the parasite.

1. *Trichophyton capitis vulgaris.**

I have selected this fungus as a type of a very extensive group. It is essentially a hair-destroying fungus, but the group includes many

* I have ventured to substitute this name for the old one "Trichophyton tonsurans:" the later is objectionable since the fungus does not always produce a "tonsure."

individuals which habitually flourish as epidermophytes, producing desquamation, but not affecting the hair itself.

The trichophyton cap. vulg. is universally distributed, but best known in the temperate zone. In the tropics it is met with on the Caroline, Gilbert and Samoa islands, and produces the disease known as Polynesian Ringworm.* There are certain parts of Europe where it very seldom attacks man. e.g. Vienna, Antwerp, and Hamburg. It is a common and virulent parasite in Paris, London, and Berlin. The scarcity of this variety of mould in certain cities is associated with the very frequent occurrence of certain other individuals of the same species or genus. In Antwerp its place is taken by achorion, in Vienna by trichophyton corporis.

The descent of the fungus has not, yet, been clearly traced out, but it seems probable that its original ancestor is a parasite or saphrophyte on the higher plants. Its first host in the ascending series is probably an animal whose temperature is considerably lower than that of man, such as the common field or barn mouse, or some other member of the cosmopolitan muridæ family. The close relations which these animals hold with vegetable produce employed by man and the higher animals would afford the parasite favourable means of ascending in the character of its host. The step between a purely saphrophytic life and a parasitic one on the mouse is not so great as might be imagined. The hair and horny epidermis are structures on the border line of the living and the dead, and, as I have proved by direct experiment, it is possible for trichophyton to attack hair when detached from the body, in which case it must be considered as a saphrophyte. When the individual fungus has become accustomed to the parasitic habit, it would transmit to its descendents those particular variations which had fitted it for this mode of life.

The next host in the ascending series would probably be the cat, which is the natural enemy of the mouse tribe. We may easily conceive that the fungus assended from this point in two ways. Rodents lie in close contact with their own dung, and this forms an excellent nidus

* Königer. Virchow Archev, Bd. 72, p. 413, 1878.

for resting or dormant spores. When moistened with water, certain fungi are capable of forming on rodent dung a small amount of thallus. This I have observed with a mucor and aspergillus, but, although direct observation is wanting, I see no reason for believing that such may not occur with the trichophytic fungi, or at least that they would remain in a dormant condition, but ready when the opportunity came to attach themselves to some host, and then to pass through their further development.

Horses and oxen probably are inoculated from straw, or rodent dung, which has been in contact with affected rodents. When we think how close is the relation into which man is brought with these animals by their domestication we need not be surprised at the fact that he himself is a host to this parasite. Man in his childhood is, indeed, the favourite host of the trichophyton. The unctious condition of his skin and the delicacy of his structures render him peculiarly liable to the attacks of the parasite.

So far as I have been able to ascertain the trichophyton is conveyed from host to host only by direct contact, and not by the medium of the air; but on this point we are in need of further evidence. When a living spore, or portion of the thallus, has been conveyed to the surface of a child's scalp, it becomes attached to the surface by means of the oily secretion with which the head of man is habitually coated. The oil and the watery saline secretion of the sweat glands afford the first nourishment for the spore, If the surface fat is scanty or too acid the spore dies. When the resting condition has ceased, the imbibition of nourishment causes the spore to enlarge, and to throw out on one side a minute bud, which, as the nutrition continues, enlarges into the primary germ-tube. The initial out-growth creeps along the surface of the scalp until a hair-shaft is encountered. Unlike the mucors and aspergilli the germ tube of trichophyton is not turned aside by this obstacle but *dissolves* an aperture in the sheath of the shaft and so gains entrance into the interior of the hair. As the germ-tube elongates, septa form behind the growing points at very short intervals (3μ—5μ), the walls between the septa, bulging outwards, give to each cell the appearance of a spore. Each mycelial filament is thus composed of a series of cells more or less round. The advancing points of the thallus proceed downwards in the direction of the greater temperature and moisture, so that the upper part of the hair is spared, while the root-portion is mainly attacked. In whatever direction the mycelial thread advances that part becomes blighted. The hair fibres are not merely separated, as in favus, they are dissolved, or partially dissolved, so that the residue is merely a mass of crumbled broken-down fibres. As the growth proceeds, branches and branchlets strike out in all directions till the whole of the portion of the shaft within the warm well-nourished follicle is filled with a ramifying thallus.

The occupation of the hair-shaft by the parasite does not, at first, paralyse the growth of the hair, but as each new portion of hair is formed it becomes a prey to the fungus, while the blighted portions are slowly elevated above the level of the follicle. These blighted portions of the hair-shaft thus exposed to view, appear to the observer as dull white corroded stumps, bearing a resemblance to the stubble in an old corn field.

The mycelial cell is sometimes considerably longer than its own breadth, and the filaments of which these longer cells are composed have the ordinary appearance of "mycelium." Both forms of filament, namely the short-celled, and the long-celled, are mycelium, the difference being merely in the relative size of their primary mycelial cells.*

None of the higher forms of development are observed in the trichophytic fungi, so long as they occupy the hair-shafts. Free gonidia or seminal formations have never yet been observed in the fungi under *purely natural conditions*. But by the simple addition of ordinary pure water I have observed the most exquisite formation of gonidia of the Botrytis type (See Plates V and VI). The description of this, however, must be referred to the chapter on cultivation, as it resulted from a change of treatment.

In general the trichophytic fungi propogate their species by vicarious methods. Instead of any special sexual apparatus, or even budding from certain branches, the primary mycelial cell tends to assume the rôle of a spore. This end seems to be attained only by a few

* See page 7.

of the cells, for most of the sporiferous cells remain sterile when placed under conditions suitable for germination.

The varieties of trichophyton will be specially considered in the chapter on variation. There are some noteworthy varieties whose action on the tissues of their host, is different from that of the trichophyton vulgaris. They are epidermophytic fungi in as much as they confine their attack to the surface of the general integument. Perfect specimens are seldom, or never, met with in this country; but in Austria and the surrounding countries they flourish well. The fungus is extremely irritating towards the cutanteous tissues of its host, for it provokes a good deal of hyperæmia and desquamation. Large tracts of skin become invaded. The fungi never form in large quantity which is surprising considering the amount of reaction. It must be sought for in the early stages, as often at later periods when the hyperæmia, or, it may be, inflammation, is well established it is difficult to find. The disease is called in Vienna *Herpes tonsurans corporis.*

A pigmented Austrian variety which I detected for the first time in the hairy scalps of three children in Vienna will be described in another place. It is worthy of being considered a variety since it produced a brown pigmented zone around the patches where it was growing.

Microsporon furfur. It is usual to describe this fungus as a separate species. I think, however, that it would be more consistent with our present state of knowledge to include it in the trichophytic group, and in the species of which trichophyton vulgaris is the type. It is an epidermophytic variety thriving, somewhat feebly, in the epidermic scales on the front of the chest of man. It belongs to the coloured races of this species, and produces a dark brown pigment, which stains the tissues of its host. It is characterised by the formation of comparatively large quantities of sporiferous cells, commonly called "spores." It provokes very little irritation, and this only amounts to a little desquamation and pigmentation. The disease is known as *pityriasis versicolor.*

2. *Achorion Vulgaris, vel Schönleinii.*

It is more consistent with the present state of our knowledge to regard achorion vulgaris (Schönleinii) as the type of a species included in the trichophytic group. Whether we regard it from the cultivator's point of view, or from the Natural Historian's, the achorion species is so closely allied to the trichophyton vulgaris species that we may reasonably consider that both are decended from one common parent. In fact the history of the two fungi is very similar. Achorion attacks rabbits, mice and horses. But it deviates more than trichophyton from the line of homologous hosts, Thus Leisering has described a variety of this species in connection with the disease of the cochin-china fowl known as "the white comb." The fungus grows at the base of the feather, penetrates into the follicle causing the feather to fall out.* Ercolani has described another variety (*Achorion Keratophagus*) which attacks the hoof of the single-hoofed quadrupeds (*Solidungula*), and the same variety has been observed by this author under the human nail where it produces the disease called Onychomycosis.†

Another variety of this species has been described by Mégnin ‡; if was found on the comb and ~~and~~ head of a cock (coq de la race de la Flèche) where it produced mealy or chalky crusts.

The growth-energy of achorion, and its varieties, is very much higher than that of trichophyton; for while the trichophyton grows in very limited quantities, the achorion is specifically distinguished by the formation of bulky masses, and the comparatively vast regions which it may cover. Thus large portions of the body of an affected rabbit or man may be covered by masses of scutula. The loss of hair in favus is much greater than in ringworm, but the actual damage which achorion causes in the hair-shaft itself is considerable less than that occasioned by trichophyton. In Favus the hair often tumbles out of the diseased follicle *en tout*, or an erect hair-shaft may be seen piercing through masses of sculuta, which hold it in its position, but in ringworm of the head the hair crumbles away, being itself the centre of the disease.

Achorion, unlike trichophyton, vegetates principally on the *surface* of the skin, The invading spore after an interval of rest, enlarges at the expense of the surface nourishment, and throws out two or three

* † Quoted by Frank in Leunis' Synopsis.

‡ Mégnin in Comptes Rendus Soc. de Biol. tome II, 1890, No. 11.

germ-tubes, septa form rapidly behind the growing point, so that the mycelial cell is a short compartment with wavy side walls. Most probably one "generation" follows another, a new thallus, developed out of a sporiferous cell, being added to the old one, until the body of the compound-colony (Scutulum) attains considerable size. The shape of this aerial portion of the thallus of achorion bears some resemblance to a cup, that is, it is contracted in the centre, and elevated at the margins. This cup-shaped form is produced probably in the following way. As the mycelial mass becomes heaped up, the parts farthest from the moisture of the skin dries by evaporation. At first the evaporation is followed by a shrinking of the whole mass, but as the loss of water continues the older central portions continue to contract, while the peripheral portions expand.*

The exact cause of the expansion is difficult to explain. Possibly it may be due to a flattening of the lateral axis of the cells, and a corresponding lengthening of the antero-posterior diameters. This selerotiod aerial portion of the thallus of achorion when dry loses all external traces of its vegetal nature, and has the appearance of a mealy or chalky concretion, with a pale yellow colour, and a "mousey" odour.

From the surface the growing points of the thallus penetrate into the follicle and ultimately into the interior of the hair. *This internal portion of the thallus* differs from the external part in some respects. The mycelial cell is not much longer than its own breadth, the side walls however may be perfectly straight and parallel, so that the series of these cells presents the appearance of "mycelium," The damage done to the hair fibre is considerably less than that perpetrated by trichophyton vulgaris, for the filaments of achorion push their way between the fibres and hence may be seen lying in straight lines more or less parallel to the long axis of the shaft. If the name "spore" be given to these intrafollicular mycelial cells, it should be borne in mind that they are not true spores of seminal or even bud formation, but merely

* This contraction and expansion may be observed in minute fragments of scutulum by the aid of the Microscope. The micro-fragment is placed in a few drops of warm water (30° c) on a warm stage. As the water evaporates the margin of the fragment contracts 12μ—18μ, in a few moments the margin is seen to expand 12μ—14μ.

a modification of the mycelial cell impressed on it by the special conditions of its environment.

Achorion in its natural habitat propogates itself, like trichophyton, by vicarious methods. Seminal organs have never been observed, nor so far as I know even bud-formation. The retrogression of the mycelial cell into a sporiferous cell is accompanied by an enlargement in size and a bulging out of the side walls. Probably it contains much undigested nourishment held as a reserve stock for the possible future development of the spore. But, just as in trichophyton, sterility is the destiny of most of these sporiferous cell. For some reason which we do not understand, the individual cells of the thallus are modified differently by the soil they pass through. And these variations become apparent when a group of these cells or spores are placed under common conditions favourable for their higher development. It seems certain, however, that none of these cells of the thallus can pass directly from the simple condition of *growth* to the conditions of a progenitor of its species. A period of uncertain length, is necessary for the transformation. But after this interval the new spore may pass through its development in another part of the *same* soil, i.e., its host. The achorion vulgaris, like trichophyton vulgaris, is difficult to eradicate, owing to the fact that both moulds penetrate below the water-proof barrier of the horny-epidermis. Achorion inflicts more damage on the tissue of its host than trichophyton, probably on account of the pressure of the masses of scutula, which lead to the scaring of the subjacent tissue.

There are several varieties included in the species achorion which will be mentioned in the chapter on Variation. Their individual life-histories have not yet been worked out, but, probably, they do not vary much from the type. I may specially mention, in this place, the achorion of Quincke* of Kiel in-as-much as the different behaviour of the skin towards it indicates that it deviates from the type of its species, and approaches still closely than achorion vulgaris to the character of trichophyton vulgaris and its species. Quincke observed it on mice and

* Quincke, Archiv. f. Experiment, Path. u. Pharmakologie Bd. XXII,

cats, and also on man whom it attacks on different parts of the general integument, such as that of the arm, thigh, or face. It produces a red circle marked by yellowish scales, a little later vesicles appear and yellow scutula are formed at the entrance to the follicles. This variety is seldom met with in Britain; and even in places where Favus is common, as in Pisa, the fungus is seldom found attacking non-hairy parts alone.

3. *The Aspergillus Genus.**

Of the three *true* Aspergilli, namely A. flavus, A. fumigatus, A. niger, only A. fumigatus possesses the power of developing upon pus, cerumen, mucous membrane, healthy epidermis, or upon nutritive substances covered with nasal or oral mucus, but otherwise favourable to their growth. A. fumigatus grows tolerably well upon slightly moist epidermis, kept at a warm temperature, but it does not penetrate into the substance of the epidermis, its growth being confined purely to the surface. Siebenmann observed it growing, spontaneously, upon some cutaneous scales in the incubator, and found a specimen developing under a living human finger nail where A. niger had previously been sown, but without effect.

All the true aspergilli attack the human ear, but more after the manner of saphrophytes than as true parasites. Their development in the ear would seem to depend on certain inflammatory conditions, their nourishment being derived from the serous exudation, rather than from the normal cells or juices of their host.

1. *Aspergillus fumigatus*:—This occurs in nature as a saphrophyte. Siebenmann observed it in Basel growing spontaneously on some nutrient substratum during the hot weather. It is closely dependent, however, on warm temperatures, on which account it is probably more abundant in tropical or sub-tropical climates. Its spores, however, are very widely distributed, and in some localities in Germany, e.g. Bern, it very frequently crops up spontaneously in the warm incubator. It so closely resembles the common blue mould in its external appearance,

* The following facts bearing on the natural history of the Aspergillus group are based on the the observations of Siebenmann, Leichtheim, Gaffky, Loeffler, Grawitz, Bezold and Delépine.

that even experts may be mistaken in its identity. They are essentially distinguished however, by their different relations to temperature, Aspergillus fumigatur flourishing well from 30°—40° c., while penicillium glaucum is sterile at these temperatures, and grows best at 15°—20° c., or the ordinary temperature of a room in temperate climates. The spores of the fungus, as already described, are formed in great abundance by a certain process of budding. They are so delicate and light that it is quite conceivable that they are conveyed by the floating matter of the air from place to place. The spores attack diseased ears, producing the disease known as *mycomyringitis aspergillina* or as *otomycosis aspergillina*. Very often there has been previously a chronic otitis media with perforation. So long, however, as the inflammatory discharge is purulent, the spores of the aspergillus do not develope, but when the purulence ceases, and the discharge becomes a thin watery liquid, saline and faintly albuminous in its chemical constitution, the spores rapidly develop into a fructifying thallus. This fact in the life-history of Aspergillus fumigatus explains the sudden appearence of this fungus in the human ear after the use of astringent lotions, such as boric acid, or zinc glycerine. The astringent succeeds in restricting the number of cells in the discharge and consequently prepares the soil for the development of the aspergillus. The mould thrives on the surface and does not penetrate into the substance of the epidermis. It has been observed by Burckhardt-Merian* to grow on a cerumen plug on removal of which it did not recur. Siebenmann believes that damp mouldy rooms have no special tendency to cause the appearance of aspergillus fumigatus in the ear. According to Wreden,† aspergillus fumigatus very seldom attacks the ears of children. This immunity is owing to the kind of aural inflammation, which is diffuse and exudative, and to the rapidity with which the discharge decomposes and becomes acid on account of the narrowness of the meatus.

The experiments of Lichtheim ‡ have revealed the fact that the aspergillus fumigatus possesses, naturally, fatal pathogenic properties.

*† Quoted by Siebenmann, l.c., 2nd Edit., pp. 43, 48.
‡ Lichtheim, l. c.

It attacks the respiratory passages of the lungs of cows and horses, and some birds, producing a fatal *Pneumomycosis.* Its development in the interior tissues of these animals never passes beyond the formation of a simple thallus,⊖ and it is only by the culture-methods recommended by Koch, that the identity of the thallus with that of aspergillus fumigatus can be accurately determined.

It is not improbable that A. fumigatus can attack the lungs and respiratory passages of man. Cohnheim* and Virchow† have described cases of human pneumomycosis, but the ~~identity~~ species of the fungus was not determined; from the character of the mycelium they were probably aspergilli. ϕ

Lichtheim‡ Gaffky,§ Baumgarten and Müller,‖ and others, have shown that pure cultivations of the aspergillus fumigatus injected into the blood stream of rabbits, produce death in three days, when the liver and kidneys are found to be pervaded by the mycelium of the fungus.= §

2. *Aspergillus niger*. The history of this fungus is similar to that of A. fumigatus, but its pathogenic properties are of a much lower order. It is a rare invader of the human ear. Siebenmann was unable to transfer the cultivated specimens to the ears of rabbits, even under various circumstances, such as scalding the ear, or when excoriated by Sarcoptes communis, or when gelatine was simultaneously introduced. He failed likewise in inducing this fungus to attack the general integument of the rabbit, either when healthy and un-wounded, or when the horny epidermis was removed, and the inoculated area had been carefully guarded by a watch-glass. Equally unsuccessful was Siebenmann's attempt to produce vegetations of this fungus on the membrana tympani of two healthy persons by insufflation of moist spores.

Aspergillus niger does sometimes appear spontaneously in the

* Cohnheim. Virch. Arch. Bd. 33, p. 157.

† Virch. Arch. Bd. IX., p. 574, p. 401.

‡ Lichtheim, l.c.

§ Mittheilungen aus den Kaiserlichen Gesundheitsamte. Bd. 1. p. 80.

‖ Baumgarten and Müller, Berl. Klin. Woch., 1882, also Baumgarten über pathogene pflanzliche mikroorganismen, sonderabdrücke der Deutschen Medizinal-Zeitung, Berlin, 1884.

⊖ Aspergilli in the respiratory passages of animals frequently form gonidia; but the aerial surfaces of the lungs and air-passages are, strictly speaking, external to the body of the animal.

= ϕ See Appendix Note B..

§. Aspergillus fumigatus produces a disturbance of the sense of equilibrium in rabbits. The animal lies on one side, with head and eyes obliquely inclined, and shows certain involuntary movements, such as rotating around the axis of its head. Dogs may be attacked, but are far more resistive, and recover without loss of equilibrium.

diseased human ear. It was not found to be pathogenic on rabbits when injected into the blood stream.

According to Delépine,* A. niger is capable of attacking human skin. He cites a case where the fungus appeared around the margin of a superficial ulcer on the leg. The skin although free from any wound was not under normal relations, being covered with splints and strapped down with ordinary soap plaster. When the splints were removed the ulcer and fungus were discovered. He established its identity by cultivation. Deléphine's conclusion regarding its pathogenicity is somewhat different from that of Lichtheim , for he found when its spores were injected into the peritoneal sac, that they showed in a few days feeble signs of germination. It is however much inferior to the Aspergillus fumigatus in pathogenic virulence, but the presence of its spores on the respiratory passages of man provoke a sort of spurious hay-fever.

3. *Aspergillus flavus.*—The history of this fungus, so far as we have yet learned, is very similar to that of the A. niger. It is much inferior to A. fumigatus in pathogenicity. It attacks the ear but seldom. Lichtheim failed to induce it to adopt a parasite life under conditions similar to those related in respect of A. niger.

4. *Eurotium Aspergillus glaucus and repens.*—These moulds are very commonly distributed in countries of temperate climate. They are often found as saphrophytic moulds on preserved jams. According to Lichtheim they are harmless saphrophytes, and provoke no disease when injected into the blood stream of such rodents as rabbits and guinea-pigs. De Bary includes them in the group of fungi which produce *otomycosis aspergillina*, but they are much inferior to A. fumigatus.

The aspergillus group approaches nearer the higher fungi than the trichophytic group; but just as the trichophytic fungi stopped in their development in the tissues of their host short of their highest form of fructification, namely gonidia, so the aspergilli have never been observed to transcend the gonidia form, in the tissues of their host. Their highest form of reproduction, namely the sporocarp, is seen only in their saphrophytic phase of life (De Bary).

* Delépine, Trans. Path. Soc. Lond., Vol. XLII, 1891, p. 423.

Doubtful Species of Moulds Parasitic on Man.

1. *Saccharomyces albicans*, Reess (oidium albicans, Robin), this fungus has been experimentally shown to be the cause of a formation of pustules and scab, known as thrush or aphthæ, on the mucous membrane of the mouth, throat and asophagus, especially in young or feeble individuals. The fungus closely resembles the Flowers-of-Wine or Saccharomyces Mycoderma (Gravitz).

Ascospores have never been observed in it. Its chief mode of existence is saphrophytic, but it is capable of exciting weak alcoholic fermentation in saccharine solutions, (De Bary). According to Cienkowski's observations,* the saccharomyces albicans may vegetate either by sprouting like the S. Cerevisiæ, or long hyphal cells may grow out of the sprouting cells, and form a thallus; ultimately however the long mycelial cell divides transversely into short cells which then vegetate simply by sprouting.

2. *Oidium subtilis cutis* (Babes).† The identity of this fungus has not certainly been made out. It was observed by Babes in connection with numerous pea-sized ulcers on the surface of the thigh of a woman. The patient was suffering from an abscess of her left thigh. The ulcers were covered with crusts, which, when examined microscopically, were seen to consist of septate mycelium, branching and forming a weft. Those filaments which were on the outer surface terminated in blunt extremities, and were seen to bear free gonidia. It was cultivated, and a rabbit inoculated with a portion of the culture, with the result of producing in three to five days the same sort of ulceration. The ulcers were round with bright red granulated bases, and extended down to the dermis and even deeper.

* Quoted by De Bary, l.c., p. 267.

† Babes, Biologisches Centralblatt, Bd. II. No. 18 p. 569.

CHAPTER IV.

THE CULTURE OF THE PARASITIC FUNGI, OR THE SAPHROPHYTIC LIFE UNDER KNOWN CONDITIONS.

Parasatic moulds are like wild plants inasmuch as they can be cultivated, and under cultivation they are found to display the same tendency to variation as the higher plants. I shall discuss these variations in another chapter, confining myself in this to the various methods employed in fungi-culture. First as to the purposes of cultivation. Wild fungi carry their resemblance to wild plants still further in showing a marked tendency to improve in type when reared under the favourable conditions of cultivation, and the cultivator takes advantage of this in order to familiarise himself with the properties and structural characters of the fungus. Cultivated moulds vegetate much more profusely than in their wild state, and this, also, is an advantage, since it affords the cultivator an extensive stock from which he can draw for microscopical or experimental purposes; one of the most valuable purposes of fungi-culture is the means it affords the cultivator of separating one fungus from another when several different varieties or species are present in the same soil.

It is usual for pathogenic fungi to retain their pathogenic properties after cultivation, though some individuals lose their pathogenicity, partially or completely, when reared through many generations under certain conditions of cultivation. Instances of this will be given in the chapter on variation. The cultivator must always bear in mind that the fungus under cultivation is not identical with the same fungus in a state of nature. The opportunities for variation are very numerous, and it is more than likely if he does not constantly bear this in mind that his conclusions, if he draw any, will be often warped by fallacies.

To obtain *pure cultivations of the trichophyton capitis vulgaris*, I have adopted for some years the method pursued in the Pasteur Institute.

The patch of ringworm is first washed with an aqueous solution of corrosive sublimate (1 : 500), and then dried with cotton wool; one of the short broken hairs is now seized between the blades of a pair of forceps, previously heated to redness in the open flame of a spirit lamp. The cultivator retains his hold of the closed forceps with his left hand and with the right severs the intra-follicular portion of the hair by means of a heat-sterilised scissors, the severed portion being allowed to fall directly into the flask containing the sterilised soil, after which the cap or plug is immediately replaced. By this method, when successfully performed, the cultivator may obtain pure cultures from the first.

The *pure culture of Achorion vulgaris* from scutula is, as all cultivators know, not always an easy matter. Different methods are employed in different countries. Some cultivators rub the sculula down with dry sand, others take minute fragments from the under surface of the scutulum. My own method is to allow the germination of minute fragments of the scutulum to take place in pure water, or in mineral water, free from organic ingredients. In this way pure cultivations can be obtained, even through precautions are not specially taken to exclude bacteria, because the water, which is sufficient to develop the germ-tube of the mould spores, and even to form minute filaments, is not favourable to the development of the bacteria. The details of this method consists in placing some ordinary spring water in a sterilised covered shallow glass dish. If the water is boiled, previously, to sterilise it, it must be shaken afterwards so that it may become aerated, as the absence of free oxygen in the liquid very considerably retards the development of the fungus. Some very minute fragments of the scutulum are now placed in the water, the cover reapplied, and the dish placed in the incubator at 30° c., only enough water should be used as is sufficient just to cover the fragments of scutulum. Germination take place more readily and more vigorously in Raulin's mineral solution, *without* the sugar. In this soil germination begins in a few hours and in twenty-four hours a minute quantity of mycelium has been formed. At the end of forty-eight hours there are several independent centres, and one or more of these may be transferred, on the point of a sterilised platinum needle, to its appropriate soil.

It is often desirable to watch the development of the mould-plants stage by stage. The methods devised to accomplish this end have all to contend with the difficulty of evaporation. With solid media the difficulty is less, and specially thin test-tubes have been constructed through which the development can be observed continuously. Quincke, Pick and Unna have employed this method. The objection to the method is the sacrifice of light by the use of the solid medium and the impracticability of using the higher lenses.

For the continuous observation of the mould fungi *in their natural habitat* I prefer a simple modification of my own of the Dallinger-Drysdale moist-stage. As this useful method of investigating the mould fungi parasitic on man has not, as far as I know, been used before, I will describe both the instrument and the method in detail, The instrument consists of a plain glass stage (Plate VIII. Fig. 1 a.) $\frac{1}{10}$ of an inch thick, and of such size as to rest steadily on the stage of a Reichert microscope. A central aperture (b) is cut through it, and a thin coverglass, larger than the aperture is fixed over it with Canada balsam. At the end of the arm which extends some distance beyond the stage to the left of the observer, is a brass socket with a one-inch brass ring attached to it by means of marine glue. The object of this ring is to hold a glass vessel about two inches deep. It simply drops in, and the top being slightly larger than the ring it is prevented from slipping through. When the instrument is in use, the well is filled with ordinary water, and a piece of clean white bibulous paper (d), shaped to the figure of the instrument, and having a central circular piece cut out of it, is then laid on the surface of the stage. The central hole in the blotting-paper must be larger, by nearly quarter of an inch, than the coverglass (c), and the slip corresponding to the arm must be long enough to reach to the bottom of the well. No special precautions need be taken to sterilise the instrument, but the surface of the stage must be washed with rectified spirits and then with distilled water. A diseased hair, preferably with a scale attached to it, taken from an untreated case of ringworm of the head, is laid on the transparent centre of the stage, and surrounded by a few drops of ordinary water, after which it must be at once protected by a clean coverglass. A clean watch-glass is now placed over the field of cultivation, and must be big enough to include a small portion of the wet blotting-paper. The whole apparatus may then be placed in the incubator. Once every twenty-four hours the well should be re-filled with water, but if the temperature of the incubator exceeds 30°c. it would require to be filled more frequently. When it is wished to make observations the instrument is removed from the incubator to the stage of the microscope, and the observations taken, the watch-glass is then re-placed, and the whole apparatus, once more, put into the incubator. The figures 1, 2 and 3 in Plate V., and figure 1 in Plate VI, are delineations of observations of a culture made on this stage. The great advantage of this method is that it enables us to observe the development of the fungi under conditions which differ but slightly from their natural conditions. For the only addition is ordinary water; the fungus continues to develop in its natural habitat. The practicability of this method however depends on a property of the trichophytic fungi themselves, namely their power of dissolving and rendering transparent the otherwise insoluble, albuminoid Keratin. At the beginning of the culture the observer will be unable to discern anything clearly owing to the opacity of the scale, but in about forty-eight to seventy-two hours the opacity begins to clear, and then the cultivator is able to discern the mycelium with its sporiferous cells, whose further development he can watch with increasing clearness from day to day. The number of bacteria which appear in the culture is too few to hinder the development of the mould, or to obscure the view of the cultivator. Other mechanical contrivances for cultivation in large quantities are numerous, I may mention the *Pasteur Flask*, delineated in figure 2, Plate VIII. The narrowness of the chimney, plugged with sterilised cotton wool, is an advantage against the risks of impurities, and the evaporation of the cultivating fluid. There is no better flask than this, for ordinary cultivation and the only drawback is its expense which is somewhat considerable when we require to use some hundreds of them.

Pick's Flask (Fig. 3, Plate VIII) is of service for culture on media which may be solidified by heat. I have used them for cultivation on white-of-egg. But their practicability for continuous observation depends entirely on the maker of the flask, for if the quality of glass is poor and not sufficiently thin, only the low powers can be used for observation. *Test tubes*, and *shallow covered glass dishes* have their advantages in certain cases. The *German one-ounce flask* I have found very convenient on account of its cheapness. I have cultivated some thousands of specimens in these simple recepticles. They are not suitable for preserving old specimens, for the width of the mouth permits of too free evaporation, and impurities are very apt to arise after some months. *Brefeld's* tube I have found of no advantage, in the cultivation of these moulds. *Kitasatos anaerobic dish* is serviceable for observing the effects of different gases on development.

CHAPTER V.

Soils, or the Nutritive adaptations of the parasitic moulds.

In the chapter on the Natural History of the moulds parasitic on man I attempted—and the attempt is the first which has been made since the time of Robin—to give some systematic account of the life of these fungi in their wild state, apart from any interference, or a minimum of such, on the part of man. But the phenomena of their vegetation may be studied to great advantage under cultivation on dead organic substances, or solutions of these. When our observations have been extended sufficiently and the conditions under which the fungi are reared, have been varied in a sufficient number of different ways, we are able to deduce this fundamental principle *that every individual fungus modifies the soil it thrives in, and every soil may impress some variation on the fungus it nourishes.* This principle is in perfect harmony with the phenomena of vegetation of the higher plants. Although among these the mutual inter-reaction of plant and soil plays only a secondary part in inducing variation (Darwin). There are the far higher influences of heredity and natural selection acting through many generations. But among the intermediate fungi, which we are now considering, the law of inter-reaction of soil and organism is pre-potent. This principle underlies some of the most vital problems of modern Bacteriology, such as that of Immunity, and the Origin of Races. For were we able to follow those inter-reactions right down to the essential chemical changes in parasite and host, we should have no difficulty in understanding Immunity and the Origin of Races. Soils, indeed, would appear to have their affinities for particular spores, just as chemical elements have affinities and dis-affinities towards other chemical elements, and probably at bottom, although unknown to us, those affinities between soil and organism are as precise as the atomic theory, or law of gravitation, and may, possibly, in the future be grouped under some law of vegetal affinities. At the present we have only obvious facts to deal with, such as that this, or that individual fungus can live as a saphrophyte only, or as a parasite only, or that both modes of existence are common to it, or that it may pass through part of its development as a parasite, but only reaches the higher stages of development as a saprophyte. Indeed our knowledge of the affinities of the moulds parasitic on man is not so extensive as this. For we are not absolutely certain that trichophyton and achorion and their varieties may exist in nature as saphrophytes, although the evidence, as it is, is not against the supposition of a pre-saphrophytic existence.

In respect of the Aspergilli we are able to affirm that they have a natural saprophytic existence, but we notice here—and the observation may guide us in considering allied groups—that the more highly pathogenic members of the aspergillus genus are seldom met with, in temperate climates, in a saphrophytic mode of existence. Thus aspergillus fumigatus the most pathogenic of the aspergilli (Lichtheim) is only occasionally found in hot weather, springing up spontaneously out-of-doors, while the least pathogenic, such as Eurotium Aspergillus glaucus, or Eurotium repens (De Bary) are widely distributed as saphrophytes, and invade the larders of every housewife. But in drawing conclusions respecting the range of their affinities, we must never lose sight of the possibility that a variety, or a species, of fungus, which in temperate climates is met with only as a parasite, or only as a saphrophyte, may flourish by the other mode of existence in a tropical or sub-tropical country. And since the parasitic moulds have not been sufficiently studied in tropical regions, this for some time to come must continue to be one of the weak points in our conclusions in respect of the range of their soil-affinities.

As relating to the Saccharomycetes, of which one member the S. albicans belongs to the group under consideration in this Thesis, we know that they have a very wide range of activity as saphrophytes and that they are capable of exciting alcholic fermentation in saccharine solutions. According to De Bary the Saccharomycetes must be considered as belonging to the Exoascus-Group, and the homologies and analogies in respect of development, are, according to this Author, certainly very numerous. The bearing of this on the queston of soil-affinities is pertinent, for the Exoasci are parasitic on living plants and we are naturally desirous of knowing whether the ancestors, or even living progenitors of the present type of our parasitic moulds were, or are, parasitic on living plants. In respect of the range of hosts in which the human parasitic fungi can vegitate, we know, at this present, of no animal outside the circle of domesticated animals which is attacked by them. We may conceive it possible that domestication produces a certain uniformity in the tissues, probably of some subtile chemical nature, so that the individuals of species within this circle are liable to the same diseases. This chemical uniformity may depend on the similarity of the food with which domesticated animals are fed, and the general sameness of the conditions under which they are domesticated. It does not seem incredible that at some future time a connection may be established between the fungi of edible plants and the moulds parasitic on the animals which feed on those plants. But these obscure and subtile points in the nutritive adaptations of these fungi must be left to the future to decide. The solution of these problems, however, must have, a pertinent bearing on the origin of races and species, and, until then, it is difficult to see how this chapter in their life-history can be written.

The nourishing ingredients in all soils whether they be living or dead, or infusions of organic matter, consist of the alimentary elements which commonly nourish plant life in general. Besides these there are other ingredients such as acids or alkalies which produce definite effects on the germination or vegitation of the fungi. The external conditions of the soil determine whether growth shall proceed or not; all other conditions may be favourable, yet growth, or even germination, cannot proceed till the circle of affinities is closed by the conjunction of these external conditions.

The external conditions of germination and vegitation, are namely, rest, moisture, oxygen, temperature, they may be included under the head of Climactic conditions. Perfection of climatic conditions for any individual fungus depends on the total balance of these four factors, but the adjustment differs for each species, if not for each variety; even for the same individual fungus readjustment of the climatic factors is required when it is transferred from one soil to another. Thus achorion growing on bread requires a moister atmosphere than when growing on soil solidified by agar-agar (Unna).

Rest is essential to growth; constant mobility of the medium, and also intermittent mobility is a great hindrance even to germination.

Oxygen is an essential factor, for the parasitic moulds are aerobic. All cultivators of the trichophytic fungi know that a more plentiful crop is obtained in a freely oxygenated atmosphere than in a confined one. I have attempted to rear trichophyton vulgaris in a liquid nutritive soil from which all air had been expelled by boiling, and which was confined in a flask containing only one or two bubbles of air, and hermetically sealed with sealing-wax, and after fifteen months there was merely a crop of feeble filamentous growths, the majority of which did not exceed a pin's head in size. A diminution in the quantity of oxygen will hasten the development of certain formations, e.g., the gemmæ of mucor forms, or the sporiferous cells of trichophyton.

Haffkine* has shown, in respect of some of the lower fungi, namely, the cholera bacilli, that an abundant supply of oxygenated air, together with a rise of the culture-temperature has a remarkable effect in reducing the virulence of the organism. It is quite conceivable that the long cultivation of the parasitic mould-fungi in freely oxygenated air would have a certain effect on their pathogenic virulence, but this, so far as I know, has never been tested.

Temperature is a very important climatic factor. I shall specially consider its influence in the chapter on Variation.

Nourishing Ingredients of Soils.

There is no circulation of nutrient particles in these fungi, but each cell is independent, though mechanically attached to independent cells like itself, for certain common mechanical ends. Nutrition is

* Brit. Med. Jour., Feb. 11th, 1893.

therefore carried on by diffusion of fresh food-particles directly through the limiting membrane into the protoplasmic substance of the cell, and the waste products resulting from their metabolism is conveyed out again through the walls. The protoplasm being destitute of chlorophyll granules, or chlorophyll pigment, is unable to effect a combination of carbon with the elements of water, to form the carbohydrate compounds necessary for the formation of protoplasm. But if the soil contain ready-made Carbohydrates, with certain mineral salts, a very minute quantity of nitrogen is required to form the proteid of the cell-substance. Indeed the presence of more than a small quantity of nitrogenous matter is, generally a distinct hindrance to the development of trichophyton vulgaris, and its varieties. Thus it avoids concentrated organic solutions and raw organic substances such as meat or bread, although I have been able to rear a feeble specimen on coagulated white-of-egg. The exact worth of each of the nutrient ingredients of the soil, in respect of its bearing on the development of the fungi, has yet to be determined. But I shall attempt to give some account of their respective value, although this must necessarily, from the present defective state of our knowledge, be very imperfect.

1. *Nitrogen compounds.*

The study of the mould-plants under cultivation has shown us that it is not necessary for the soil to contain *organic* nitrogen compounds provided some organic carbon compound, such as sugar, be present. The nitrogen may be taken up from inorganic nitrogen compounds such as ammonia, or ammonia-compounds, or nitrates. Thus the fungi belonging to the the trichophyton and achorion species may be reared excellently on soils absolutely free from proteid matter, such as mineral water with two per cent. of sugar (glucose), or on common soils such as beef tea prepared in the ordinary way. According to Pasteur, Fritz and Raulin, Mucor racemosus and aspergillus niger take up nitrogen from ammonia-compounds as well as from nitrates* Whether this or that particular nitrogen compound will afford its nitrogen for the benefit of

* Quoted by De Bary, l.c. p. 354.

any particular variety of fungus cannot be decided, a priori, but must be ascertained by direct experiment.

According to Nägeli a large number of compounds may serve as sources of nitrogen, if they are in a soluble state or if they can be made soluble by the fungus. Free nitrogen and cyanogen cannot by themselves supply nitrogen; some compounds containing nitrogen may serve at the same time as sources of nitrogen and of carbon, while others such as oxamide and urea, can serve only as sources of nitrogen.* Verujski† has estimated the amount of the nitrogenous substance which disappeares from 10 cb. cm. of different nutritive liquids during the growth of achorion. His results are given in the following table:—

Fungus.	Soil.	Duration of Growth.	Loss of Nitrogenous Substance.	Weight of Fungus.	Relation between weight lost and weight of crop.
Achorion.	Barley water, neutralised.	70 days.	0.110 gram.	0.055 gram.	2
do.	Barley water, acidulated.	70 ,,	0.150 ,,	0.076 ,,	2
do.	Barley water with 2.5 °/₀ glycerine.	72 ,,	0.240 ,,	0.118 ,,	2
do.	Barley water with 5 °/₀ glycerine.	72 ,,	0.170 ,,	0.085 ,,	2

This table shows that when an increase in the weight of the crop is obtained it is accompanied by a *corresponding* loss of nitrogenous substace, for the ratio between the gain of the one and the loss of the other, in these, and more extended experiments, is, approximatly, a constant one. Trichophyton, as already mentioned, has a feebler capacity for assimilating nitrogenous substances than achorion, and both these species are inferior to the other moulds which display higher vegetative powers, such as the aspergilli, or the mucorini, just as these are much inferior to the lower fungi in respect of this property, Cultivators who have attempted to rear trichophyton and achorion side by side in urine will have found another illustration of this relativity, for the achorion-crop gets in advance of the trichophyton-growth, though both are of a feeble sort. Excellent crops of these fungi can be raised on soils free from all traces of albumins and globulins, provided that certain carbo-

* Nägeli, Ernährung d. niederen Pilze (Untersuch) etc., 1879, I: and Sitzber. d. Munchener Acad. juli 1879. I have quoted these facts very nearly in the words of De Bary's summary, l.c. p. 354.

† Annales de L'Institut Pasteur vol. I. No. 8 p. 369.

hydrates and mineral salts be present in suitable quantities. But the fact must not be lost sight of that the principal epidermal and trichopytic fungi, in their natural habitat feed on an albuminoid, namely Keratin, and when we compare this fact, with the higher affinity of, at least, the trichophytic moulds for carbohydrates under cultivation, we may, perhaps, be justified in regarding this as another link in the evidence which encourages us to believe that the present parasitic moulds of man are derived from ancestors which were parasitic on plants.

2. *Carbon and Carbohydrates.*

Although, from the want of chlorophyll, carbohydrates cannot be formed out of simple carbon, hydrogen and oxygen through the reduction of simple, widely distributed compounds of these, yet the assimilation of the carbohydrate probably underlies to some extent the formation of the nitrogenous ingredients of the protoplasm. In this the fungi show their affinity with plants, of which the same principle is true (Goodale). The carbohydrates are converted into cell-substance, and probably cell-membrane. According to Nägeli almost all compounds of carbon afford nourishment, with the assistance of oxygen, provided they are soluble in water and not too poisonous. Nägeli mentions urea. formic acid, oxalic acid, and oxamide as exceptions to the rule. Cyanogen and carbonic acid are likewise incapable of affording their carbon to fungi (De Bary). Verujski* had studied particularly the relation of some of the carbohydrates to achorion and trychophyton, and he found that glucose was consumed by trychophyton, but not saccharose, and that oxalic acid was one of the products. Duclaux† has observed this acid in glucose soils, in which aspergillus niger had been cultivated. The oxalic acid results, no doubt, from the breaking-up of the sugar. Possibly the action of these fungi on sugar is of the nature of a fermentation, for traces of oxalic acid is found in the alcoholic fermentation of glucose, by the action of yeast‡, My experience is in harmony

* Verujski, l.c., p, 385.

† Annal. de l' Institut Pasteur, vol. I, No. 8, p. 305.

‡ Halliburton, Physiological Chemistry, 1892, p. 72.

with Verujski's in respect of trichophyton capitis being able to assimilate glucose, but not any of the saccharoses. Thus I could obtain no apparent growth where cane sugar, or lactose, or maltose, were, respectively, the only sugars present. Even some of the glucoses are unacted on by trichophyton, e.g., levulose, although, according to Unna, levulose is a useful addition to proteid soils for the cultivation of achorion.

These and other facts show that the nutritive affinities, or hydrolytic actions, of trichophyton, are considerably more limited than those of the common saphrophytic moulds, such as penicillium glaucum, or Eurotium Aspergillus glaucus. The sensitiveness of trichophyton to the quantity of glucose is wellknown to cultivators, for the crop inveriably suffers when the percentage rises above 2 per cent. I have been able to rear some very feeble crops of the fungus in Raulin's solution, which contains about 6 per cent. of sugar-candy with a liberal supply of mineral salts. If the cane sugar is assimilated at all under these circumstances Verujski and myself will require to alter our conclusions in respect of the non-assimilativeness, of the saccharoses.

Some of the fungi parasitic on man can thrive on starch or starchy solutions. Delépine* has shown that glucose is formed when aspergillus niger is grown on rather dry starch. I have reared a French variety of trichophyton capitis in a starchy solution consisting of filtered pea-soup, from which all albumins and globulins had been separated by boiling, but on examining the solution, after the growth had developed, I was unable to detect the presence of any glucose. I removed the fungus from the soil and allowed it to remain for fifteen hours under running water, to wash away all traces of the solution; the fungus substance itself give no reaction of glucose with Fehling-solution but developed a *blue* colour in the presence of free iodine. When this coloured mycelium was examined under the miscroscope, some of the filaments were seen to be uniformly stained, but in others the colour was concentrated in round bodies occurring here and there in the cell-substance. Delépine† has shown that glucose is found in pure cellulose and gum arabic after the cultivation of aspergillus niger.

* Delépine, Trans-Path Soc. Long, vol. XLII. 1891, p. 437.

† Delépine. l.c p. 451.

3. *Mineral Ingredients.*

Exact information is wanting regarding the peculiar influence of each of the mineral salts found, usually, in the natural soils of this group of fungi. It is certain that they are essential to the well-being of the fungi, even though they do not become actual cell-substance. Distilled or absolutely pure water cannot support life. This statement appears to be contradicted by an observation, which I have made more than once, namely, the germination of spores of achorion and trichophyton in distilled water. But in both these experiments a sufficient number of spores were present to afford the proper mineral and organic ingredients necessary for the germination of the few mature spores.

It seems probable that the inorganic elements and their compounds, necessary to the life of the fungi, are the same as those found in the higher plants. We are told that trustworthy analysis of the ash of flowering plants shows that certain elements are always present, namely, potassium, calcium, magnesium, and phosphorus; that others, e.g. iron chlorine, sulphur, and sodium, are nearly always constant but often exist in proportions too minute for estimation.* According to Nägeli the moulds and Schizomycetes can attain full development with fewer elements.† There are certainly no ingredients of soil which are so potent in producting variation in moulds as the inorganic ingredients, as was pointed out long ago by Schleiden in respect of the higher plants. Raulin found that a very trifling alteration in the mineral ingredients of his standard soil led to the wasting of Aspergillus niger, and gave rise to the appearance of other fungi. In my own cultures I have observed that the trichophytic fungi are reared best in soils in which potassium is the prevailing base, and phosphoric acid (in combinations) the prevailing acid, and under these conditions, it is of secondary consequence whether carbohydrates predominate over proteids, or proteids over carbohydrates. The aspergilli, according to Siebenmann, are closely dependent for their well-being on the presence of iron in the soil; the

* Goodale, in Physiological Botany, p. 247.

† Nägeli, in Sitzungsbericht der bayer, Akademie, and quoted by Goodale, l c., p. 247.

‡ Siebenmann, l:c.

absence of this metal in otherwise good soils led to the pining away of some species of these fungi which had grown well in a ferruginous soil.

The following analysis by Sieber* of the Eurotium aspergillus glaucus, gives the relative quantity of organic and inorganic substances found in this fungus. It may be taken roughly as an example of the quantitative relativity of the mineral ingredients in the fungi of the parasitic group, although minute differences will be found to exist between one variety and another.

After drying the fungus, Sieber, estimated that one hundred parts of the dried residue contained:—

Matter soluble in Ether	11.19
,, ,, Alcohol	3.36
Ash....	28.95
Albumin	0.73
Cellulose	55.77

No doubt can be felt concerning the relativity of this analysis, for the amount of ash probably varies with the age of the fungus, just as it is found to do with the age of the higher plants. It will vary no doubt in different fungi, and perhaps more than we might anticipate. From the minute quantity present we may be sure that the inorganic ingredients of these fungi have a physiological rather than a substantive function to perform.

Non-nutritive Ingredients.

As De Bary observes, the amount of available food material in the substratum is not the only point of importance; its chemical nature, also, has to be considered. Dutrochet† discovered some time since that the development of moulds was affected by the acid, or alkaline reaction of the soils in which they grew. The discovery has been verified by every cultivator of moulds since that time. Duclaux and Verujski's‡ investigations have shown that trichophyton capitis and achorion capitis are far more sensible of the presence of small quantities

* Sieber, quoted by Siebenmann, l.c., p. 15.

† Dutrochet, in Ann de Sc. Nat. Sér. 2, I, p. 30, quoted by Dr Bary l.c. 354.

‡ Verujski l.c. p. 383.

of acids than alkalies. Thus the spores or these fungi remain inert for many months in the presence of 1.33 gramme of tartaric acid, and 1.19 gramme of acetic acid per litre, but can develop if the acids be neutralised. Verujski found that the presence in the soil of 12 grammes per litre of tartaric acid sufficed to permanently arrest the development of the spores. The effect of the alkalinity is given in the following table:—*

Alkalinity	1.87gr.	Weight of Crops		0.080 gr.
,,	4.07 ,,	,,	,,	0.050 ,,
,,	6.27 ,,	,,	,,	0.018 ,,
,,	10.77 ,,	,,	,,	insignificant.

The acid, or acids, which result from the hydrolytic action of these fungi naturally tends to diminish the alkaline reaction of the soil and to some extent may account for their power of developing better in alkaline substrata than in acid soils. For, as the table above shows, the presence of 10.77 grammes of alkali (carbonate of soda) in a litre of soil is very much against the development of the fungus, yet this acidifying property of their growth enables them to gradually improve the soil, so that the weight of the final crop amounted to 0.063 gramme. The common moulds flourish in nutrient solution which are more or less acid, and their growth is feeble, or even ceases entirely, in soils with alkaline reaction. This is true also of the mucorini, and the aspergilli. But shades of difference in respect of their behaviour to acids and alkalies vary from species to species, and probably from one variety to another.

* Verujski, l.c., p. 384.

CHAPTER VI.

On Variation.

In considering this important part of our subject it will be well for us to remind ourselves of the distinction which was drawn by so high an authority as Darwin between the terms "species" and "varieties."

"When a young naturalist commences the study of a group of organisms quite unknown to him he is at first quite perplexed to determine what differences to consider as specific and what as varieties; for he knows nothing of the amount and kind of variation to which the group is subject; and this shows at least how very generally there is some variation. . . . . His generally tendency will be to make many species, for he will become impressed . . . . with the amount of difference in the forms which he is constantly studying, and he has little general knowledge of analogical variations in other groups and in other countries by which to correct his first impressions. As he extends the range of his observations he will meet with more cases of difficulty, for he will encounter a greater number of closely allied forms. But if his observations be widely extended, he will in the end generally be enabled to make up his mind which to call varieties and which species, but he will succeed in this at the expense of admitting much variation. I look at individual differences as highly important, as being the first step towards such slight varieties as are barely thought worth recording in works on Natural History. And I look at varieties which are in any degree more distinct and permanent as *steps* leading to more strongly marked and more permanent varieties, and at these latter as leading to species and sub-species The passage from one stage of difference to another and higher stage may be, in some cases, due merely to the long continued action of different physical conditions in two different regions, but I have not much faith in this view; and I attribute the passage of a variety from a state in which it differs very slightly from its parent to one in which it differs more, *to the action of natural selection in accumulating differences of structure* *in certain definite directions.* Hence I believe a well-marked variety may be justly called an *incipient species* . . . . From these remarks it will be seen that I look upon the term species as one arbitrarily given for the sake of convenience, to a set of individuals closely resembling each other, and that it does not essentially differ from the true variety which is given to less distinct and more fluctuating forms. The term variety, again, in comparison with mere individual difference is also applied arbitrarily and for mere convenience."*

I have quoted this passage at length because there is a tendency at present to multiply unduly the *species* of parasitic moulds.

Observers have been impressed with the amount of difference which appears in individuals of the same species, but from insufficient acquaintance with the phenomena of variation and want of knowledge of the laws which govern them, they have attached undue importance to these variations, and have raised them to the rank of species. Some of the differences which we have passed over as insignificant, may possibly, be of much importance, as leading by natural selection to the development of new races or new species.

The extensive cultivatiou of these moulds in different countries is constantly bringing new forms to light. But as our observations become more extended we shall be able to correct the impressions made on our minds by the mutability in one group of fungi by comparison with those of another, and so arrive at some fair conclusions as to the categorical worth of any particular variation.

We shall be greatly helped in this undertaking if we extend our observations to the higher plants. It is notorious that variations are extremely numerous in plants under cultivation The laws which govern their variation are the same as those which controle the variation of the fungi. If there is any difficulty in the study of the higher plants in determining what forms are specific and what only variations, the difficulty of this task is much greater for the parasitic moulds. A large proportion of the cultivated plants are known in their wild state, and as the type of structure of the cultivated plant is more advanced and precise, the

* Origin of Species, p. 50.

botanists has a definite standard wherewith to compare his variations.

The higher plants moreover are reproduced by a seminal apparatus or by bud formation and the product of these is a *new individual.* The new individual always tends to reproduce the parent-character although this may be disguised by the pecular circumstances of its growth or by the potent influence of natural selection acting through a long series of generations.

But the mould fungi parasitic on man (with the exception of the Aspergilli and some Mucor forms) have no sexual apparatus of reproduction. The reproductive faculty is never by any means solely confined to a particular part of the body of the fungus. The nearest approach of the purely parasitic moulds to the formation of a new individual is the formation of free gonidia: and this, among the trichophytic fungi, is resorted to only under the most favourable circumstances.

This indefiniteness of form, or comparative rarity of the more differentiated forms, in the parasitic moulds deprives us of a standard type with which to accurately compare the several deviations. This at the outset is a difficulty. To apply this to concrete examples, how, we may ask, are we to fix the standard type for the varieties of Trichoptyton or for the varieties of Achorion? If our knowledge of the life-history of the fungi were complete the type should be taken from the character displayed by the fungus in its natural state, where these are only imperfectly known, as is the case with all the human parasitic fungi, we must chose a provisional standard type. It is usual for bacteriologist to make a cultivation of the fungus on some soil such as beef broth before deciding what are the typical characters of the fungus. The great advantage of this method is the strong tendency towards perfection in development which the fungus displays under cultivation. The cultivated fungus exceeds in its growth-proportions many hundreds of times the growth-proportions of the parasitic prototype, There is even an advance in organization, so that there is some difficulty at first in recognizing the cultivated mould as the direct decendant of the parasitic fungus. For the lower pathogenic fungi, this is the only method possible, because very little is known of their *natural* life-history beyond what can be inferred from clinical observation. Our knowledge of the natural life-history of the parasitic moulds themselves is fragmentary and incomplete: and it is only now that we are beginning to trace the course of the parasites through their several stages of evolution.

But in choosing this artificial standard we must inevitably fall into error of judgment if we do not recognize the fundamental fact that *individual spores of the same species of fungus differ in their affinities for the same soil,* just as certain pathogenic fungi are pathogenic to some individuals of a species and not to others of the same identical species.

This difference in affinity—for at bottom suitability of a particular fungus to a particular soil is always a question of chemical affinity—manifests itself in differences of form, growth-energy, pigment-formation, duration of the dynamic period, gregarious or agregarious tendencies of the several plant-colonies, etc. Nothing is more natural than that these difference should occur in the different individuals of the same species. If it were not so then would these fungi have been excluded from the laws which govern the rest of the plant-world.

Darwin in his "Plants and Animals under Domestication" supplies us with a large number of instances of varieties quite analogous to those which occur in the different individuals of parasitic mould fungi obtained from different sources.*

But, strange as it will appear to our more enlightened descendants, the recognition of these variations has come upon some observers with the ardour of an original discovery. Every new form is put down categorically as a new species. The observer however should guard himself against errors of this sort by a comprehensive study of the laws of variation as laid down by Darwin.

It may be convenient to give first a summary of the kinds of varations which we observe in the different individuals of the same species when cultivated under the same conditions.

* As an example of one of these analogical instances I may be allowed to quote the following from "Animals and Plants," vol, I. p. 331. "Dr. Anderson procured seed from an Irish purple potato, which grew far from any other kind. so that it could not, at least in this generation, have been crossed, yet the many seedlings varied in almost every possible respect so that scarcely two plants were exactly like.'

We meet with variations of:—

1. Form of the primary mycelial cell.
2. Form of fruit and fruit-bearing branches.
3. Pigment formation, or chemical alteration in the protoplasmic contents of the cell.
4. Length of the dynamic period, or period of active growth.
5. Gregarious or agregarious tendencies of the plant-colonies.
6. Multiplication-powers.
7. The static or resting period.
8. Pathogenic virulence.

The different forms of the primary mycelial cell have already been given in the chapter on "Comparative Morphology." In the typical trichophytic fungi, reared under what we may call the common conditions of cultivation, the primary mycelial cell is very much longer than its own breadth (about 50—60 times when nourishment is abundant), and confined by straight parallel walls. But there are individuals in which the cells are much longer than this, and consequently the septa much farther apart, and others in which the septa are much more evident and more closely situated to each other. There are some individuals in which the tendency of gonidia or bud formation is greater than in others. I have watched certain individual specimens through many generations without observing this mode of reproduction, while in a French specimen the most beautiful gonidia or gonidiophores were observed by me, even on the third day after the sowing. The length of the dynamic period also varies: in some it has ceased by the time the colony has reached the size of a pea, while in others it allows the single colony to assume bulky proportions, and to fill the whole of the soil-place. Again some individuals grow from beginning to end as a single colony, while others multiply and produce numerous secondary colonies.

In some individuals these secondary colonies have a strong gregarious tendency and unite at their margins to form a compound colony, while others are agregarious and remain throughout their whole life as isolated plants.

The composition of the cell walls differs probably in different individuals. In some the aerial filaments are strong enough to remain erect and so project freely into the air, while in others the cell walls are weak and the filaments consequently creep along the surface of the soil.

Notable differences are observed among the several individuals of the same species with regard to their pathogenic virulence. I have observed some recent specimens of hair-destroying fungi, which after artificial cultivation through four generations had no parasitic effect upon guinea-pigs, while another individual of the same species attacked the skin and hair of a guinea pig after lying dormant for over one year.

Notorious differences are observed between individuals in different countries. Thus the hair-destroying fungus of the scalp (trichophyton capitis vulgaris) can seldom be induced to attack the hair of children in Vienna, whilst it flourishes abundantly as an epidermophytic fungus of the body generally. This is not due to the absence of the trichophyton vulgaris in that region. I myself have observed it in the hair of three children in that city; and of all the varieties I have met with none can equal it in the vigour of its growth-energy. In Paris as in London and Liverpool, and other large towns, individual specimens are met with, which vary much in their pathogenic virulence, and in the amount of damage they inflict upon the hair. In Liverpool I have observed specimens which would spread in a scattered agregarious fashion over the whole scalp, while others were gregarious and localized in the damage they occasioned.

Even in the same family notable differences are observed. Thus is a family of five children, some of whom I attended professionally for ringworm, scarcely two of them were alike, yet in all the disease had sprang, probably, from the same original source, namely, a cat affected with ringworm. Thus from direct observation as well as from the study of analogical variations in other groups among the higher plants, we are led to believe in the existence of distinct races of fungi belonging to the same species.

Unna, Quincke, Neebe and others have described variations in individuals specimens of Achorion obtained from different sources, Unna and Neebe describe nine "species."*†

Unna and Neebe have arranged all the forms they have met with in to two main classes. (1) Those which develop upon the upper surface a rich aerial mycelium (the "aerophilic species.") (2) Those which produce only a scanty aerial mycelium (the "aerophobic species.") They describe three aerophilic "species." (1) Growths with a diffuse downy white surface; formation rapid and uniform, with rich, aerial, mycelium (*Achorion euthythrix*, *Favus griseus*). (2) Fungi with diffuse white downy surface, growing quickly, aerial filaments rich, membrane dimishing towards the periphery (*Achorion atacton*, *Favus sulfureus celerior*). (3) Fungi forming flat while isolated growths, aerial mycelium radiating out forming zones. (*Achorion radians*, *Favus Sardiniensis*.) These observers include six "species" in the *Aerophobic* division which they classify according to the character of the ampulliform swellings of the mycelium. (1) Fungi in which these swellings are "acromegalic" and "rosary-like" (*Rosenkränze*) but which form no terminal swellings and no yellow masses. *Achorion dikroon*, *Favus sulfureus tardus*. (2) "Acromegalic" growths with few terminal swellings and yellow masses but no "rosary-like" arrangements. (*Achorion akromegalicum*, *Favus Scoticus*). (3) "Acromegalic" growths with many swellings and yellow masses forming heaped-up hollow white masses with a zone of radial mycelium. (Achorion demergens, Favus Batavus). (4) Fungi forming "immense masses of swellings and yellow masses," also "acromegalic" swellings (*Achorion cysticum*, *Favus Hamburgensis*). (5) "Acromegalic" growths, with terminal swellings and yellow masses and numerous rosary-like" arrangements of swellings, growth masses heaped up in convex masses, or flat with "moss-like" radiating filaments (*Achorion Moniliform* *Favus Bohemicus*). (6) Forms similar to No. 5 but

* It is to be regretted that that the word "*Art*" is employed by Unna and other German writers in such combinations as "*Favusarten*," as it leaves the English reader undecided as to whether the author intends to signify "Species of Favus," or "Varieties of Favus." I am inclined to think Unna regards his different forms as species.

† Monatshefte für prak. Dermatologie Bd. XVI, Nrs. 1, 2, pp. 17, 57.

with fewer and less regular "rosary-like" arrangements. (*Achorion tarsiferon, Favus Polonicus.)*

Although I am unable to agree with Unna and Neebe in their mode of classifying these different varieties, yet their researches are useful because they show that the Fungus Achorion varies a good deal according to its origin. We might have presumed this from analogy with the variations of the higher plants, but they have demonstrated these variations by collecting specimens from different sources and cultivating them on a common soil. In the chapter on Classification, I shall give my reasons for objecting to the inferences they draw from these variations.

Laws of Variation.

Besides these *natural* variations, there are variations which we are able to originate and control. The study of these throws much light on the natural variations, and they must be carefully considered before we attempt to classify the fungi.

1. *The soil modifies the plant-fungus according to the quantity of nourishment which it contains.*

Andrew Knight* long ago regarded excess of food, whether or not changed in nature, as probably the most potent cause of variability of plants. Schleiden† afterwards held the same view, but he considered the inorganic ingredients of the soil to be more powerful in inducing variation than the organic compounds. This principle, in the main, holds true for both the lower and higher fungi. When the spores of the common hair-destroying fungi (trichophyton vulg. or achorion vulg.,) are allowed to germinate in spring water, at 25°—30°c. temp., the *form* of mycelial cell departs from the type given by standard cultures on common soils such as beef broth. They become as short almost as they are broad, measuring from 2—3μ in diameter and 3—4μ in length. The walls between the septa at first are straight and parallel. Thus the form of the cell approaches very near to the form of the cell in its

*A treatise on the culture of the Apple. p. 3; quoted by Darwin, "Animals and plants under domestication, vol. II., p. 256.

†"The Plant," by Schleiden, translated by Henfrey, 1848, p 169; quoted by Darwin, l.c.. p. 257.

natural parasitic habitat, the epidermis and hair, because the condition of the artificial and natural soil closely resembles each other.

I have observed that when a new generation of a trichophytic mould (trich. vulg.) cultivated under ordinary standard conditions, is passed through pure white-of-egg (coagulated) the mycelial filaments become extremely attenuated, and there develops a very beautiful and umbelliferous arrangement of gonidia, (see plate VI. fig. 3.) When the soil contains the necessary ingredients for the life of the fungi, the quality of the organic compounds (saccharine or proteid) has very little influence on the form of the mycelial cell. Much more potent in this respect are the inorganic ingredients as already mentioned in the chapter on "Soils, or nutritive adaptations." Raulin found in the cultivation of Asp. niger that a very trifling alteration in the mineral constitution of his typical soil led to the *wasting* of the fungus. The absence of *iron* in soils, as Siebenmann showed, stunted or even prevented the development of certain species of aspergillus.

? umbellate

But, although, various kinds of food-stuffs, and the various conditions of chemical reaction, neutral, acid and alkaline, have each their own effect in hastening or retarding the development of the thallus, and the formation of fruit, yet it is doubtful whether they have any special tendency to induce variations, which might be considered as *racial* distinctions.

In support of the principle that soil is capable of inducing variations I may mention some instances among the lower Fungi where the addition of an organic ingredient, or even an organic compound is capable of inducing notable variations capable of persisting for a longer or shorter time. In an admirable paper, Adami has collected numerous instances of this sort. He cites Vincent's* experiments on the effect of carbolic acid on the growth-forms of the Eberth-Gaffky bacillus Under the influence of the carbolic acid the long motile bacilli become short and non-motile. Charrin† has shown some striking alterations in

* Vincent Comptes Rendus de la Soc. de Biol.. 1890, No. 5, quoted by Adami Med. Chronicle, 1892, p. 370,

† Quoted by Adami, ibid.

the form of the small bacillus of blue pus when this fungus is grown under the influence of β-Naphthol. The short bacillus becomes a long straight bacillus. A 4% solution of alcohol has a still more striking effect in increasing the length of the organism.

But there are other conditions of soil besides quantity of nourishment which must be considered as causes of variation. One of these is the presence of free acid, and free alkalies. The effect of these upon the variation of the lower fungi has been more precisely demonstrated, than their influence upon the higher fungi parasitic on man. While every cultivator of these fungi knows that the weight of his crops depends on the percentage of free acid or free alkali, I am unacquainted with any researches which show how cell-forms and pigment formation is influenced by these agents, or what share they may have in producing racial differences.

It has been shown among the lower fungi that the presence of free acid or free alkali strongly affects the pigment formation.* I may cite, for the purpose of analogy, Gessard's experiments on the culture of the bacillus pyocyaneus. This organism developed a green pigment on solid white-of-egg (alkaline) and a blue colour on glycerine-pepton-agar-agar (acid). There can be no doubt that this peculiar pigment-secreting property of the moulds and lower fungi is closely linked to the constitution of the soil which nourishes the fungus, This has been worked out more precisely in some of the lower fungi, than in the parasitic mould, we cannot therefore lay down categorically the lines of physical and chemical conditions under which this or that pigment is developed in the trichophytic fungi. Nor am I aware of any systematic researches which have been attempted to throw light on the subject. I have myself made only a few casual experiments on this point, and in the frontispiece, figure 2, may be seen the appearance of an English trichophytic fungus reared in a common soil to which some acid

* Schottelius, Kölliker's, Zeitschrift. Leipzig, 1887, quoted by Adami, l. c.
Laurent, Annales de l' Inst. Pasteur, IV, 1890, p. 465.
Gessard, ibid. IV, 1891, p. 737.
" ibid. V, 1891, p. 65.
Wesserzug, ibid. II, 1888, p. 75.

tartrate of potash had been added. It was derived from colourless ancestors and the modification consisted in the secretion of a brown pigment. Close as this pigment formation is linked to the constitution of the soil, it is, generally, an *inherited* property. These are naturally coloured races of moulds, and herein lies the whole importance of this subject of pigment-formation. If the colour-variation has been stamped on a race by natural selection, what, we may ask, are the race-advantages which it confers? There are many facts, to which Darwin himself has directed our attention which would lead us to believe in a certain correlation between colour and immunity or (in the language of chemistry) between the formation of pigment and the display, or the want of display, of chemical affinities.

Thus, for example, to cite one of these facts quoted by Darwin :* "fifteen cart-horses were turned into a field of tares which is part swarmed with black aphides, and which no doubt were honey-dewed and probably mildewed; the horses, with two exceptions, were chesnuts and bays, with white marks on their faces and pasterns; and the white parts alone swelled and became angry scabs. The two bay horses with no white marks entirely escaped all injury." Here, there was a certain correlation between the fungus and the absence of colour, and the analogy suggests that the presence of pigment, or colouring matter, in fungi may serve certain ends useful to the race. I have observed that the presence of black pigment is sometimes associated with a great increase in growth-energy, in the reproductive power, and, probably, even in parasitic activity. This was the case with the black Austrian trichophytic mould, and with an English white mould, which by a change of treatment (described in another place) was converted into a coloured variety. Possibly the colouring matter of the tissues, or soils, which nourish the fungus is complimental to the colour of the fungus, itself, and in some way is associated in modifying the virulence of the parasite. I observed out of 48 inoculations of four successive generations of a white trichophytic mould, on a series of guinea pigs, that when the parasitic affinities of the fungus were weak, the white-haired

* Animals and Plants under Domestication, Vol. 2, p. 337.

guinea pigs "took" more decidedly than the coloured pigs, and when the latter were attacked, the white animals were attacked more vigorously. On the other hand when a white parasitic mould had passed through the short black and curly hair of a negro-child, I noticed that the dried spores of the fungus retained their parasitic affinities, after a full year of dormancy. May we not account for this by the principle that the virulence of an organism increases in proportion to the resistance offered to its passage through its host, in the same way as the virulence of the anthrax bacillus is raised by passing through a series of hosts, whose resistance is in the ascendency? We are however far from understanding the exact relationship of colour to the parasitic affinities of the fungi, and these facts shew that here is a new avenue of research which may lead to the discovery of some useful laws.

2. *Temperature is a factor of prime importance in the causation of Variation.*

I observed when an English white trichophytic mould, cultivated at 25°—30°c., was reared in the same soil at the temperature of the body, 37°—40°c., that notable changes occurred, and that they appeared even in the first generation.

The first cultivated generation (25°—30°c.) which sprang directly from the parasitic spores developed into a fine specimen of a white mould. There was a single plant-colony, which enlarged at the margin; its upper surface was covered with strong, erectile filaments of a snow white appearance; its under surface displayed a yellow brown colour. Microscopically the mycelium had the typical-characters of the species. The *second generation* was reared at 37°—40°c in another portion of the same, sterilised, soil; but the crop which resulted from the culture was so strikingly different from its predecessor that no one, primâ facie, would have included it in the same species. Instead of a single, or compound, plant-colony, there sprang up 200 to 300 minute separate colonies most of these no larger than a pin's head and having no gregarious tendency. The centre of each plant was coloured dark brown or almost black. The combined effect of a multitude of these black centred moulds was very striking. The brilliantly clear liquid appeared crowded with minute black fungi each fringed with rays of grayish white filaments. When one of these minute plant-colonies was magnified I was able to observe the following notable changes of structure. (see fig. 2, pl. VII.

1. Shortening of the mycelial cell, septa 40—50μ apart.
2. Extreme tortuosity of the cell walls.
3. Irregular, pathological, swellings of mycelium.
4. Modification of branch-formation.
5. Secretion of a black granular pigment, both in the cell and around it.

The shortening of the cell was not due to secondary partitioning-off, such as occurs in all old specimens; for it was observed in the extreme marginal young mycelium. The tortuosity was a striking feature, and also the irregular, pathological, (?) swellings. One of the distinguishing features was the black granular pigment which was identical in appearance with that of the black Austrian mould. It lay in abundance all around the filaments, it was not affected by caustic potash, but was partially dissolved by cuprammonia.

The *third generation* was reared on a fresh portion of the same soil but at a lower temperature (25°—30°c.), and the progeny was found to revert, to some extent, to its original characters, (see fig. 3, pl. VII.) Instead of a multitude of seperate single colonies, there sprang up a single colony which enlarged at its margin and was white in appearance. But, when magnified, I observed that the reversion was only partial, the formation of pigment granules had ceased almost entirely, there were fewer irregular swellings, although the mycelial cells were still short. But the most distinguishing feature of all was the curious formation of spiral branches. I am not sure that these "branches," whose very limited growth alone distinguished them from all other branches, were not of the nature of a reproductive apparatus. This is suggested by their manner of dividing by division into short globoid bodies.

In the *fourth generation* the reversion to the original type was interrupted in one feature, namely, in the multiplication of plant-colonies;* but in other points the reversion was continued, there was no pigment secretion, the walls of the filaments were straight and parallel, or slightly curved; the mycelial cell was much lengthened, and approached the typical size; the spiral branches were still present but less numerous. In this culture I observed the curious appearance represented in fig. 5, pl. VII. A branch from a filament was seen to twine round a neighbouring filament, both filaments giving off a bud a very little distance above the line of contact. Whether this was a sexual apparatus I cannot say, as no coalition of the two buds, or any subsequent formation was observed.

These culture experiments have seemed to me worthy of detailed description, on account of the principle of variation which underlies them. The features of the black variation were characteristic enough to confer on the fungus the position of a distinct variety, if not of a species, and yet these changes had been induced simply by an alteration in the temperature under which the two varieties had been reared.

The culture of the black Austrian mould is another example of this modifying influence of temperature. The first generations were cultivated by me in Vienna, and they did not present that striking appearance which the later generations presented. A living specimen of this fungus was carried in a sterilized and protected flask from Vienna to England. The flask was placed in my breast-pocket, the temperature of which was 37.7°c. By the time of my arrival in England (a week later) the fungus presented a coal-black appearance. Further cultivations of it, even at the ordinary temperature 25°—30°c., produced progenies of the same black variety, and resembling my English black variety in its formation of numerous colonies. In this instance, the increased temperature seems to have intensified the black colouring matter, in as much, as the original fungus, even in its natural habitat, the skin, displayed a brown colour.

Before any general statement can be made regarding the correlation of colour and temperature in the *mould* fungi, we would require to test a

* This may have been due to the accidental, temporary, elevation of the incubator temperature to 35°c.

large number of coloured and colourless moulds by rearing them at different temperatures. The typical instance which I have illustrated can be taken as a fact *per se*; and it acquires special interest by contrast with the effect of temperature on the *lower* fungi.

Thus Adami* has caused the colour of Bacillus ruber (Plymouth), Bacillus ruber (Kiel) Microbacillus prodigiosus, Bacillus indicus, and Sarcina erythromyxa, to disappear by rearing them at temperatures above those at which they commonly grow. For example, the Bacillus ruber of Kiel developes a crimson colour when cultivated upon sterilised potato at 15°—25° c.; but if the culture be kept for two days at a temperature of 37° c. its progeny or succeeding generation, is *colourless.* If the first colourless generation be reared at the higher temperature (37° c.) a colourless race of these organisms may be started. Adami found that a similar result could be obtained by heating a coloured specimen for a few minutes to a point approaching that at which life is impossible (55°—57°c.). When these culture-experiments are compared with mine, the relation of temperature to colour must be admitted to be widely different for some of the higher and lower fungi. The contrast is made still more striking by an observation I made on the black Austrian mould, for, after heating a specimen of this fungus, in its liquid soil, to the temperature of ebullition, for a few seconds, I was able to obtain a scanty and feeble regrowth in fresh soil, but the new growth was as deeply pigmented as the preceding generations.

Temperature may induce changes in the parasitic virulence of fungi. That the virulence of some of the lower pathogenic fungi may be profoundly modified by rearing them at elevated temperatures has been proved, conclusively, by the researches of Pasteur, Chamberlain, Roux, Metchnikoff, and Haffkine. Thus M. Haffkine† has shown that the cholera bacillus loses its power of exciting gangrene in the skin of the animal into which it is injected, when it is reared through a series of generations at 39°c. temperature, and in a freely oxygenated atmosphere.

The effect of high temperatures on the pathogenity of the moulds

* "Med. Chronicle," vol. XVI., Sept., 1892, p. 374, et. seq.

† Haffkine, Brit. Med. Jour., Feb. 11th, 1893.

has yet to be worked out. The well-known experiments of Gravitz,* by which he attempted to show that the common non-pathogenic aspergillus glaucus, when reared at the temperature of the body 37°c—40°c., acquired pathogenic properties have been proved incorrect by Koch,† Gaffky‡, Loeffler, Lichtheim,§ and Leber.‖ We have no instance, at present, of a saphrophyte, in itself harmless, becoming malignant simply as a result of artificial treatment. All the pathogenic moulds, without known exception, are pathogenic *in themselves.* This does not prevent our believing that they may commonly exercise an innocent saphrophtic life before becoming attached to an animal or vetegal host. We know that this is the case with the aspergilli. Thus the aspergillus fumigatus, the most pathogenic of all the aspergilli, springs up spontaneously in certain nutrient soils (Lichtheim). Many of the microphyton of the human skin are, indeed, of the nature saphrophytes, being perhaps intermediate between the true saphrophytes and the true parasites. This is true of the aspergilli of the ear. It is well known by Aurists that the aspergilli never attack the healthy epidermis, but depend for their sustenance on the serous or fatty secretion of certain morbid states of the ear. (Siebenmann.) But in all these instances the mould fungus must be exposed to a change of temperature when it passes from its saphrophytic existence to its habitation on the warm human skin. What effect has this elevated temperature on the fungus? Does it merely "force" the growth without modifying its character, just as hot-house plants are made to grow faster and more luxuriantly than out-of-door specimens of the same plants? Or is it able to raise a feeble and dormant pathogenic property into virulent activity. These questions apply also to the trichophytic fungi which have, probably, a pre-parasitic life as saphrophytes.

* Grawitz, Virch. Archiv. Bd. 81, 1880.
ibid, Berl. Klin. Woch. Nos. 45, 46, 1881.
† Koch, Berl. Klin. Woch. No. 52, 1881.
‡ Gaffky, Mittheilungen aus dem Kaiserlichen Gesundheitsamte, Bd. 1. p. 80, 1881.
§ Lichtheim, Berl. Klin. Woch. No. 9, 1882.
‖ Leber, Berl. Klin. Woch. No. 11, 1882

The present state of our knowledge, however, will not permit us to answer these questions, nor, indeed, to frame any general law of the correlation of temperature and pathogenity.

Natural selection, a factor in the formation of Natural Races and Species.

We have seen that individual fungi may be induced to depart from the common features of their class by slight changes of treatment, which we have the power of controlling. We are, hence, naturally led to the question, do such changes of treatment bear any relation to those spontaneous causes which produce, in course of time, races and species? Apart from any interference on the part of man, there are many species so closely allied, that we are compelled to base the distinction on physiological rather than or anatomical grounds, and within these species there is a large number of individuals which differ in certain points from the type of their class. Indeed the differentiation is carried much farther than this. Take the spore-progeny of any one fungus, such as Achorion vulgaris, and it will be found that one spore differs from another spore in certain subtle chemical respects. For if a group of spores, or sporiferous cells, say a minute fragment of Favus scutulum, be placed in a nutritive soil such as spring water, or mineral water, so that all the spores are equally exposed to the influence of the soil, only one here and there will germinate, while the others remain dormant, and may be consumed by the germinating spores.

In passing through a soil, whether it be animal or vegetable, the fungus is modified, so that a difference, sometimes minute, sometimes very important, may originate in the individual. We can find many illustrations of this law among the lower fungi. Thus when a quantity of a pure culture of the cholera bacillus larger than is necessary to produce death, is injected into the peritoneal sac of a rabbit, the bacilli remaining in the peritoneum, after the death of the animal are found to to possess a much higher degree of virulence than those injected (Haffkine). It is a notorious fact that the virulence of the pathogenic lower fungi is increased by passing through a host (i,e. soil), which resists its development, while those hosts which are extremely susceptible to the fungus, have no exalting influence on the organism. There can be no doubt that the pathogenic mould fungi are also modified, in their passage through different hosts, although complete evidence of this is wanted.

According to M. Basquet* the achorion of the mouse (achorion arloini) deviates in form from the achorion found on the body of man. The evidence of cultivators of achorion is strongly in favour of this modification of form of the fungus by passage through different hosts. When specimens were collected by Unna from different sources and reared upon a common soil, they manifested considerable variations of form. It must, however, be borne in mind that one individual may differ from another physiologically without much, or any alteration of form. Thus—to cite once more the case of the two sisters suffering from ringworm—cultivation of the two individual fungi in a common soil, manifested no noteworthy differences, and yet the virulence of the fungus in the elder sister was considerably higher than in the younger sister; the history of the case leaves no doubt that the disease was acquired from the same source.

How are we to account for these variations? I think that the doctrine of natural selection offers the best, it not the only explanation of them. When an individual fungus passes through a certain host, the modification may be very slight, but when this variation is accumulated by natural selection through countless generations, it may end in determining a new species. In this way, I believe, achorion has become differentiated from trichophyton, both being derived from common ancestors.

* Annales de Derϕmatologie, March, 1893.

CHAPTER VII.

Classification—Conclusion.

The very large number of different forms which the naturalist is constantly meeting with in the study of fungi makes him desirous of arranging them into groups of species and genera. His tendency at first, as Darwin assures us, is to make many species, but as his observations extend the number of his species becomes smaller, while the number of his varieties increases. A mistaken notion very often lies at the bottom of a too-active classification, in respect of what should constitutes a species-making difference, and what merely a variety. It seems to me that those variations, however minute, which prove serviceable to the species in its struggle for existence, by rendering it more successful in vegetating and propogating its species, are to be selected, above all, for purposes of distinction. Thus the species which includes all the trichophytic or hair-destroying fungi is characterised by a certain peculiarity of nutrition, which enables them to dissolve the, otherwise insoluble, albuminoid keratin. This variation is of benefit to the species since it provides it with the means of gaining access to a habitat which suits it, and affords it a suitable substratum for existence. It is by a selective variation of this sort that the aspergillus fumigatus is naturally distinguished from penicillium glaucum, for the former fungus has the power of vegetating at the temperature of the human body, and even in its interior, whilst penicillium has not this power. We feel sure that here we are dealing with distinctions which nature recognises, and we can confidently place them in distinct species. Such natural variations as these are transmitted from generation, to generation; in fact they are permanent variations. Permanency, seems to me, to be one of the essential conditions of a species-making difference. But when the cultivator is attracted by some peculiarity of form or colour, and allows himself to be misled into building up his species upon them, he is sure to end in error and confusion. This is specially true of that immense collection of fungi which are entitled *fungi imperfecti,* because they are all distinguished by their want of fixity and precesion of type. The hyphomycetes, alone, include far more than four hundred species in Germany itself (Frank), and they are notorious for their pleomorphism, and fickleness of character; and to this group belong the mould-fungi parasitic on man and the higher animals. Cultivators of achorion, and trichophyton, the most prevalent and destructive of the pathogenic moulds, are in the habit of referring to the size of the "spores" or to the erectile aerial filaments, as means of distinguishing one species from another. Thus Unna and Neebe have classified the varieties of achorion into those that form erectile aerial filaments, and those that do not. And they have associated this physical feature with the affinity of the two classes of fungi for oxygen, which they designate, respectively, as "aerophilic" and "aerophobic." Now it is very misleading to designate any variety of achorion as aerophobic, because they are, one and all, peculiarly dependent upon oxygen for their development. In no other variety of the pathogenic moulds is there so great a disproportion between the internal and external thallus, for the great mass of the vegetation is on the free surface of the skin, in contact with the oxygen of the air. The erection of free filaments on the aerial surface of the fungus depends upon the composition of the cell-membrane, and those moulds which, like the aspergilli, have the property of forming resinous material, show a very beautiful display of erectile filaments. The resinous substance gives strength and elasticity to the filaments, but the formation of this resinous substance does not depend entirely on the presence of free oxygen, for the formation of a certain hydrocarbon, must, probably first take place, by the oxidation of which the resinous substance is formed. And cultivators know that this hydrocarbon is not always formed even in the same individual fungus, when slight changes of treatment are introduced. It is surprising that Unna has not thought of this, for his own cultivations showed him that the variety of plant, which he burdens with the name of achorion euthythryx, produced erectile filaments in one soil, and in another none at all, or only a very trifling number of them. And yet both soils were provided with an equal amount of free oxygen. But it was the alteration of the nourishing ingredients in the soil, or a change in its chemical reaction, which hindered the formation of the resin-forming hydrocarbon. It is, therefore, very unsatisfactory to classify fungi according to variable qualities of this sort, although all cultivators are aware that some groups display this property as a feature charasteristic of their class.

Classification based on the size of the "spore," in respect of the commoner parasitic moulds, trichophyton and archorion, and their varieties, is equally unsatisfactory. I have pointed out more than once in this Thesis that the contrast between spore and mycelium cannot be drawn, so long as the parasite occupies the tissues of its host under absolutely natural conditions. The "spores" are short-celled filaments, taking their shape from the peculiar features of their surroundings. They are cells with a vicarious function, not a permanently transmitted variation.

Unna has endeavoured to make a sub-classification of the varieties of achorion, according to their ampulliform swellings; which is very remarkable, since he regards these swellings as *pathological*. But a principle such as this is as misleading, as if we were to classify oak or willow trees by the variety of their gall nuts!

I think it possible that *colour* may become a means of distinguishing races from one another, because we generally find in nature a correlation between colour and various forms of energy. Thus the black variety of trichophyton, which might be called *trichophyton nigrum austriæ*, shows some natural differences, e.g. it very seldom attacks the hair of children in Vienna, although its power of attacking English hair is not a whit less than that of other varieties, and its vegetative energy and powers of enduring heat are far beyond any English or French variety yet observed by me.

But our observations of coloured races are too limited to base any generalisation upon them; and it would be rash, at this present, to make colour the basis of a classification. The imperfection of development which characterises all the *fungi imperfecti*, is an *insuperable* obstacle to a rigid classification. We must be content with broad distinctions. The impossibility of a minute classification is shown in the following Table, which I have modified, from Frank's classification of these fungi, in accordance with our present knowledge of the subject. A glance at this table will show that different varieties of trichophyton are not included in one group; and it is impossible to do so, because forms which are subject to extreme variations have been made the basis of classification. The fruit and fruit-bearing branches of the trichophytic group, for example, are not precise formations, and are subject to many variations, some of which I have delineated in plate I. On which of these fruit formations shall we base a classification, when we are ignorant of the conditions under which they appear, and fail to appear? We can distinguish, however, four broad groups of moulds parasitic on man, namely: the aspergilli, the muccrini, (?) the sprouting fungi, and a group which I propose to call the *Haplomycetes domestici*, in which I would include all the simple filamentous fungi, which propagate their species by vicarious methods, and which are parasitic on man and domesticated animals. The following scheme* shows the method of arrangement which I think most consistent with the present state of our knowledge.

* shown in the Second Table.

FUNGI IMPERFECTI,

(modified after Frank in Leunis's Synopsis).

- **I.** ungi which commonly form Pycnidia and permogonia.
- **II.** Fungi which commonly form Stromata, and have gonidia-bearing branches.
  GYMNOMYCETES.
- **III.** Fungi which commonly form free filamentous mycelium, and sometimes gonidia-bearing branches.
  HYPHOMYCETES.
  - **I.** Fruit-branches simple or branched, ending in an ampulla from which alone the buds, or gonida, arise.
    - Fruit branches simple, or slightly branched.
      - Aspergilli.
    - Fruit branches repeatedly branched dichotomously.
      - No known species parasitic on man.
  - **II.** Fruit-branches not ending in ampullæ; buds, or gonidia, in groups at the simple extremity of the fruit-branch, or attached to the branch by short stalks.
    - Fruit-branches unbranched.
      - Some forms of Botrytis Bassii.
    - Fruit-branches branched, the branchlets ending in a tuft of spores.
      - Botrytis Bassii.
        - Some varieties of Trichophyton.
  - **III.** Fruit concatinate, arising vicariously, not by bud-formation, but by abjunction: Fruit arranged simply, umbelliferously, or paniculately.
    - Fruit-branch grows beyond spore chains.
      - Oidium.
    - Spore-chains arising from fruit branches distinguishable from the mycelium.
      - Penicillium.
    - Fruit-branches not specially distinguished from mycelium, the spore-chains arising directly from mycelium, or by the segmentation of the mycelium itself.
      - Epidermophytic fungi.
      - Some forms of Trichophyton.
      - Some forms of Achorion.
  - **IV.** Fruit attached individually to the fruit-branch, or in very small groups; not concatinate.
    - Trichophyton cap. vulg. (under cultivation)
    - Achorion vulg. (under cultivation)

THE AUTHOR'S PROVISIONAL ARRANGEMENT

OF THE MOULD-FUNGI PARASITIC ON MAN.

Fungi parasiti hominis.

- Aspergilli.
  - A. fumigatus.
  - A. niger.
  - A. flavus.
- Mucorini ?
  - M. racemosus ?
  - M. mucedo ?
- Saccharomycetes.
  - S. albicans.
- HAPLOMYCETES DOMESTICI.
  - Achoria. (species)
    - A. muridarum. (variety parasitic on rodents).
    - A. hominis corporis. (variety).
    - A. hominis capitis vulgare. (variety).
  - Trichophyta. (species)
    - T. muridarum (Group parasitic on rodents)
    - T. album austriæ (variety).
    - T. fuscum. (Microsporon furfur). (variety).
    - T. nigrum austriæ: (variety).
    - T. hominis capitis vulgare. (variety forming type of species.)

APPENDIX.

Note A.

On the pathogenic Mucors.

The pathogenity of the mucors in respect of warm blooded animals is certainly much more limited in scope, than that of the aspergilli. I have included Mucor mucedo and M. racemosus among the human parasitic fungi, but, strictly speaking, their presence on man is always in the capacity of saphrophytes. No species of mucor has been observed to grow in the interior human tissues. Lichtheim* has studied, in cultivation, two mucor forms, which were named by Cohn of Breslau, *M. rhizopodiformis* and *M. Corymbifer*. They grow well at the temperature of the body, and are distinguished by the delicacy of their structure. When injected into the vein of a rabbit they produce lassitude, ending fatally on the third day. M. rhizopoiformis is far more pathogenic than M. Corymbifer, but there is no difference in the nature of their effects. After death, the kidneys are found much swollen and congested, and pervaded by non-septate mycelium. Dogs are refractory to these mucor forms. An exact description of their morphology is given by Cohn in Lichtheim's article.

Note B.

On the pathogenity of the Aspergilli.

The capacity of the Aspergilli to develop in the living tissues of warm-blooded animals cannot be equalled by any other species of fungus. They have observed in the lungs and respiratory passages of the Jay, Swan, Stork, Raven, Eider-duck, Parrot, Hens, and Doves, in the Snowy Owl, in the Hawk, Green-plover and Pheasant, and also in some

* Lichtheim, Zeitschrift, f. klin, Med. 1884, p. 140.

waterfowls, e.g. the Flamingo, Auk, and Cormorant. References to the origin observations may be found in an article by Virchow in his Archives, Vol. IX, p. 557. Probably all the recorded cases of human pneumomycosis are due to the Aspergilli, but the imperfection of description of most of them makes it impossible to identify the fungi. Exact descriptions have been given by Virchow (l.c.) and Conheim (l.c.); it is very doubtful if pneumomycosis can always, if, indeed, ever, be considered as a *primary* disease of the lung. Generally some other disease is present which ends fatally.

Note C.

Literature on the mould-fungi parasitic on Man and the higher animals.*

General Literature.

1. De Bary's Comparative Morphology and Biology of the Fungi, 1887. English version, by Prof. Balfour.
2. Siebenmann's Die Fadenpilze, Wiesbaden, 1889.
3. Leunis, Synopsis der drei Natturreiche zweite theil, Botanic.
4. Goodale, Physiological Botany, London, 1890.
5. Darwin, Animals and Plants under Domestication, London, 1868.
6. Trouessart, Microbes, Ferments and Moulds, London, 1889.
7. Tyndall, Essays on the floating-matter of the air.
8. Flügge, Micro-organisms, New Sydenham Society, 1890.
9. Halliburton, Chemical Physiology and Pathology, London, 1891.
10. Robin, Vegetaux Parasites, Paris, 1853.

Special Literature.

11. Grawitz, Beiträge zur systematischen Botanik der pflanglichen Parasiten, mit experimentellen Untersuchungen, etc., Virch. Arch. vol. LXX, 1877.

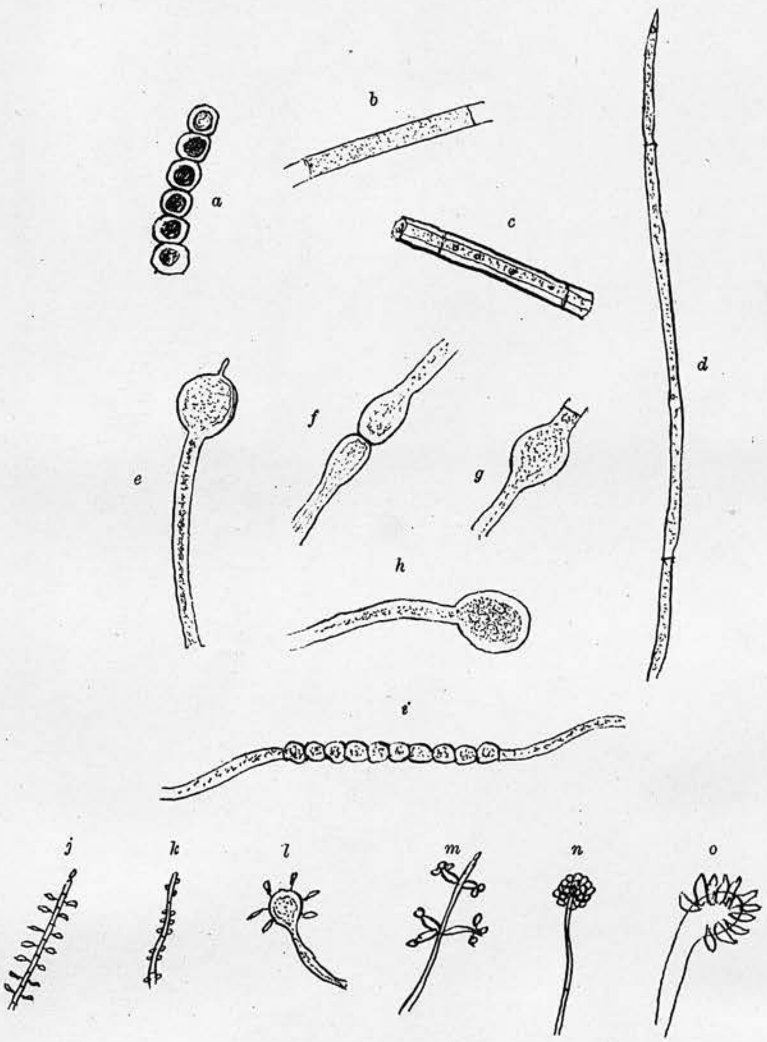
* This list is far from exhausting all the literature of the subject, but many works, which might have been useful, were beyond the resources of any English Medical Library, to which the Author had access.

12. Grawitz, über Schimmelvegetationen im theirischen organismus: Experimentelle Untersuchung, Virchow's Archiv. Bd. 81. 1890.
13. Grawitz Experimentelles zur Infectionsfraga, Berl. Klin. Woch. No. 14, 1881.
14. Grawitz, die Anpassungstheorie der Schimmelpilze, und der Kritik des Kaiserlichen Gesundheits Amtes. Berl. Klin. Woch, Nos. 45, 46, 1881.
15. R. Koch, Entgegnung auf den von Grawitz in der Berliner med. Gescellschaft gehaltenen Vortrag über die Anpassungstheorie der Schimmelpilze, Berl. klin. Woch. No. 52, 1881.
16. Lichtheim, über pathogene Schimmelpilze, Berl. klin. Woch. No. 9, 1882.
17. Lichtheim, über pathogene Mucorineen und die durch sie erzeugten Mykosen des Kaninchens, Zeitschrift für Klinische medicin, Berlin, 1884.
18. Leber, über die Wachsthumsbedinungen der Schimmelpilze in menschlichen und thierischen Körper, Berl. klin. Woch. No. 11, 1882.
19. Eberth, Zur Kenntniss der mycosen bei Thieren. Virch. Archiv. Bd. 80.
20. Schultze. Die Theorien über die Area celsi Virch. Arch. Bd. 80, p. 193,
21. Koniger, Beobachtungen zur geographischen Pathologie. Virch. Archiv. Bd. 72, 1878, p. 413.
22. Babes, oidium subtile cutis, Természettudományi, Közlöny XIV. Reported in Biologisches centralblatt, Bd. II, No. 18, p. 569.
23. Grawitz, Stellung des Soorpilzes in des Mycologie der Kahmpilze, Virch. Arch. Bd. 73, 1878, p. 147.
24. Delépine, Aspergillus niger (a case of melanomycosis of the skin) Trans. Path. Soc. London, 1891, p. 423.
25. Baumgarten, Die pathogenen Hyphomyceten, sonderabdrücke der Deutschen Med. Zeitung, Berlin, 1884.
26. Verujski; Trichophyton tonsurans et Achorion Schöneinii, etc., Annales de l'Institut Pasteur, vol. I, 1887, p. 360.

27. LESLIE ROBERTS, Artificial cultivations of the Ringworm fungus, Brit. Jour. of Dermatology, vol. I, 1888, p. 359.
28. QUINCKE, über Favus, Monatshefte f. prak. Derm., vol. VI, 1887, p. 981.
29. ibid Archiv. f. exp. Pathologie Bd. 22, 1886, p. 62.
30. DUCLAUX, Trichophyton tonsurans, Comptes Rendus de la Soc. Biol. Jan. 1886.
31. SCHWENGERS, über Einwirkung von medikamenten auf Kulturen von Favus und Trichophyton, Monatsthefte, f. prak. Derm., Vol. IX, 1890, p. 155.
32. UNNA (von Sehlen) Flora dermatologica, Monatshefte f. prak, Derm. vol. XI, 1891, p. 485.
33. UNNA, Flora dermatologica, Monat. f. p. Derm. vol. XII, 1891, p. 249.
34. NEEBE and FURTHMANN, Vier Trichophytonarten. Monat. f. p. Derm. vol. XIII, p. 477.
35. UNNA, Flora dermatologica, Monat. f. p. D. vol. XIV, p, 303.
36. SABOURAUD, Reference to incompleted work on the different species of Trichophyton; Monat. f. p. D. vol. XV, p. 619.
37. UNNA and NEEBE, Die bisher bekannten neun Favusarten, Monat. f. p. D. vol. XVI, pp. 17, 57.
38. For literature on Pneumomycosis up to 1856, see Virchow's article, Beiträge zur Lehre von den beim Menschen vorkommenden pflanzlichen Parasiten, in Virch. Arch. vol. IX, p. 559.

The following authorities, referred to by Flügge, may also be consulted:
39. NÄGELI, Die niederen Pilze, München, 1877.
40. FRIEDREICH, Virch. Arch, Bd. 2.
41. COHNHEIM, Ibid. Bd. 33.
42. FÜRBRINGER, Ibid. Bd. 76.
43. ROSENSTEIN, Berl. Klin. Woch, 1867.
44. WAGNER, Jahrb. f. Kinderheilkunde, 1868.
45. ZENKER, Jahresb. der Ges. für Natur-u-Heilk, in Dresden, 1861-62.
46. GROHE, Berl. Klin, Woch, 1871.

47. Block, über Pilzbildung in thierischen Geweben. Diss. Stettin, 1871.
48. Bezold, In den Vorträgen im ärztlicken Verein zu München, 1881.
49. Gaffky, Mitheilungen des Kais. Ges.-Amts, Bd. 1, p. 80.
50. Löffler, Ibid, p. 134.
51. Schütz, über das Eindringen von Pilzsporen in der Athnungswege, Mitt, a.d. Kais Ges.-Amts., Bd, 2.
52. For the position of the trichophyton question in 1883, see article by Malcolm Morris, and G. C. Henderson, Jour., of the Royal Microscopical Soc., April. 11, 1883.
53. For the clinical aspects of the several trichophytic and epidermomycoses, consult the writings of Kleinhans, F. von Hebra, Kaposi, H. von Hebra, Besnier, Vidal, Quinquard, Pick, Thin, Malcolm Morris, Jamieson and Brocq.



H. L. R., del.

a. Form of mycelium of trichophyton in the interior of hair.
b. Young mycelium, cultivated.
c. Older mycelium, cultivated, showing double contour, and fat-globules.
d. Form of mycelial cell when nourishment is abundant.
e. h. End-ampulla.
f. g. Intercalary ampulliform swellings.
i. Resting gemmæ or sporiferous cells.
j.—o. Different forms of fruitification.

T. Dobb & Co., Printers, L'pool.

Fig. 1.

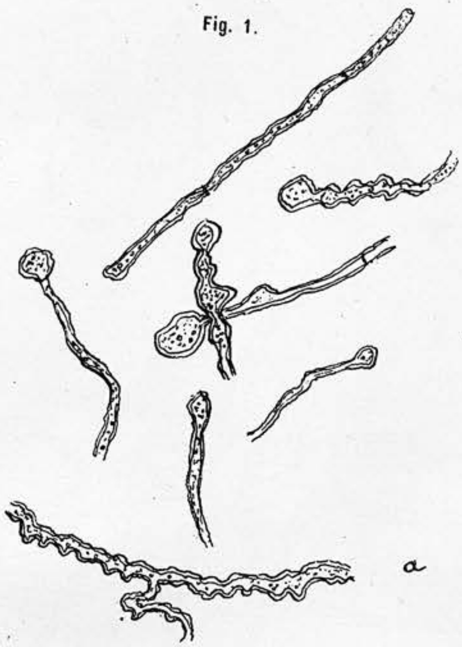
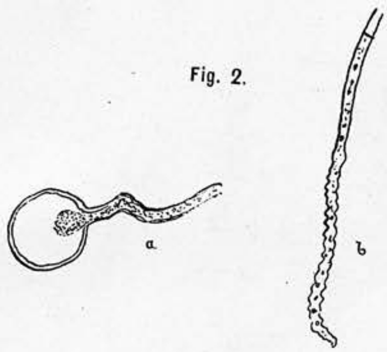


Fig. 2.



H. L. R., del.

Fig. 1.—Showing senile degeneration of mycelium of a 3-year-old specimen of Trichophyton Capitis Vulgaris.

a. Represents deeper mycelial filament, above are drawings of white aerial filaments.

Fig. 2.—*a*. End-ampulla 4 years 4 months old. The protoplasm has shrunk from the walls.

b. Showing effect of distilled water on mycelial filament.

T. Dobb & Co., Printers, L'pool.

Plate III.

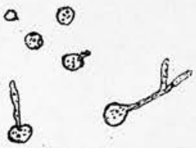


Fig. 1

Figs. 4

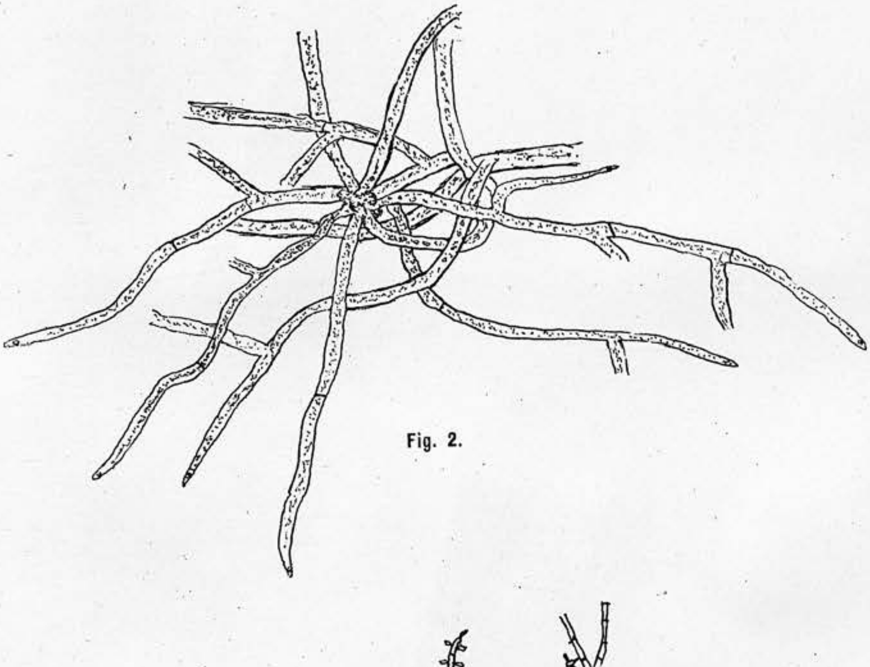


Fig. 2.

H. L. R., del.

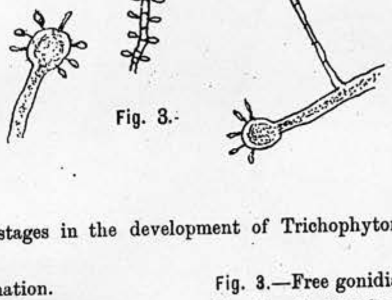


Fig. 3.

Showing the several stages in the development of Trichophyton Capitis Vulgaris.

Fig. 1.—Germination.

Fig. 2.—Thallus.

Fig. 3.—Free gonidia.

Fig. 4.—Resting Spores.

T. Dobb & Co., Printers, L'pool.

Fig. 1.

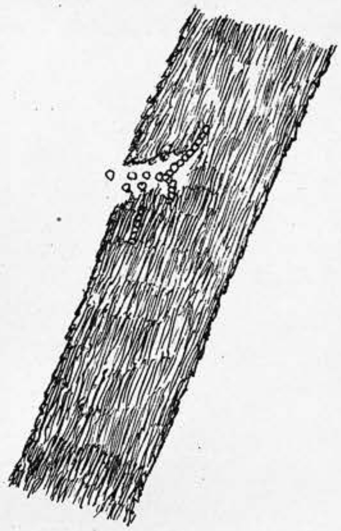
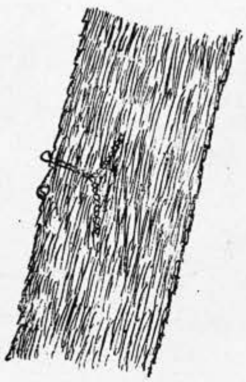


Fig. 2.



H. L. R., del.

Fig. 1.—Trichophyton Capitis Vulgaris attacking human hair.
Fig. 2.—An earlier stage.

T. Dobb & Co., Printers, L'pool.

Fig. 1.

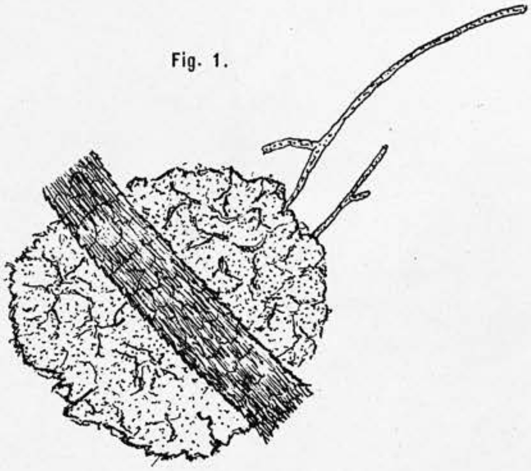
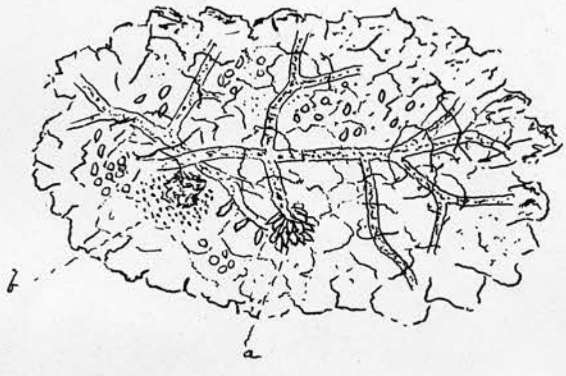


Fig. 2.



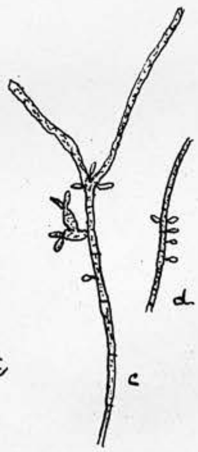


Fig. 3.

H. L. R., del.

Fig. 1.—A trichophytic fungus growing in epidermal scale from human ringworm.

Fig. 2.—Further development of same fungus.

Fig. 3.—Fruit-bearing branches of same fungus. (See Page .)

T. Dobb & Co., Printers, L'pool.

Fig. 1.

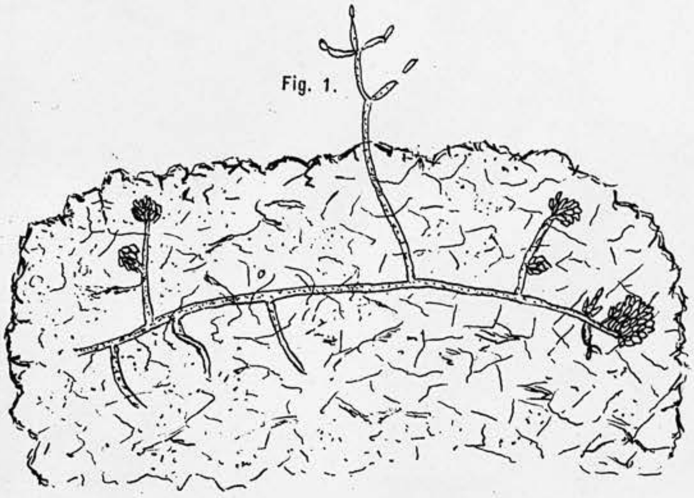
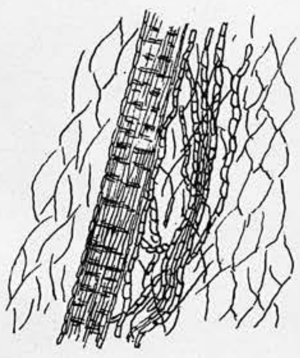


Fig. 3.

Fig. 2.



H. L. R., del.

Fig. 1.—Same epidermal scale as in Figs. 1 & 2, Pl. V., showing another portion of same fungus.

Fig. 2.—An English trichophytic fungus (parasitic on man) cultivated on white-of-egg.

Fig. 3.—Hair-Shaft of guinea-pig attacked by human Trichophyton capitis vulgaris, artificially inoculated.

T. Dobb & Co., Printers, L'pool.

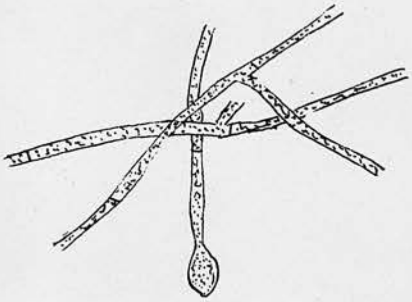


Fig. 1.

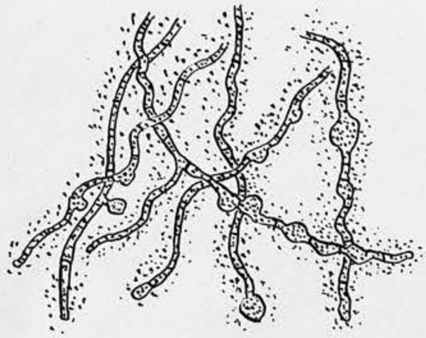


Fig. 2.

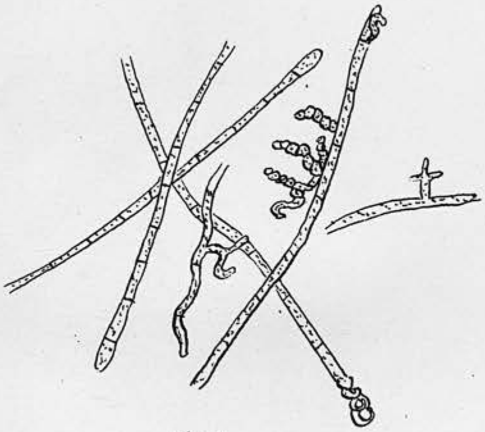


Fig. 3.

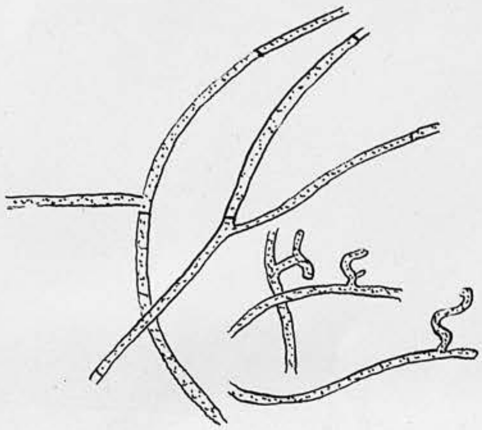


Fig. 4.

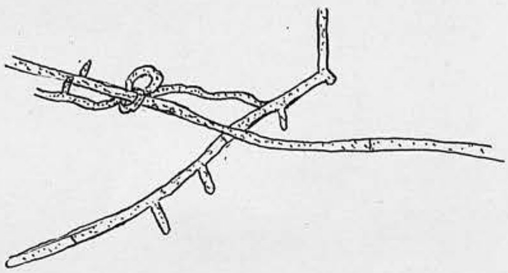


Fig. 5.

H. L. R., del.

Showing Variations induced by change of temperature.

Fig. 1.—1st generation.
Fig. 2.—2nd generation.
Fig. 3.—3rd generation.
Figs. 4 & 5.—4th generation.

T. Dobb & Co., Printers, L'pool.

Fig. 1.

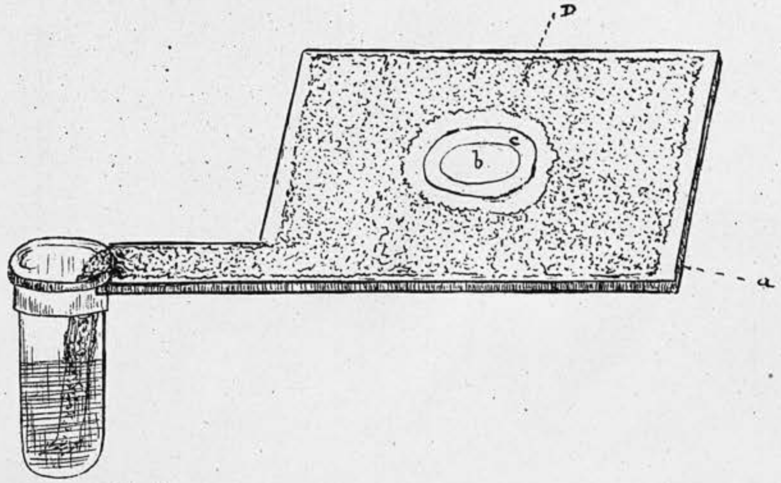


Fig. 2.

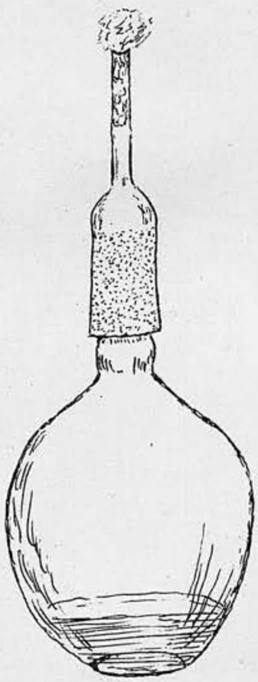
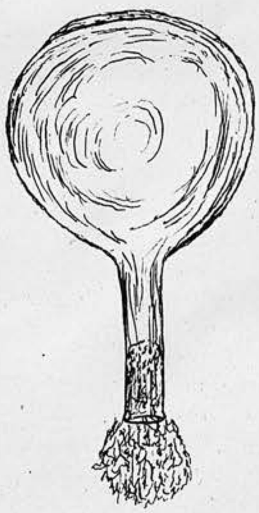


Fig. 3.



H. L. R., del.

Fig. 1.—Dallinger-Drysdale moist stage.

Fig. 2.—Pasteur Flask.

Fig. 3.—Pick's Flask.

T. Dobb & Co., Printers, L'pool.