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U N I V E R S I T Y O F M I S S O U R I

Department of Botany



C H E M O T R O P I S M O F F U N G I

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## CHEMOTROPISM OF FUNGI.

Dr. Bary (1881) advanced the supposition that the oogonia of certain Phycomycetes not only attract the antheridia-producing branches, but determine the formation of these branches as well. The same writer (1884) later raises the question as to whether the bending of a germ-tube toward the epidermis of its proper host, but not toward every membrane or moist surface, may not be brought about by a specific reaction in the parasite induced by physical or chemical stimuli which may be supposed to operate through unknown secretions from the host plant.

This was written, though not published, before Pfeffer's (1883) first studies on the reactions of motile unicellular organisms to chemical stimuli. During the progress of his studies with Saprolegnia swarm-spores, he observed that the hyphae of the fungus turned toward the nutrient substances, and he supposed that chemical agents might in many cases determine the direction of growth of these and other hyphae.

Kihlman (1883) observed that if a germinating ascospore of Melanospora parasitica lies at a distance of not more than four or five spore diameters from a growing hypha of its host, Isaria farinosa, the direction of growth of the latter is deflected toward the spore of the parasite until there is contact with its germ-tube.

According to Brefeld (1883), the fact that neighboring sporidia of Ustilaginaceae conjugate in pairs, the connecting



tubes taking the shortest course between two sporidia, may be explained by assuming a directive chemical influence.

Wortmann (1887) observed the turning of young germ-tubes of Saprolegnia species, and concluded that these are very sensitive to chemical stimuli, especially to those concerned in nutrition; a most energetic turning was observed toward flies' legs.

Marshall Ward (1888) mentions two factors as mainly influencing the direction of growth of "lily-Botrytis", namely, the contact of hyphae with one another or with solid bodies, and the direction in which food lies in relation to the growing hyphae.

Woronin (1888) holds that it is through chemical influence that the conidial germ-tubes of Peziza baccarum reach the wounds of the host plant.

Strange (1890) made careful observations on growing hyphae of Saprolegnia ferax for the purpose of confirming their reported turning toward nutrient substances. That the hyphae turn toward a region of diffusing nutriment was regarded as very questionable; there was noticed, however, a stronger growth of the hyphae in the nutrient region, and the hyphae, by their branching, became more abundant here than elsewhere. The germinating conidia sent their tubes in all directions provided enough nutriment for growth was present, and not markedly toward any area containing a greater amount of nutriment. Similar results were obtained with germ-tubes from Penicillium spores; these showed no chemotropic turning, but a much better growth when they reached the diffusion region around the





openings of the capillary tubes containing the test solutions.

Reinhardt (1892) found that the direction of growth in Peziza species is affected by chemical influences. Gelatine containing sugar and spores of Mucor species have an attractive effect. Spores of Aspergillus niger, A. flavus, and Penicillium glaucum, as well as colonies of various bacteria, cause a cessation of growth which is followed by a reversal of the direction of growth.

Büsgen (1893) observed chemotropism in the case of Botrytis cinerea, and supposed that a chemical stimulus might cause the germ-tubes in the dew-drop to seek the host epidermis; but he considered that penetration is brought about by contact influence. He asserts the possibility of chemotropism in the case of germ-tubes from uredospores and from the conidia of Peronosporaceae, and of chemotaxis in the case of Cystopus swarm-spores.

Many of the foregoing are merely opinions or passing observations made in the course of investigation of other phenomena. Miyoshi (1894), however, made chemotropism of certain fungi the subject of systematic and extensive experimental study. The principal fungi used by him were Mucor stolonifer, M. mucedo, Phycomyces nitens, Penicillium glaucum, Aspergillus niger and Saprolegnia ferax. The tests were made with the aid of perforated membranes, such as strips of epidermis, celloidin films and mica plates, of capillary tubes, and of injected leaves and petioles of Tradescantia species. He concludes that in the case of the fungi enumerated molecules of many substances diffusing from the openings cause diversion of the hyphae from their original direction of growth, the turning being either



toward the diffusing substance (positive chemotropism) when the substance is attractive to the fungus, or away from the substance (negative chemotropism) when the substance is repellent. Some substances are wholly or almost wholly neutral in effect. The direction and amount of turning are dependent upon the concentration. Chemotropism is most marked at an optimum concentration which varies for the substance and the fungus. The concentration just sufficient to cause turning is for most attractive substances very low. Repellent substances are acids, alkalis, alcohol, certain neutral salts and toxic compounds; also very strong solutions that are neutral or ~~or~~ attractive at lower concentrations. Generally attractive substances are ammonium nitrate, ammonium chloride, ammonium malate, ammonium tartrate, potassium phosphate, sodium phosphate, ammonium phosphate, meat extract, peptone, sugar, asparagin, etc. For chemotropic phenomena Weber's Law holds. The effect of an attractive substance may be overcome by the presence, in sufficient quantity, of a repellent substance.

The same investigator (1895), in connection with his study of the penetration of natural and artificial membranes by fungi, found that hyphae of Botrytis cinerea and Penicillium glaucum would not grow through the membrane unless it was placed on a nutrient substratum; there would be, however, no penetration through the membrane to ~~the~~ substratum if the fungi were grown in a nutrient medium, although the mycelial growth was more vigorous.

Swingle (1896), in explanation of the effects of Bordeaux mixture, advanced the hypotheses, which he based on the studies





of Reinhardt, Büsgen and Miyoshi, that copper hydroxid may prevent the germ-tubes of parasitic fungi from entering the host plant through negative chemotropic action.

Nordhausen (1898) accepted Miyoshi's conclusions, and investigated the biological bearing of chemotropism upon the penetration of plant tissues by certain fungi, without bringing forward any additional evidence in favor of chemotropism.

No further investigation seems to have been made with relation to fungi, until Clark (1902), in his investigation of Swingle's hypothesis, had occasion to test the chemotropic reactions of certain species, especially Mucor stolonifer, to toxic substances. For the most part he followed Miyoshi's methods closely. In every case it was found that the hyphae would turn from a nutrient medium and grow into media containing such toxic substances as salts of copper, cobalt, nickel, zinc, etc., until a concentration sufficient to cause death was reached. The hyphae turned toward non-nutrient media and distilled water as readily as toward nutrient media. His conclusion is that Mucor stolonifer is negatively chemotropic to some secretion of its own mycelium, and that this negative chemotropism is much greater than any positive chemotropism it may have for food substances or oxygen.

Massee (1904) found that fungi are attracted to their hosts by specific stimuli from substances in the cell sap. In the case of saprophytes and facultative parasites, the attractive substance is saccharose; the facultative parasites may, however, be repelled by more potent negatively chemotropic substances in the cell sap. In the case of obligate parasites, the cell-sap of the host plant is the strongest positive chemotropic agent; malic acid is the specific attractive substance for the germ-tubes



of Monilia fructigena, and the enzyme pectase for Cercospora cucumis. Immune plants owe their immunity to the absence of the chemotropic substance.

Other factors affecting the direction of growth of fungous hyphae have received but little attention, while the causes of the bending of sporangiophores, especially of certain Mucorineae, have been carefully studied.

A negative hydrotropism for the sporangiophores of Phycomyces nitens was first experimentally established by Wortmann (1881), and was later confirmed by the more extended studies of Errara (1881) and of Steyer (1901). Molisch (1883) showed that the sporangiophores of Mucor stolonifer and Coprinus velaris are negatively hydrotropic; while Klebs (1898) made similar observations for Sporodinia grandis, which Falck (1901) has failed to confirm.

Wortmann (1881) observed what he supposed to be negative hydrotropism in the case of the mycelium of Mucor species, which would grow towards water but would turn aside and branch profusely on reaching its surface. The conditions of experiment were such as to make his explanation of the phenomenon doubtful. Steyer (1901) concludes that moisture plays an unimportant role in determining the growth and spreading of the mycelium of Phycomyces nitens.

Jönsson (1883) grew mycelia of Mucor stolonifer, Phycomyces nitens, and Botrytis cinerea on sloping filter paper strips having their two ends dipped in vessels of water at different levels. Phycomyces and Mucor showed negative rheotropism under these conditions, while Botrytis showed positive rheotropism.





Hofmeister (1867), Wortmann (1881), Dietz (1888), Klebs (1898), and Steyer (1901) have established for various Mucoraceae a negative reaction of their sporangiophores to gravity and to strong light; but there is a positive reaction to contact and to moderate intensities of light.

Kny (1881) holds that gravity has no effect upon the growth of the mycelium of Mucor mucedo, M. stolonifer, Trichothecium roseum and Eurotium repens. Miyoshi (1894) concludes from his tests that neither gravity, light, contact nor moisture affected the turning of the six fungi used in his investigations. Steyer (1901) in his study of the reactions of Phycomyces, found that the mycelium is indifferent to light, contact, and gravity.

#### MATERIALS AND GENERAL METHODS.

To a greater or less extent fourteen species of fungi have been used; these, with respect to nutritive adaptations, may be classed as follows:

##### Strict Parasites

Uromyces caryophyllinus

##### Facultative Saprophytes

Sphaeropsis malorum

Cercospora apii

Monilia fructigena

##### Facultative Parasites

Botrytis vulgaris

Daedalia quercina

##### Strict Saprophytes

Mucor stolonifer

Mucor mucedo

Phycomyces nitens

Penicillium glaucum

Monilia sitophila

Sterigmatocystis nigra

Coprinus micaceus

Agaricus fabaceus

With exceptions as noted below, spores from pure cultures one to two weeks old of the various fungi were used in making inoculations. Sphaeropsis malorum was not ready in pure culture as soon as needed, and inoculations for the first experiments



with this form were made directly from infected apple twigs; the spores were found to germinate quickly and the hyphae grew rapidly, so that the observed bacterial and mould contamination in these cultures was slight. Cercospora apii obtained from celery leaves was grown in artificial culture on pieces of sugar beet; spores were not produced, but satisfactory inoculations were made with small portions of detached mycelium. Inoculations in the case of the three Hymenomycetes were made with portions of mycelium from pure cultures which had been made from sporophores by the "tissue-culture" method (Duggar, 1904). Spores of Uromyces caryophyllinus taken directly from carnation leaves were used to some extent. The germination of these was not certain under all conditions, and the growth was limited; the use of the fungus was soon abandoned. All other spores gave perfectly satisfactory germination in gelatine and agar-agar media. Even such species as Penicillium glaucum and Sterigmatocystis nigra, which have been found (Duggar, 1901) not to germinate in distilled water, were able in gelatine and agar made up with distilled water to give a germination of practically 100%.

Precautions were taken to have all apparatus chemically clean and thoroughly sterile. Glassware and mica plates were boiled in alkali and in acid, and again, after a thorough rinsing, in distilled water. Covers were rinsed in 95% alcohol, then wiped with a sterile cloth. Heavier glassware and mica plates were sterilized with dry heat at a temperature of 140° to 150° C. Celloidin films were sterilized by being boiled in redistilled water; strips of epidermis, by being rinsed in al-



cohol, and afterwards soaked in two changes of sterile redistilled water. The media used were sterilized, whenever possible, under 15 lbs. pressure of steam, or by fractional sterilization at 100° C. Except in those experiments in which capillary tubes were used, contaminations were of rare occurrence; whenever contamination was apparent, the experiment was disregarded in tabulating results. Most of the chemicals used were the chemically pure preparations of reliable manufacturers. The water used in making up test solutions was redistilled in glass apparatus. All experiments were made in duplicate, and were repeated when occasion demanded. A fairly constant temperature of 28° to 29° C. was maintained for the cultures. Beet decoction, made by boiling 450 grams fresh weight of sugar beet root in 1000 cc. tap water, was the usual basis of nutrient agar and gelatine media. The stock decoction was diluted two to ten times for use.

#### TESTS FOR CHEMOTROPISM.

##### The Capillary Tube Method.

In the first tests Pfeffer's (1888) method with capillary tubes was used. These tubes were filled with the chemical solutions under the air-pump, were rapidly rinsed in sterile distilled water, and were placed on the cover glasses in drops of the culture medium while the last was still liquid. The cover glasses were then inverted over Van Tieghem cells made up in the way described by Clark (1899), and were sealed to the cell rim with vaseline. A small quantity of liquid the same in composition as that used in making up the culture medium, was



placed in the bottom of each cell. Observations were made when the hyphae were 40-75 spore diameters in length, and again three or four hours later when growth had become indefinitely great. In estimating the effects of the chemicals, regard was had for the hyphae from spores lying within a radius of one lumen diameter from the opening of the tube, and such other hyphae from more distant spores as entered this area. Only those were held to be chemotropically affected that showed a turning out of their former courses toward or away from the tube opening. In recording the observations Miyoshi's method was used; to denote a turning away of 12% to 37%, the symbol  $r$  was used; for a turning away of less than 12% and an attraction of less than 12%, the symbol  $o$ ; for an attraction of 12% to 37%,  $a_1$ ; for an attraction of 37% to 62%,  $a_2$ ; etc.

This method was used in the preliminary testing of a large number of representative chemical substances with Sterigmatocystis nigra and Mucor mucedo; Botrytis vulgaris was used with a few that gave decided effects. The series included the sulphate of sodium, of magnesium, of calcium, of ammonium, of potassium; the normal phosphate of ammonium, of potassium; the dibasic phosphate of ammonium, of potassium; the monobasic phosphate of potassium, of sodium, of calcium; the chloride of potassium, of ammonium, of calcium, of magnesium, of sodium, of lithium; the nitrate of potassium, of ammonium, of calcium, of magnesium, of sodium; the oxalate of magnesium, of potassium; the tartrate of magnesium, of potassium, of sodium-potassium; the bitartrate of potassium; the lactate of magnesium; the malate of magnesium, of ammonium; the acetate of potassium;





acetic, lactic, tartaric, malic, oxalic acids; cane sugar, dextrose, galactose, maltose, lactose, starch; glycerine, ethyl alcohol; active pepsin, boiled pepsin, trypsin, peptone, mannit, agaracin casein, asparagin, urea; meat extract, beet decoction, bean decoction, distilled water; mercuric chloride, copper sulphate, copper acetate, lead nitrate, zinc nitrate, iron sulphate, iron chloride, potassium ferrocyanide. In the case of such of these compounds as were tested by Miyoshi, concentrations were used that would give the maximum effect of attraction or repulsion. With nearly all compounds two, and with many, three concentrations were tested.

The hyphae showed a tendency to turn, in small numbers, toward the tubes in the majority of tests; but in only a few instances were more than about 37% of the hyphae in the observed area affected. The repellent effect was much less marked, never affecting more than one fourth of the hyphae, and even in the most marked cases some of the hyphae would grow toward the tubes and enter them. The following table gives the most marked instances of attraction and repulsion noted in the series; the symbols have been explained above.

TABLE I.

	$\text{Ba H}_2(\text{PO}_4)_2$ 2%	$\text{K H}_2\text{PO}_4$ 2%	$\text{Mg SO}_4$ 2%	$\text{Na NO}_3$ 2%	$(\text{CaH}_2)_2\text{HPO}_4$ 2%	$\text{NH}_4\text{NO}_3$ 2%	Acetic Acid 5%	Malic Acid 5%	Pepsin (solid) 2%	Pepsin (liquid) 2%	Trypsin 1%	Meat Extract 10%	Ammonium Malate 2%
<i>Sterigmatocystis nigra</i>	r		r			r							r
<i>Mucor mucedo</i>		a <sub>2</sub>		r	a <sub>2</sub>		r	a <sub>2</sub>	a <sub>2</sub>	a <sub>2</sub>	a <sub>2</sub>	a <sub>2</sub>	a <sub>2</sub>
<i>Botrytis vulgaris</i>									a <sub>2</sub>	a <sub>2</sub>			

In Table II is given a comparison of the effects of a number of representative compounds as observed by Miyoshi and



in the present investigation. Leaving out of consideration Saprolegnia ferax, Mucor mucedo and Aspergillus niger represent the extreme effects in Miyoshi's table; the values given by him are to be found in columns I and III below. It was impracticable to use Aspergillus niger in the present study, and Sterigmatocystis was substituted with the idea that it may really be the form used by Miyoshi. The results for Mucor mucedo and Sterigmatocystis nigra are given below in columns II and IV respectively.

TABLE II.	<u>Mucor mucedo.</u>		<u>Aspergillus niger.</u>	<u>Sterigmatocystis nigra.</u>
	I	II	III	IV
Monobasic potassium phosphate, 2%	a <sub>3</sub>	a <sub>2</sub>	a <sub>1</sub>	a <sub>1</sub>
Monobasic sodium phosphate, 2%	a <sub>2</sub>	a <sub>2</sub> ?	a <sub>1</sub>	a <sub>1</sub>
Neutral ammonium phosphate, 2%	a <sub>4</sub>	a <sub>1</sub>	a <sub>2</sub>	a <sub>1</sub>
0.2%	a <sub>1</sub>	a <sub>1</sub>	a <sub>1</sub>	a <sub>1</sub>
0.1%				
Ammonium nitrate, 2%	a <sub>3</sub>	a <sub>2</sub> ?	a <sub>2</sub>	r
Potassium nitrate, 2%	r	a <sub>1</sub>	r	o
Calcium nitrate, 2%	r	a <sub>1</sub>	r	o
Magnesium sulphate, 2%	r	o	r	r
Ammonium chloride, 3%	a <sub>3</sub>	o	a <sub>1</sub>	o
Potassium chloride, 2%	r	o	r	o
Sodium chloride, 2%	r	o	r	o
Sodium-Potassium tartrate, 2%	r	a <sub>2</sub> ?	r	o
Cane sugar, 20%	a <sub>1</sub>	o	a <sub>1</sub>	o
10%	a <sub>4</sub>		a <sub>3</sub>	
5%		o		o
1%	a <sub>3</sub>	a <sub>1</sub>	a <sub>2</sub>	a <sub>1</sub> ?
0.1%	a <sub>1</sub>	o	a <sub>1</sub>	o
Lactose, 2%	a <sub>1</sub>		a <sub>1</sub> ?	
1%		a <sub>1</sub>		a <sub>1</sub> ?
Maltose, 2%	a <sub>1</sub>		o	
1%		o		a <sub>1</sub> ?



TABLE II (Continued).	I	II	III	IV
Dextrose, 10%	a <sub>4</sub>	o	a <sub>2</sub>	a <sub>1</sub> ?
5%	a <sub>4</sub>	r?	a <sub>3</sub>	o
2%	a <sub>3</sub>	a <sub>2</sub> ?	r	a <sub>2</sub> ?
1%	r	o	a <sub>1</sub> ?	a <sub>1</sub> ?
Ethyl alcohol, 1%	a <sub>1</sub>	a <sub>2</sub> ?	a <sub>1</sub>	a <sub>1</sub> ?
Glycerine, 10%	a <sub>2</sub>	o	o	a <sub>1</sub> ?
Asparagin, 2%	o	o	a <sub>2</sub>	-
Urea, 2%	a <sub>3</sub>	a <sub>1</sub> ?	a <sub>2</sub>	a <sub>1</sub> ?
Peptone, 2%	a <sub>1</sub>	o	a <sub>1</sub>	a <sub>1</sub> ?
1%	a <sub>3</sub>	o	a <sub>1</sub>	a <sub>1</sub> ?
Meat extract, 10%	a <sub>1</sub>	o	o	a <sub>1</sub> ?
1%	a <sub>3</sub>	o	o	a <sub>1</sub> ?
0.1%	a <sub>1</sub>	o	o	a <sub>1</sub> ?
0.01%	a <sub>1</sub>	o	o	a <sub>1</sub> ?

In the control cultures, where distilled water was used in the tubes, the effect was the same as for the majority of chemical substances; that is, from 10% to 30% of the hyphae turned toward the tubes. The same amount of positive turning was observed in the case of all the strongly toxic compounds used. Even with a 0.05% solution of mercuric chloride and a 1% solution of copper sulphate, which completely inhibited germination within a radius of eight to twelve tube diameters from the openings, the hyphae not only grew across the diffusion areas, but 10% to 30% of those approaching the openings turned toward them and grew for a considerable distance into the tubes.

Although four concentrations of cane sugar ranging from 20% to 0.1%, and four concentrations of meat extract ranging from 10% to 0.01% were used, no definite relation between the strength of stimulus and that of response was apparent.



Two corresponding series were made with ten representative compounds; in one sugar beet agar was the culture medium; in the other, distilled water agar. No difference in the behavior of the hyphae due to a difference in the media in which they grew could be observed.

#### Tests with Mica Plates.

Thin sheets of mica were cut into pieces about 25X16 mm.; these were perforated with a needle, the holes being about 0.1 to 0.15 mm. in diameter, and about 2mm. apart. Covers of suitable size were cut from glass 1mm. thick. A layer of gelatine or agar was placed on a cover, a mica plate was placed on this just before it hardened, and a second layer was placed above the plate. The chemical to be tested was made up in double strength solution in redistilled water, and one volume of this was added to one volume of gelatine or agar also made up double strength in redistilled water. It was usually found convenient to have the layer containing the chemical next to the cover. The cover was inverted over a stender dish of 36mm. diameter containing distilled water, and was sealed with a coating of vaseline around the rim of the vessel.

This method seemed to possess distinct advantages over the one with capillary tubes: the numerous perforations made it possible to make a large number of observations from a single preparation; the medium containing the chemical and that containing the spores could be more nearly equalized in amount and in consistency; fewer hyphae would take a course that would lead them through the openings without apparent turning; better sterilization could be secured; and there was less difficulty in making up the preparations.

In making the counts, hyphae within a radius of one open-





ing diameter from the margin of each opening were considered; the hyphae within such an area were classed in the counts as those turning toward the openings, those turning away from the openings, and those apparently indifferent. After an examination of the entire preparation in each case, those holes were selected for the counts which represented the average condition. In calculating the percentages from the counts, the difference between those attracted and those repelled was made the dividend, and the total number within the observed area was made the divisor, the results are as follows:-

TABLE III.	<i>Monilia frutigena</i>	<i>Monilia sitophila</i>	<i>Stenogasteria nigra</i>	<i>Botrytis vulgaria</i>	<i>Cercospora apii</i>	<i>Sphaeria malorum</i>
	I	II	III	IV	V	VI
Neutral potas. phosphate 2%	12	19	24	22	33	22
	21	20	18	25	36	25
Monobasic potas. phos. 2%	29	22	24	26	36	23
	20	26	23	20	25	21
Dibasic ammon. phosphate 2%	13	20	21	20	15	23
Calcium phosphate 0.2%	49	41	34	31	35	29
Phosphoric acid 0.2%	48	30	35	39	23	30
Malic acid 1%	^^	^^	24	^^	38	^^
	21	21	26	21	17	^^
Tartaric acid 1%	^^	20	43	22	19	18
	25	27	34	25	24	22
Oxalic acid 1%	^^	17	23	^^	17	^^
	23	22	23	34	11	40
Cane sugar 5%	38	32	24	31	20	48
	30	37	20	30	20	55
	30	35	24	33	23	42
Glucose 5%	39	39	20	34	30	37
	40	24	23	39	20	30
Peptone 2%	14	28	21	13	20	19
	26	23	20	21	13	22



TABLE III (CONTINUED)

	I	II	III	IV	V	VI
Pepsin (active) 2% 0.2%	24	20	19	42	23	30
	25	30	22	27	31	22
Pepsin (boiled) 2%	26	31	36	33	40	37
Asparagin 0.1% 0.1%	19	23	30	23	30	15
	15	23	30	20	30	24
Copper sulphate 0.005%	29	21	21	25	34	31
Dibasic sodium phosphate 2% 0.2%	35	25	26	26	15	20
	38	30	27	20	26	23
Starch paste 0.4% 0.4%	34	24	20	21	27	25
	44	27	22	20	26	15
Maltose 2% 0.2%	33	30	22	21	25	14
	36	29	28	31	30	28
Control gelatine made up with distilled water	28	30	29	24	22	18

Note: The symbol ^ indicates that germination was inhibited. Uromyces caryophyllinus was tested with the majority of these substances, in the few instances in which growth was

sufficiently great for a determination, the turning was practically the same as for the other fungi.

If the percentages of turning toward distilled water gelatine as determined by the control experiments, be deducted from the percentages of turning toward the various chemical compounds, it will be found that in only three instances would the difference, which would be supposed to indicate the amount of turning due to chemical influences, approximate 25%, or about the average of the lowest of the several degrees of positive chemotropism recognized by Miyoshi. Most of the values, even for supposedly highly attractive substances, are very near the controls.

Evidently the results thus far have not been favorable to the theory of chemotropism. But it was thought that the fungi, all of which grew perfectly normally in 8% gelatine made up in distilled water, might turn more strongly from some other medium in which there was less of available nutriment, to one



having an abundance. In agar made up with distilled water, the fungi germinated after a greater length of time and grew more slowly; but from this non-nutrient agar the turning toward a nutrient salt solution favorable for fungous growth was no more marked than from agar containing the same proportions of the nutrient salts to the nutrient salt solution; nor did the nutrient solution seem to attract from either medium more strongly than did distilled water. This test was made with Sterigmatocystis nigra, Mucor mucedo and Mucor stolonifer by the capillary tube method.

It is possible, although there is evidence against it in some of the previous experiments, that the diffusion of the solutes might be rapid enough to bring about practical uniformity in the media before the germination of the spores. To determine whether the time at which the stimulus is applied has an influence upon the reaction, series were made up with two of the more slowly growing species, Botrytis vulgaris and Monilia fructigena, the spores being distributed in non-nutrient gelatine, and this placed above the mica plates. Four duplicate series were arranged. In one the layers of gelatine containing the substances to be tested were placed below the plates at the time of making up the cultures; in another series these layers were added just as the spores began to germinate; in a third, after the most vigorous hyphae had attained a length of 15 to 20 spore diameters; <sup>in a fourth, when the length was 40 to 50 spore diameters.</sup> The final counts were made a little later when the growth was about 75 spore diameters. The results are given in table IV for the five compounds tested and the control. The numbers in columns I indicate the average number of hyphae in the area around each hole; those in columns II, the percentages of turning toward the holes.



TABLE IV

	Hours until application of chemical.	Dextrose 4.5%		CuSO <sub>4</sub> 0.01%		Oxalic Acid 0.05%		KH <sub>2</sub> PO <sub>4</sub> 2%		NH <sub>4</sub> NO <sub>3</sub> 2%		Non-nutrient Gelatine	
		I	II	I	II	I	II	I	II	I	II	I	II
		<i>Botrytis vulgaria.</i>											
	0	11	35%	11	38%	6.3	34%	4.0	37%	5.5	36%	9.2	34%
	10	9.0	35%	6.0	30%	7.8	36%	5.4	30%	7.2	36%	7.6	34%
	13	11	33%	5.5	34%	6.4	25%	6.4	31%	11	33%	7.2	35%
	16	3.3	30%	7.6	34%	5.5	30%	5.8	35%	5.0	32%	5.6	36%
<i>Monilia fructigena.</i>													
	Hours until application of chemical.	Dextrose 4.5%		CuSO <sub>4</sub> 0.01%		Oxalic Acid 0.05%		KH <sub>2</sub> PO <sub>4</sub> 2%		NH <sub>4</sub> NO <sub>3</sub> 2%		Non-nutrient Gelatine.	
		I	II	I	II	I	II	I	II	I	II	I	II
	0	9.3	38%	10	30%	11	28%	3.7	31%	8.2	34%	4.4	26%
	10	9.5	36%	11	30%	11	30%	3.7	28%	6.8	25%	11	32%
	15	13	33%	7.5	27%	10	32%	4.3	30%	5.4	27%	13	27%
	20	17	32%	8.7	27%	9.3	29%	5.3	29%	5.1	26%	8.3	30%

It is evident that the time of application has little or no effect upon the amount of turning. It will also be seen by comparing these results with former ones, that it is immaterial, as far as the percentage of turning is concerned, whether the spore-containing layer is above or below the one containing the test substance.

Other culture media were used, such as 10% and 25% glycerine, gelatine made up to contain 10% of glycerine and to contain 5% of cane sugar. With none of these were there more distinct indications of chemotropism than in former tests in which non-nutrient gelatine was the culture medium. This would indicate that the available nutriment and the concentration of the medium have no effect.

The final test along this line was made with silica jelly,





a medium free from organic matter and of suitable consistency. The method of preparation was that used by Moore (1903), except that dialysis was accomplished satisfactorily with parchment paper. In order to secure the proper coagulation of the medium, it was necessary to add mineral salts to all media used. A nutrient salt solution containing 1 gram of ammonium nitrate, 0.5 gram of monobasic potassium phosphate, 0.25 gram of magnesium sulphate, a trace of ferric chloride, and 5 grams of glucose in a volume of 100cc, was made the basis of the work. It would seem that if fungi show chemotropism under any conditions it would be by turning from a medium lacking some one or more of the elements necessary for full development, toward the diffusion centers of compounds supplying the missing element or elements, or toward a full nutrient solution.

In the tests each compound in turn except ferric chloride was omitted from the silica jelly containing the spores, and in each case jelly containing the omitted compound in proper proportion on the one hand, and full nutrient jelly on the other were used on the opposite side of the mica plate from the above-mentioned spore-containing layer. Control tests were carried on with jelly lacking one and the same compound on each side of the plate, and also with full nutrient jelly on each side of the plate. In each case the lower layer was covered with an unperforated mica plate. The results are given in the following table.



TABLE V.

	<i>Mucor stolonifer.</i>	<i>Stigmatocystis nigra.</i>	<i>Monilia sitophila.</i>	<i>Botrytis vulgaria.</i>	<i>Monilia fructigena.</i>
From full nutrient jelly to full nutrient jelly.	35%	32%	32%	33%	29%
From nutrient jelly lacking glucose to nutrient jelly lacking glucose.	32%	36%	37%	30%	33%
From nutrient jelly lacking glucose to jelly containing glucose.	27%	30%	34%	30%	26%
From nutrient jelly lacking glucose to full nutrient jelly.	30%	36%	33%	30%	30%
From nutrient jelly lacking $\text{K}_2\text{HPO}_4$ to nutrient jelly lacking $\text{K}_2\text{HPO}_4$ .	33%	30%	29%	33%	26%
From nutrient jelly lacking $\text{K}_2\text{HPO}_4$ to jelly containing $\text{K}_2\text{HPO}_4$ .	36%	36%	31%	30%	28%
From nutrient jelly lacking $\text{K}_2\text{HPO}_4$ to full nutrient jelly.	32%	30%	33%	36%	28%
From nutrient jelly lacking $\text{NH}_4\text{NO}_3$ to nutrient jelly lacking $\text{NH}_4\text{NO}_3$ .	35%	35%	33%	36%	28%
From nutrient jelly lacking $\text{NH}_4\text{NO}_3$ to jelly containing $\text{NH}_4\text{NO}_3$ .	34%	32%	32%	33%	29%
From nutrient jelly lacking $\text{NH}_4\text{NO}_3$ to full nutrient jelly.	34%	33%	33%	31%	32%
From nutrient jelly lacking $\text{MgSO}_4$ to nutrient jelly lacking $\text{MgSO}_4$ .	33%	31%	33%	33%	27%
From nutrient jelly lacking $\text{MgSO}_4$ to jelly containing $\text{MgSO}_4$ .	39%	34%	33%	33%	29%
From nutrient jelly lacking $\text{MgSO}_4$ to full nutrient jelly.	29%	33%	32%	35%	30%

There is a very striking uniformity in the percentages, and this under conditions that would be presumed to be most favorable for chemotropic reaction.



Tests with Epidermis and Celloidin Films.

To test the effect of physically different perforated sheets as well as to effectually repeat the methods used by former investigators, use was made of celloidin films which had been perforated, and of strips of epidermis of Yucca aloifolia. This gave, with reference to physical properties, a range from the wholly impermeable mica plates on the one hand to the semipermeable celloidin films on the other.

The tests with epidermis were made with Monilia fractigena, Sterigmatocystis nigra, Botrytis vulgaris, Sphaeropsis malorum, and Mucor stolonifer, the spores of which were distributed in non-nutrient 8% gelatine above the epidermis in its final position; gelatine layers containing 5% cane sugar, 4.5% dextrose, 0.01% copper sulphate, 0.1% oxalic acid, 0.2% phosphoric acid, and non-nutrient gelatine, were spread below the epidermis. Under these conditions the penetration of the stomates or turning toward the stomates was practically zero. When no culture medium was used, the spores being merely spread on the under surface of the epidermis, hyphal growth was good. A few hyphae of each species grew through the stomates; but there was no evident turning toward them, and in no case was there penetration of more than 1% or 2% of the stomates.

In similar series with celloidin films, the turning from one gelatine layer to another was about equal to that obtained with mica plates. When the spores were spread on the film without a culture medium, very few of the hyphae grew through the holes, the percentage of turning being negligible. The hyphae in these cases were surrounded by a distinct film of condensed moisture.



## TESTS FOR OTHER FACTORS.

The tests thus far have failed to give evidence of the existence of any **marked** chemotropism. There has been at the same time a considerable and fairly constant turning of the hyphae from a medium containing spores to a sterile medium when these were separated the one from the other by any one of several partitions. Since this turning is apparently unaffected by the chemical relationships of the media employed, the cause of the turning must be sought in other factors concerned. Two possibilities at first present themselves: the mechanical partitions may have a thigmotropic or other influence, or the germinating spores themselves may affect the direction of growth.

Tests without Mechanical Partitions.

A slight modification of the method employed by Clark (1902) was used. A large drop of agar 8 mm. in diameter was placed in the center of a sufficiently large square of glass; this drop was surrounded by four drops of about 5 mm. diameter, placed equidistantly around the first, and with a space of about 3 mm. between each smaller drop and the larger one. Nonnutrient agar and 10% sugar beet agar were used for the drops, and were arranged in four combinations: the central drop was of nutrient agar and two small drops diagonally opposite each other were of nutrient agar, the other two being non-nutrient; the central drop was of nutrient agar, two small drops adjacent to each other of nutrient agar, and the other two of non-nutrient agar; two similarly arranged combinations with the central drops of non-nutrient agar. The fungi used were Monilia sitophila, Rhizoglyphus stolonifer and Botrytis vulgaris. A few spores were sown with the platinum needle at the center of the large drop in each preparation; the cover was inverted over a stender dish containing distilled water and was sealed to its rim. The growth was watched until the





hyphae had grown about two thirds of the distance from the center to the margin of a large drop; the preparations were then opened, and with a sterile needle the small drops were pushed up until their edges came in contact with the larger drops. Later observations showed that the hyphae of the three fungi grew readily from either medium into a similar or a dissimilar medium, and with the same percentage of turning. An equal amount of turning toward the agar drops was observed in the case of those hyphae which had grown beyond the bounds of the larger drops on the moist glass; whether the agar was nutrient or non-nutrient seemed immaterial. The turning was apparent at a considerable distance from the surface film in so large a percentage of cases as to negative the supposition that the physical condition of the film has an influence.

A further test without mechanical separation was made by placing small crystals of cane sugar, copper sulphate, oxalic acid, monobasic potassium phosphate and ammonium nitrate in the center of layers of non-nutrient gelatine on cover glasses. Spores of Monilia fructigena, Botrytis vulgaris, Sterigmatocystis nigra, Mucor stolonifer and Monilia sitophila were used; in some instances they were evenly distributed in the gelatine, in other instances the gelatine was inoculated by being touched with the needle at several points varying in distance from the crystal. In no case was there any distinct turning toward or away from the diffusion center.

#### The Effect of Hyphae upon the Direction of Growth.

Clark (1902) explains his results by supposing that the fungus secretes some substance to which the growing hyphae are negatively chemotropic. While this hypothesis would very well explain his results, he seems not to have made it the subject of experimental study.



It may be reasonably assumed that if a fungus is negatively chemotropic to its own secretion, the stimulus to turn away from an area containing the fungus would, in early stages of growth, be in some degree proportional to the amount of mycelium in that area.

To determine whether the amount of mycelium has an affect, inoculations were made with differing numbers of spores; the growth was from non-nutrient gelatine and gelatine containing M/4 solution of dextrose, and was toward similar as well as different media as indicated in Table VI, where the results are given.

TABLE VI.

	<i>Stenigmatocystis nigra</i>		<i>Monilia sitophila</i>		<i>Botrytis vulgana</i>		<i>Monilia fructigena</i>		<i>Mucor stolonifer</i>	
	I	II	I	II	I	II	I	II	I	II
Non-nutrient gelatine to gelatine containing M/4 dextrose.	4.1	34%	5.0	24%	6.8	27%	5.0	20%	15	31%
	15	32%	16	25%	22	37%	7.3	26%	54	36%
Gelatine containing M/4 dextrose to gelatine containing M/4 dextrose.	8.5	24%	4.2	25%	8.3	29%	4.8	26%	5.0	25%
	2.2	36%	2.3	38%	2.3	37%	2.1	36%	2.0	38%
Gelatine containing M/4 dextrose to non-nutrient gelatine.	8.0	25%	7.7	26%	6.7	28%	6.0	28%	6.3	31%
	19	35%	17	36%	23	37%	18	29%	14	31%
Non-nutrient gelatine to non-nutrient gelatine.	3.0	33%	5.0	30%	8.0	34%	4.5	22%	7.1	28%
	21	40%	16	40%	18	37%	17	34%	36	41%
Gelatine containing M/4 glycercine to gelatine containing M/4 dextrose	3.9	30%	4.3	27%	6.3	30%	7.0	28%	17	29%
	2.2	38%	1.9	39%	1.8	36%	1.8	34%	3.1	35%
Non-nutrient gelatine to gelatine containing 0.01% Cu S <sub>2</sub> O <sub>4</sub> .	3.1	24%	5.3	28%	6.3	28%	5.0	27%	18	33%
	2.0	39%	1.5	39%	2.1	39%	1.6	32%	4.3	39%

Note: Columns I give the average number of spores per hole.

Columns II give the percentages of turning toward the holes.



There is indication that the number of hyphae in a given area and the amount of turning from that area are correlated. It may be said of this, as of succeeding tests, that it is at best only relative. It is manifestly impossible to eliminate growing hyphae from the experiments, and their effect is the very factor to be tested.

A test was made by comparing preparations in which only one layer of gelatine was inoculated with preparations in which both layers were inoculated. Two parallel series were prepared; in one the spores in the lower layer were about twice as numerous as in the other series. An examination of Table VII, which gives the results, shows that the percentage of turning toward a layer containing hyphae is less than toward a sterile layer; and in either case the abundance of mycelium in the layer influences directly the turning of the hyphae from that layer.

TABLE VII.

<i>Monilia sitophila.</i>			<i>Stigmatocystis nigra.</i>			<i>Mucor stolonifer.</i>			<i>Mucor mucedo.</i>			<i>Phycomyces nitens.</i>		
I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
6.5	0	26%	6.5	0	32%	5.5	0	32%	11	0	29%	7	0	28%
12	0	35%	15	0	38%	14	0	39%	23	0	34%	14	0	37%
4.5	7	20%	5.0	8	23%	4.5	7	15%	11	19	25%	9	16	24%
9	6	22%	12	8	24%	9.5	7	24%	19	11	30%	16	9	29%

Note: Column I gives the average number of hyphae per hole in the layer from which the turning takes place.

Column II gives the average number of hyphae per hole in the layer toward which turning takes place.

Column III gives the percentage of turning.



To determine whether this negative turning is due largely to chemical changes in the culture medium, beet decoction in which a fungus had grown was tested with that fungus. The sugar beet decoction was diluted to one third the strength of the stock solution.

When a good amount of mycelium had been formed, the liquid was filtered under sterile conditions at room temperature. The filtrate was replaced in the incubator in the case of those species which had fruited, and was allowed to remain for twenty-four hours in order that any spores that had passed through the filter might germinate; it was then refiltered. In this way a practically sterile medium was obtained. One volume of this decoction was added to one volume of double strength gelatine, and tests were made with this in comparison with control beet decoction in which there had been no fungous growth.

The results are as follows:

TABLE VIII.	<i>Botrytis vulgaris</i>		<i>Stigmatocystis nigra</i>		<i>Penicillium glaucum</i>		<i>Monilia sitophila</i>	
	I	II	I	II	I	II	I	II
	Growth toward gelatine made up with beet decoction in which fungus had grown.	6.2	29%	10	29%	8.0	23%	8.0
	15	31%	19	27%	18	22%	23	25%
Growth toward gelatine made up with fresh beet decoction.	5.3	33%	9.0	34%	12	31%	9.0	26%
Control.	15	35%	17	37%	28	33%	20	33%
	<i>Mucor stolonifer</i>		<i>Agaricus fabaceus</i>		<i>Aspergillus niger</i>		<i>Macrodia quercina</i>	
	I	II	I	II	I	II	I	II
Growth toward gelatine made up with beet decoction in which fungus had grown.	19	32%	49	24%	31	24%	46	26%
Growth toward gelatine made up with fresh beet decoction. Control.	22	38%	50	32%	43	30%	35	31%

Note: Column I gives the average number of hyphae per hole.  
Column II gives the percentage of turning toward the holes.

The percentages for all of the fungi in this table except Mucor stolonifer, have been computed from four distinct series. In this way erratic results have been nullified, and the difference in effect,





although not marked, may be regarded as constant. There is certainly a lessened attractive influence in the case of the decoction in which the fungi have grown; this might be due to the mere abstraction from the decoction of nutrient substances, or to the conversion of compounds occurring in the decoction into compounds which are repellent in their effect, or to the secretion of products by the fungus which have a repellent effect. That the first is probably not the case is to be inferred from the fact that in previous tests these fungi have been found to grow as readily toward distilled water and other non-nutrients, as toward nutrients; a mere decrease, therefore, in the amount of available nutrient could hardly have a pronounced effect. We must therefore conclude that a medium in which a fungus has grown may become less attractive, or more repellent, to the fungus through the agency of some undetermined substance or substances which are secreted or otherwise formed by the growing fungus; this reaction would be negative chemotropism.

Miss Ferguson (1902) found that germinating spores of Agaricus campestris, or bits of older growing mycelia, which have a very marked effect in causing the germination of spores of this species; at the same time there seems to be a retarding effect upon the growth of the protruded germ-mycelium. Mycelium that is not growing, masses of ungerminated spores, or growing mycelia of other fungi do not have the same influence over germination. Her observations leads her to suppose that oxygen or carbon dioxide is not the determining factor, but that some secretion is formed which stimulates or makes possible the emission of the germ-tubes. Other observations relative to the influence of germinating spores upon the growth of fungi have been made by Kihlman (1883) and Reinhardt (1892);



these have already been referred to in this paper.

Numerous instances of the influencing by various plant cells of the direction of movement and of growth of other cells of the same or different kind, have been recorded, and the general terms cytotaxis and cytotropism have been applied to this peculiar phase of chemical influence. In the cases enumerated by Pfeffer (1904, sec. 155) the effects seem to be due either to the excretion of a hypothetical specific substance which furnishes the stimulus, or to changes in the relative proportions of oxygen and carbon dioxide through respiratory or photosynthetic activities. Since these phenomena seem to be analogous in a general way to the above-described turning of fungous hyphae, and since the term cytotropism indicates nothing as to the exact nature of the ultimate stimulus-substance, this term might be found a convenient one for designation in the present instance.

#### The Effect of Moisture.

The circumstance that a number of preparations in which the culture medium had become evidently dry, gave large percentages of turning, suggested that the hyphae might react to a hydrotropic stimulus. Layers of agar containing spores of several fungi were spread on coverglasses, and sterile strips of filter paper were placed in contact with the agar and allowed to dip into the water of the Van Tieghem cell. The average percentage of turning toward the strips for those spores within a distance of 0.35 mm. from their edges was 40% for Penicillium glaucum, 50% for Mucor stolonifer, Monilia sitophila and Sterigmatocystis nigra, 55% for Mucor mucedo, 57% for Botrytis vulgaris, and 60% for Monilia fructigena and Sphaeropsis halorum.



The percentages given are less than would be the case if a smaller area about each strip had been considered; this is doubtless because the spores were rather thickly sown, and the hyphae from those nearest the strip, being numerous, exerted an effective repellent influence on those more distant, causing them to grow away from the strip. Notwithstanding this, the evidence of positive hydrotropism for these fungi was quite conclusive.

As a further test, mica plates were cut to fit Van Tieghem cells and were perforated; a drop of non-nutrient gelatine was placed on each and covered with a perforated plate small enough to fit inside the cell; upon this was placed another layer of non-nutrient jelly containing the spores; another perforated plate was added, and a third layer of gelatine sterile like the first; the lower surface of this was left uncovered. The mica cover was inverted over a cell containing water, during the time required for the proper growth of the fungi, evaporation took place from the now uppermost layer to the surrounding atmosphere, as was apparent from the dry condition of the gelatine around the holes in the uppermost plate; water diffused from the lower layers to supply the deficiency. In this way it came about that the middle layer which contained the spores, was moister than the uppermost layer but dryer than the lowermost. There was observed a very decided turning of the hyphae toward and through the openings in the third plate which separated the middle and lowermost layers, while comparatively few grew toward the uppermost layer. The estimated percentages are as follows:-



TABLE IX.

	<i>Monilia fructigena</i>	<i>Monilia sitophila</i>	<i>Strigomoniliopsis nigra</i>	<i>Botrytis vulgaris</i>	<i>Penicillium glaucum</i>	<i>Sphaeria malorum</i>	<i>Mucor mucedo</i>	<i>Mucor stolonifer</i>	<i>Phycomyces nitens</i>
From middle layer to lowermost layer.	43%	70%	80%	55%	68%	65%	73%	63%	65%
From middle layer to uppermost layer.	20%	20%	20%	30%	18%	15%	18%	15%	18%

Experiments were set up in which very firm gelatine (16%) containing the spores was covered with mica plates having a few perforations. The plates were sealed to the covers by an application of vaseline around their margins. The covers were then inverted over stender dishes level full of sterile distilled water. In this way the water came in contact with the gelatine only through the perforations, and diffused from these through the gelatine layer. Hyphae of Mucor stolonifer grown under these conditions showed a tendency to grow toward the openings from a distance of 1.5 mm., but on coming within 0.5 mm. of the openings the course was changed and the hyphae circled the openings in lines more or less concentric with their margins. The majority of those nearer the openings than 0.5 mm. grew in a radial direction away from them. In a few instances hyphae grew into the water. Mucor mucedo showed a quite decided turning toward the holes, and about 65% of the hyphae within a radius of three hole diameters turned through them and grew into the water. With Botrytis vulgaris about 40%, and with Penicillium glaucum, 85% of the hyphae within a corresponding area were affected hydrotropically. In every case the growth in the water was in all directions, directly downward as well as radially in a horizontal plane. The value of the control cultures, which were duplicates in all respects excepting that the dishes were only partly





filled with water, was vitiated by the accumulation of condensed moisture in comparatively large drops about the openings in the plates. This caused an unmistakable turning toward the holes, which, however, was not so decided as in the test cultures.

It is evident from these results that all of the fungi tested in this regard are, under the conditions of experiment, positively hydrotropic; but Mucor stolonifer may under certain conditions show a negative hydrotropism. This response to a hydrotropic stimulus probably accounts in large measure for the constant turning toward protected layers from those more exposed, which latter may have become dryer through evaporation.

A sharp distinction between hydrotropism and rheotropism on the one hand, or between hydrotropism and osmotropism on the other, can not in all cases be made, although these phenomena in typical cases are quite distinct. The phenomena here reported are probably due primarily to differences in the moisture content of the layers, and not to water currents either molar or molecular. For this reason the term hydrotropism has been applied, which, however, is not in agreement with the current opinion (Pfeffer, 1904:592) that in the case of the fungous mycelia heretofore studied, osmotropism and rheotropism, but not hydrotropism, have been established. It is further recognized that chemical rather than other properties of water furnish the effective stimulus, in which event hydrotropism would properly be regarded as a special kind of chemotropism.

#### Aerotropism.

Under the conditions prevailing in some of the experiments above described, there was doubtless an inadequate supply of oxygen, as when a medium poor in oxygen was enclosed between impervious plates. There was then a very decided tendency for the



hyphae to turn toward the edges of the plates. The observance of this phenomenon from time to time suggested that the fungi might show an aerotropic sensibility either as a positive reaction to oxygen or as a negative reaction to carbon dioxide.

In order to definitely test the matter, experiments were arranged in which the growth toward holes in mica plates could be observed when the plates separated normal non-nutrient gel-  
 atine <sup>from non-nutrient gelatine</sup> saturated with carbon dioxide on the one hand, and from normal non-nutrient gelatine on the other. The fungi used were Penicillium glaucum, Sterigmatocystis nigra Mucor mucedo, Botrytis vulgaris, Monilia fructigena, Monilia sitophila and Phycomyces nitens. In no case was the percentage of turning toward the carbon dioxide gelatine greatly different from that toward the control gelatine.

As a further test, a layer of gelatine containing spores was placed below a perforated mica cover for a Van Tieghem cell, and a perforated mica plate, small enough to fit inside the cell was placed below the gelatine. A layer <sup>of normal gelatine was spread below this</sup> ~~of carbon dioxide~~  
 and a layer of carbon dioxide gelatine above the cover. This preparation was sealed to the cell rim, and the whole placed under a bell-jar practically filled with carbon dioxide and kept at a room temperature of 21° to 24° C. Efforts were made to have the moisture conditions equal within and without the cells; and the exposed gelatine layers, which served very well as indicators, showed no difference in this respect until after the observations on the majority of the preparations had been made, although there was drying of the outer gelatine layer by the time the more slowly growing fungi had reached the proper stage of growth. The same fungi were used in this experiment as in the former one, with the addition of Mucor stolonifer. In most in-



stances the turning toward the gelatine containing carbon dioxide and exposed to an atmosphere of carbon dioxide, was as great as toward normal gelatine; the growth, however, was less vigorous in the former case. In these preparations in which there was less turning toward the carbon dioxide gelatine, this gelatine had become evidently rather dry.

It is to be concluded, therefore, that the observed turning toward the edges of preparations is not due primarily to aerotropic sensibility. The experiments also negative the supposition that the observed repellent influence of growing hyphae may be due to the consumption of oxygen or <sup>to</sup> the production of carbon dioxide by the fungus, or to both.

#### Osmotropism:

Notwithstanding the fact that osmotropism is intimately associated with chemotropism, and that many of the tests for the latter are in equal measure tests for the former, direct tests were made by growing the fungi in media of higher osmotic pressure and of lower osmotic pressure than the test media, as well as in an isosmotic medium; glycerine, a good nutrient substance, and yet a substance reported by Miyoshi to be neutral in its chemotropic effect, was used to give the desired concentration to the culture media. The series failed to show that the concentration of the culture medium has an effect upon the amount of turning. No excessively high concentrations of mineral salts, however, were used.

#### Other Tropic Phenomena.

Under conditions that would favor a manifestation of geotrop-



ism and of thigmotropism, there was no indication that these are concerned in determining the direction of growth of the fungi. The effects of light and of heat were in no way tested, but they probably do not enter as factors.

#### Biological Significance.

The conclusions reached in these studies may be found to have a somewhat important bearing upon the biological problem of infection by parasitic fungi. In the absence of any experimental investigation, nothing definite can now be said. It would seem, however, that the drying of dew and other surface moisture in which spores had germinated, might be a favoring condition for the hydrotropic turning of the germ-tubes toward the stomates, especially if the cells within are over-distended with water, which has frequently been observed to be a condition favorable for infection; if the germ-tubes are numerous in the vicinity of a stomate, the repellent influence of these upon one another would cause some to seek the unoccupied region within the stomate. At all events the phenomenon of the entrance of germ-tubes, ~~At all events the phenomenon of the entrance of germ-tubes, whether~~ by stoma or through cuticle, is a complex one, many factors of which remain undetermined. That mere entrance is probably not due to specific peculiarities either of host or of parasite, is evidenced by the recent work of Miss Gibson which has been mentioned by Marshall Ward (1905). Miss Gibson found that spores of various members of the Uredineae sent their germ-tubes readily into the stomates of plants widely different from their hosts, and which they were unable to infect. Marshall Ward met with numerous instances of the same phenomenon. This would indicate that the entrance of a hypha into a stoma is merely a preliminary act,





distinct from infection proper, and controlled by general conditions while the fate of the hypha after its entrance is determined by complex reactions between parasite and host, which are largely specific in their nature. In the light, then, of known facts, no simple explanation such as the theory of chemotropism due to the presence of specific chemical compounds, is adequate. Chemotropism may possibly be one factor in the complex phenomenon, but it is certainly not the predominant factor.

#### CONCLUSIONS.

Various tests upon a number of fungi failed to indicate the existence of any definite chemotropic sensibility to nutrient substances or other chemical compounds in solution. If positive chemotropism exists, it is less prominent than other tropic phenomena involved, and was obscured by them.

Those substances which furnished nutriment to the fungi caused a decided growth, often with thickening of the hyphae and an increased branching; but they did not cause a more marked turning of the hyphae toward the diffusion centers than did non-nutrient and toxic substances.

All of the fungi tested show a tendency to turn from a region in which hyphae of the same kind are growing toward one destitute of hyphae, or in which the hyphae are less abundant. The turning toward a medium in which mycelium has grown, but from which the mycelium has been removed, is less marked than that toward a medium in which no mycelium has grown. This may be regarded as a negative reaction to stimuli from chemical substances which owe their origin in some way to the growing fungus.



Various fungi show a positive hydrotropism; but an overabundance of moisture may cause a negative reaction in certain fungi.

The changing of the direction of growth of fungous hyphae is a complex phenomenon in which at least two factors, cytotropism and hydrotropism, ~~and aerotropism~~, are concerned. Since the complete elimination of neither of these factors is possible, all tests must be relative, and to that extent unsatisfactory.

It would seem that the reactions of mycelium to various stimuli are not necessarily the same as the reactions under similar conditions of sporangiophores, gametophores, and other specialized parts.



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