T H E S I S

A STUDY OF SURFACE TENSION AS INFLUENCING SPORE GERMINATION.

Submitted by

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A Study of Surface Tension as Influencing Spore Germination

I N T R O D U C T I O N

The surface tension of the infection drop has been thought by some experimenters to play a part in governing the behavior of germinating fungous spores. Duggar (1) in 1901 made brief attempts to stimulate germination in spore cultures by changing the surface tension with oil. The results of his specific experiments were negative though several substances which he used successfully to influence germination are known to affect surface tension. Noble (2) in working with Urocystis tritici gives considerable attention to the possible effect of surface tension on the germination of spores of that fungus. By the use of sodium oleate and sodium ricinoleate he lowered the surface tension of the germination drop to 38 dynes but without apparently stimulating germination. Very dilute solutions of soaps, on the other hand produced marked effect. Sodium stearate, 1 part in 4,000,000 stimulated germination. Noble failed, however, to measure the tension of these solutions and makes no mention of their pH but suggests that the minute amounts of soap added ~~was~~ were not sufficient to materially influence the surface tension and that the stimulation is due to the soap

itself or the fatty acid radical.

Melhus and Durrell (3) obtained stimulation of germination of the spores of Puccinia coronata Corda. by the addition of petrolatum and by contact of the culture drop with neutral paraffin oil. The possibility of chemical influence here, as in the case of the soaps, is obviated and the writers state that the stimulus is apparently due to surface tension only.

In the present study not only thick-walled spores but those having very thin walls were used. The spores of several fungi were studied and conditions of germination were reduced to standard in an effort to eliminate all factors influencing germination except that of surface tension.

It is the purpose of the following discussion to study the influence of neutral paraffin oil on the germination of fungous spores and to consider if the action is due to surface tension effects or to other factors.

MATERIALS AND METHODS

Previous studies by Duggar, Noble and Melhus and Durrell have been with thick-walled spores. In the experiment at hand it was thought advisable to also use spores having walls of a different character. The following fungi were therefore used in the experiments under

discussion: Puccinia graminis tritici, Erysiphe graminis, Botrytis allii, Cephalothecium roseum, Aspergillus niger and Penicillium expansum, Basisporium gallarum.

The spores of these fungi were germinated in the following media: tomato juice, Pfeffer's solution, gelatine, tap water and distilled water. The tomato juice was prepared as follows: the ripe tomatoes were crushed filtered and the filtrate diluted with twice the volume of water and then fractionally sterilized. The Pfeffer's solution was prepared from the following formula given by Duggar (4), Calcium nitrate, four grams, potassium nitrate 1 gram, magnesium sulfate 1 gram, potassium chloride five-tenths gram, potassium dihydrogen phosphate 1 gram, ferric chloride a trace and three to seven liters of distilled water. The one percent gelatine was prepared from pure commercial flake gelatine and distilled water. The distilled water used for germination purposes was glass distilled to avoid any toxic action due to foreign matter. The tap water contained the following in traces - Silic acid, sulfuric acid, carbonic acid, calcium hypochlorite, sodium, potassium, magnesium and iron.

The various hydrogen-ion concentrations of the different media was determined electrically with a Wendt

apparatus and by colorimetric methods as described by Clark (5).

The germination of the spores of the various fungi was carried on in Syracuse dishes on the above mentioned media Fig. (1) , also in drops of the same media on slides in moist chambers. All cultures were kept in moist chambers at 20-23°C which was a general average for all fungi.

During the readings on surface tension a constant temperature of 20°C was maintained to obviate possible error due to the temperature factor. All glassware used in the experiments were thoroughly cleaned in "cleaning solution" and washed in distilled water.

Method of Determining Surface Tension.

Several methods of determining surface tension have been devised in years past. Some of the prominent ones have been: Searles tortion apparatus, Quincke's stalagnometer and the Standard Ring Method of T. H. MacDougal (6). In the present work a modification of the ring method as recently proposed by Durrell, Person and Rogers (7) was used. This method is as follows: a chainomatic balance Fig. (2) served as a modification of the standard ring method of determining surface tension. Wires were suspended from the beam ends and two copper rods were

Fig. 1. Desiccator used as moist chamber showing method of spore germination in Syracuse dishes.

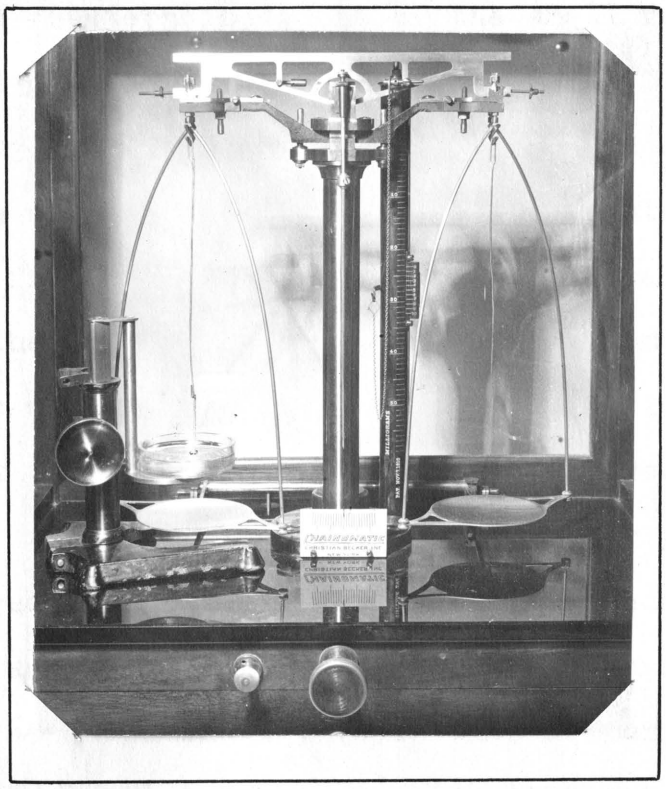

Fig. 2. Picture of "Chainomat" used for testing surface tension

attached to the lower ends of these. Both wires and rods were counterpoised. One of the small rods had the lower end ground to form a circular base of known size (Fig. (3) and was suspended from the left-hand wire. The other pin, used merely as a counter balance, was hung from the right-hand wire. The scale pans were left on the balance and an old dissecting microscope stand was used to carry the vessel of liquid to be tested Fig. (4). Where the surface tension of drops was to be measured, the drops were placed on a cover glass which was lowered into a glass container, two inches in depth and one inch in width and about one-fourth full of mercury, as shown in Fig. (3). This mercury provided a perfectly level surface on which to float the cover glass containing the drop of media to be tested. The depth of the vessel practically eliminated the possibility of any convexion currents influencing evaporation.

In using this device the balance beam is lowered and the vessel containing the liquid raised by the rack and pinion of the dissecting microscope so that the surface of the liquid comes in contact with the polished drop pin, of known diameter, hanging from the balance arm (the drop pin was cleaned between readings with ether). By adjustment of the rack and pinion, equilibrium is established with the pointer end at zero. The sliding

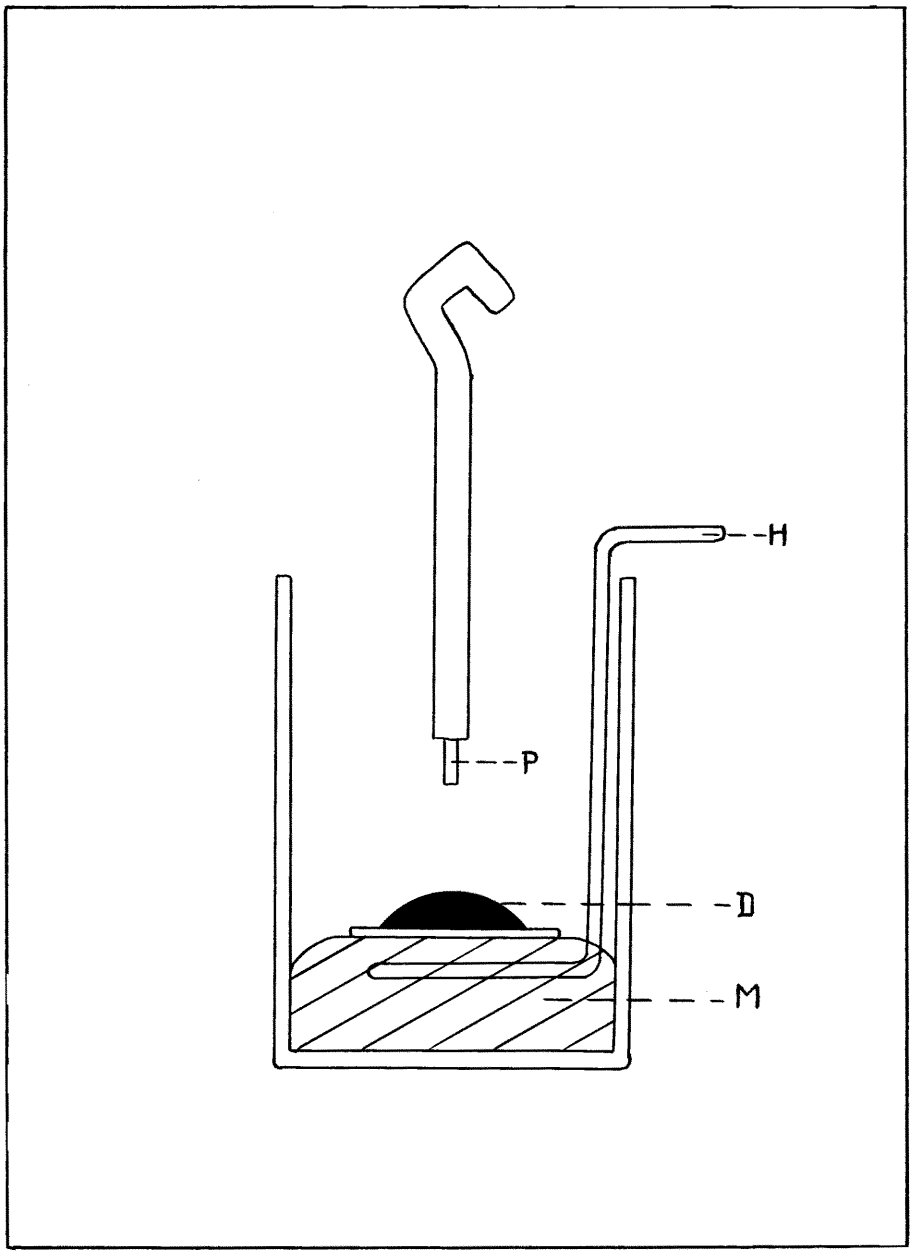

Fig. 3. Detail of apparatus for measuring tension in culture drop.

M - Mercury in small glass vial
D - Drop of medium on cover slip floating on mercury
H - Wire to remove cover slip for cleaning
P - Pin of known diameter to measure tension.

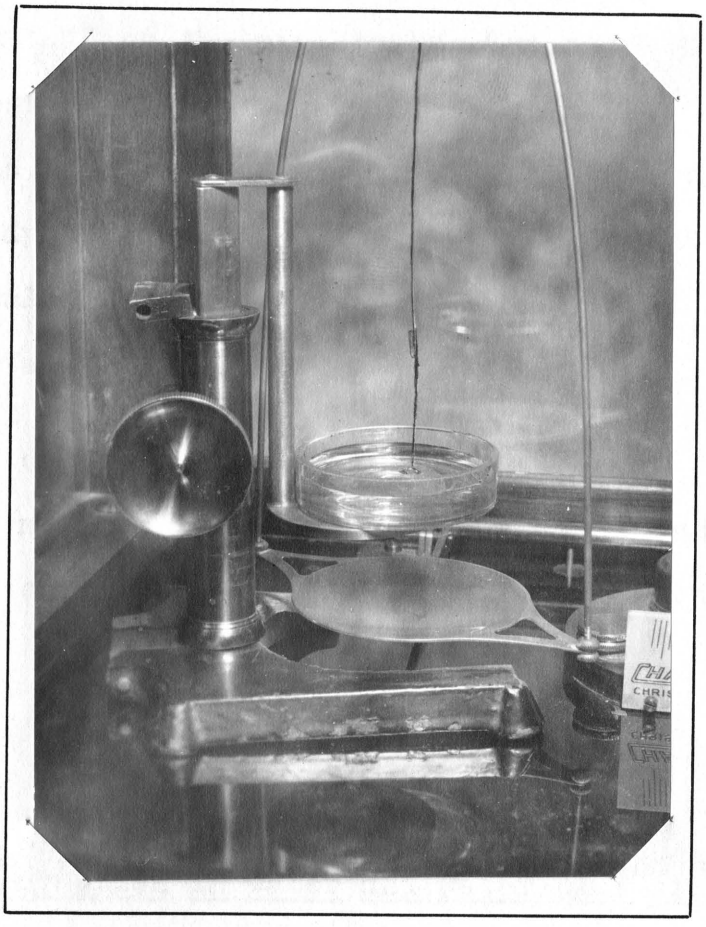

Fig. 4. Detail of old dissecting stand and dish used with "chainomat" in testing surface tension.

scale on the "chainomat" is then lowered slowly until the film holding the pin breaks. The reading of the scale indicates the weight necessary to break the surface film. From this and the known size of the drop pin, the surface in dynes may be calculated by the use of the following formula

$$\text{Surface tension} = \frac{981\ M.}{C}$$

981 dynes - one gram weight

M - mass in grams read on the sliding scale

C - circumference of the drop pin in contact with the film of liquid.

EXPERIMENTAL DATA

Preliminary experiments on surface tension and spore germination in water and water plus oil.

Preliminary experiments to gather further evidence that the surface tension of water is effected when oil is added were made by placing a ring of oil around a drop of water as shown in Fig. (8). Langmuir(8.) and Adam (9) state that under these conditions a film of oil is formed over the surface of the liquid and is one to two molecules in thickness. By the use of the apparatus described above, a number of readings were made and the surface tension in dynes was calculated for water, water

plus oil and oil alone. In Table I is presented data illustrating the effect of paraffin oil on the surface tension of water in drop culture at 20°C.

Table I. Surface Tension of Water Drops With and Without Oil.

Number of Readings	Pull in grams of water	Pull in grams of oil and water	Pull of oil alone.
1	.00119	.00888	.0074
2	.00122	.0089	.0072
3	.00125	.0085	.0078
4	.00127	.0084	.0071
5	.00119	.0087	.0073
6	.00126	.0085	.0076
7	.00124	.0088	.0073
8	.00122	.0089	.0080
9	.00127	.0085	.0072
10	.00119	.0084	.0070
Av.pull in grams	.0123	.00846	.00739
Surface tension in dynes	69	48.2	41.1

In the above table it may be noted that a tension of 69 dynes was obtained for water under the conditions of the experiment. This appears to be a fairly accurate reading and comparable to standard tests. Further, it will be seen that the tension of oil alone is but 41.1

dynes. Most interesting to observe in this connection is the tension on the water in contact with oil where a tension of 48.2 dynes is manifest. While the number of ~~the~~ tests in this experiment ~~are~~ not large the average difference in dynes under the three conditions ~~are~~ marked and it would seem that in the case of the tests of oil and water, the water is strongly influenced by the oil. In fact, the results for oil and water and oil alone are so alike that it appears that measurement is not being made of oil and water but of oil in each case - rather substantiating the statements of Langmuir (8), and Adam (9).

When oil was added to water in which spores were sown an increase in germination resulted. Dissimilar spores were used, Puccinia graminis uredospores comparatively thick-walled and Botrytis allii, thin-walled.

In the case of Puccinia graminis the percentage of germination was increased from 55 to 68 percent and a similar condition was evidenced in the case of Botrytis allii which was increased from 39 to 82 percent when oil was added. This correlates with the findings of Melhus and Durrell in the case of Puccinia coronata Corda.

The Optimum Condition for Spore Germination

In order to more fully verify the previously recorded

results with oil on spore cultures, it was thought advisable to consider other factors envolved which might effect the results in studying the effect of oil and surface tension. Such factors as acidity of media, viscocity, osmotic pressure and specific gravity were considered and an attempt made to reduce these to constancy.

Acidity.

Various workers have shown that acidity of media had a marked effect on spore germination. In order to standardize the media where-in spores were to be germinated, it was thought necessary to maintain a constant hydrogen-ion concentration of the culture media.

Series ranging from pH 2 to 8.5 were made of the various media in which spores were germinated so as to locate the optimum to be used in subsequent tests. The results of these tests are given in Table II.

Table II. Percent of Spore Germination of Different Fungi on Different Media.

Puccinia graminis tritici

	Hydrogen-ion Concentration												
Media	2.	3.	3.5	4.	4.5	5.	5.5	6.	6.5	7.	7.5	8.5	
Tomato	2	18	28	34	41	45	51	46	30	10	2	0	
Pfeffer's	5	61	64	60	65	50	30	58	31	20	7	5	
Gelatine	5	25	28	31	35	51	2	21	20	6	5	0	

Table II (continued)

Erysiphe graminis												
Tomato	0	0	3	5	8	12	15	13	4	2	0	0
Pfeffer's	0	0	0	0	0	2	3	5	6	7	6	0
Gelatine	0	0	3	5	7	5	5	3	3	0	0	0
Botrytis allii												
Tomato	30	35	67	70	78	80	78	60	54	40	43	31
Pfeffer's	0	3	5	11	15	15	8	5	6	4	0	0
Gelatine	0	4	8	30	36	2		18	7	0	0	0
Cephlothecum roseum												
Tomato	0	0	30	47	52	56	58	50	42	31	28	25
Pfeffer's	0	0	0	0	5	7	8	2	1	0	0	0
Gelatine	0	10	21	30	31	28	6	5	0	0	0	0
Basisporium gallarum												
Tomato	0	31	32	40	42	44	31	28	30	20	21	10
Pfeffer's	0	0	3	5	12	21	18	5	8	0	0	0
Gelatine	2	4	11	17	18	12	6	2	1	0	0	0

In the above table, the results with spores of Puccinia graminis, Erysiphe graminis, Botrytis allii, Cephlothecum roseum and Basisporium gallarum, while varying with different media show quite constant optimum between 4.5 and 5.5 pH, graphically expressed in Fig. (5). It was therefore decided to use a pH of 5 throughout the experiments thereby reducing this factor to constancy.

With all spores, except Puccinia graminis, the more colloidal media as tomato juice and gel gave constantly higher germination than the more liquid media as Pfeffer's. As the pH of these media was known some other factor as viscocity, osmotic pressure, specific gravity or the anions involved were thought to play a part.

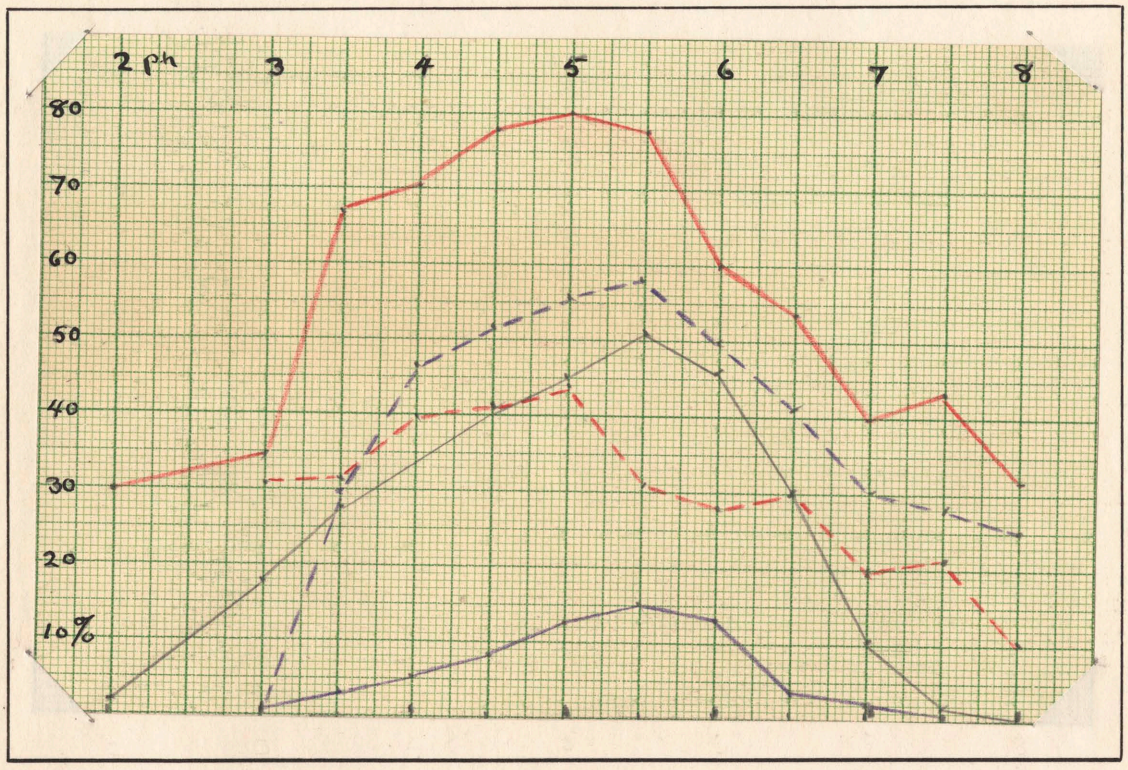

Fig. 5. Graph showing germination of fungus spores in tomato juice of various hydrogen-ion concentrations.

Puccinia graminis
Botrytis allii
Erysiphe graminis
Cephalothecium roseum
Basisporium gallarum

In order to eliminate these factors from consideration in our study of the effect of oil a brief study was made of them as recorded in the following paragraphs.

Effect of Different Acids on Percentage of Germination.

After determining the optimum pH for germination of the different fungi under consideration, the behavior of different acids is of interest.

Dunn (10) found that certain acids affected the germination of spores of Sclerotinia cinera and that different acids behaved differently in regard to spore germination.

Lille (11) further emphasizes this idea by showing that the organic acids have greater penetration than the inorganic acids.

In order to determine the effect of different acids upon the germination of the spores of Basisporium gallarum and Cephalothecium roseum they were germinated in media acidified with the inorganic acids, HCl, HNO_3, H_2SO_4, and with the organic acids, formic, acetic, malic, oxalic, succinic, tartaric, lactic and citric, all of which were made up to the optimum hydrogen-ion concentration of 5 pH in water and also in 1 percent of gelatine.

In Table III will be found the results of these tests.

Table III. Germination of Spores of *Basisporium gallarum* and *Cephalothecium roseum* in organic and inorganic acids of different hydrogen-ion concentrations.

Spores	pH						
	5.5	5.	4.5	4.	3.6	3.2	Acid
Basisporium gallarum	1	13	15	8	2	0	HCL
	3	18	21	15	5	0	HNO_3
	7	12	13	1	0	0	H_2SO_4
Cephalothecium roseum	2	33	49	15	0	0	HCl
	6	42	35	10	0	0	HNO_3
	15	46	65	53	1	0	H_2SO_4
Basisporium gallarum	1	2.5	2	1	0	0	Oxalic
"	1	3.	4	3	2	0	Succinic
"	1	1.5	3	2	1	0	Lactic
"	2	12.	7	2	1	0	Malic
"	1.5	3	4	4	2	0	Tartaric
"	1	6	4.5	1	.5	0	Salcylic
"	3	8	5	4	2	1	Citric
Cephalothecium roseum	22	42	20	7	0	0	Oxalic
"	21	48	53	11	0	0	Succinic
"	17	43	31	2	0	0	Lactic
"	27	38	11	2	0	0	Malic
"	2	66	34	27	6	0	Tartaric
"	5	18	2	0	0	0	Salcylic
"	5	6	5	3	0	0	Citric.

With the use of a number of acids at constant pH better germination was obtained with the thin-walled spore, *Cephalothecium roseum* in the time allowed for germination

than in the case of thick-walled spore, Basisporium gallarum. It is also shown in Table III that no striking differences exist between the results with organic and inorganic acids under the conditions of the experiment.

Some of the acids used as for instance, tartaric show a greater effect on permeability of the spore wall of Cephalothecium roseum. Likewise, malic and succinic apparently increase permeability and subsequent germination of the same fungus spores.

Hydrochloric acid, with the same fungus, gave lower germination than tartaric, malic and succinic. These acids are the chief ones present in tomato juice and their presence may account for the greater germination generally manifest in that medium as shown in Table II.

It is realized that several descrepencies exist in the above data but time was not available and it is beyond the scope of the present discussion to analyze the effect of these molecules or ions of the acids involved or the effect of the buffers present in the media. It seems sufficient for the present discussion to call attention to the optimum germination of the spores ~~under~~ at a pH of 4.5 to 5.5 with the various acids used. In the tests on the effect of oil on the spore culture this factor was kept constant.

Reaction of Spores to Different Dilutions of Culture Media.

In previous experiments with germinating spores it was repeatedly noted that in general, better germination was obtained in colloidal media than in water or solutions.

This involved a consideration of the viscocity and specific gravity of the media. Tests made on viscocity of Pfeffer's solution and on tomato juice and 3-percent of gelatine at different dilutions showed slight difference, at least not in accord with the differences in germination in the same media. The specific gravity of the media used in tests with oil was also measured by means of the volume weight method, with the result that little difference was found in the various dilutions ofmedia. Those slight differences that were manifest seemed out of all proportion to the resulting germination.

Osmotic pressure of the media determined by means of a Beckmann thermometer yielded results showing no outstanding difference in the media used.

It might be stated at this point that tests on the pH of the various dilutions of the tomato juice and gelatine media showed them to be practically identical in hydrogen-ion concentration.

Nothwithstanding the similarity of the media used in respect to the above mentioned factors, a marked

difference in the germination of spores was manifest as shown by the following results.

Tests were made using spores of Aspergillus niger, Penicillium expansum, Cephalothecium roseum, Botrytis allii and Erysiphe graminis in different concentrations of tomato juice as follows: 33%, 16%, 8%, 4%, 2%, 1% and H_2O. The results of this test are shown in Table IV and more graphically in Fig. (6).

Table IV. Percent Germination in Different Concentrations of Tomato Juice.

Organisms	Percent Tomato Juice						
	33	16	8	4	2	1	H_2O
Botrytis allii	95	86	78	60	49	34	20
Cephalothecium roseum	85	68	74	65	78	58	27
Aspergillus niger	90	90	56	54	18	14	10
Penicellium expansum	83	81	69	41	39	22	9

It was found as shown above, that the maximum of germination occurred at the highest concentration of the medium and that a decrease in germination occurred as the dilution approached water.

This work was then supplemented by similar tests with gelatine. Starting with a 10-percent concentration the gelatine was diluted to 5, 2½%, 1¼%, 5/8 and 5/16%.

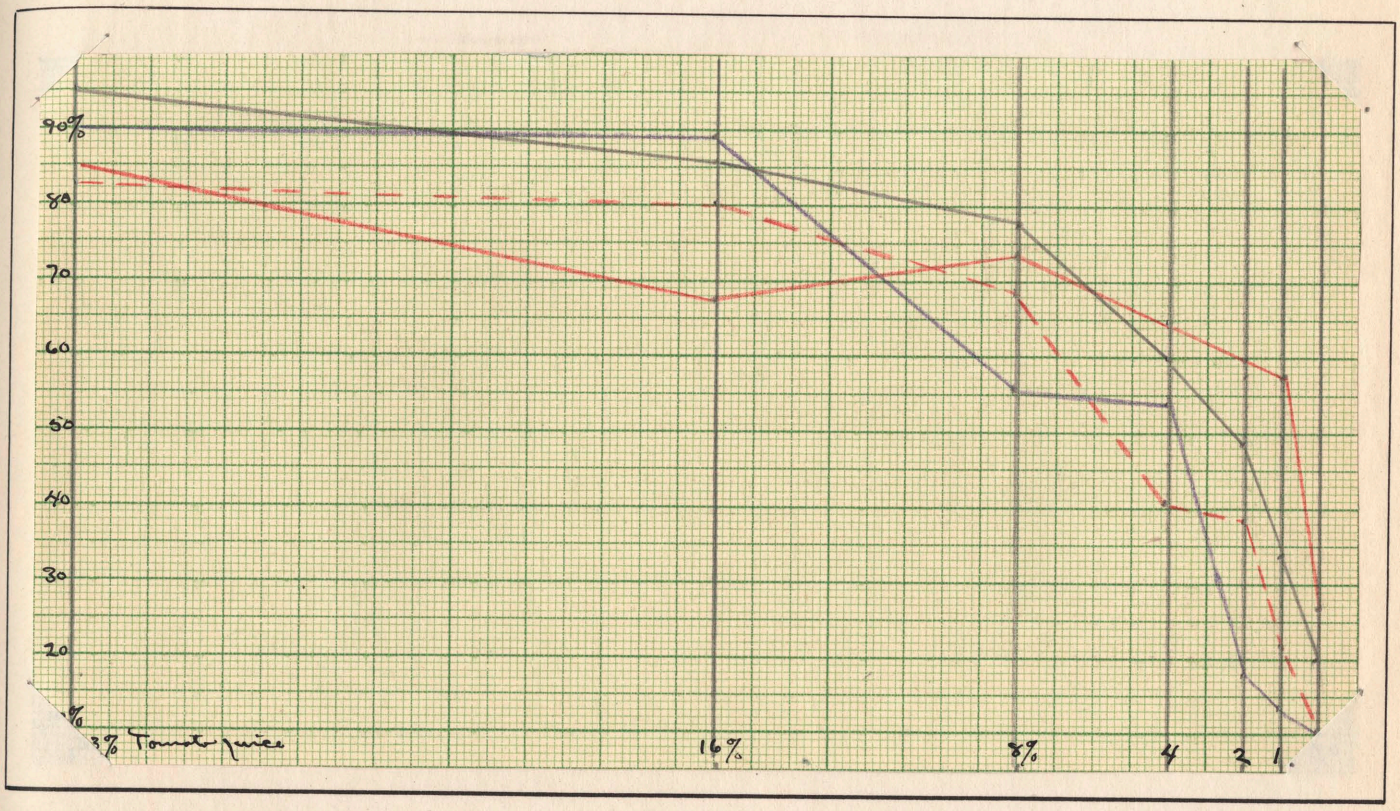

Fig. 6. Graph showing germination of spores in dilutions of tomato juice.

Botrytis allii
Cephalothecium roseum
Aspergilli niger
Penicillium expansum

That the results were quite similar to those of different dilutions in tomato juice are shown by Table V. and Fig. (7).

Table V. Percent Germination in Different Concentrations of Gelatine.

Organism	Percent Gelatine						
	10	5	2½	1¼	5/4	5/16	H_2O
Botrytis allii	92	82	39	27	24	15	22
Cephalothecium roseum	92	90	70	35	29	23	20
Aspergillus niger	93	89	80	80	20	10	5
Penicillium expansum	93	90	80	70	18	10	10

In the above tables and accompanying graphs, a marked decrease in germination is shown in the more dilute media. In the case of gelatine, solutions containing from 5 to 10 percent gelatine solidify and on these solid media the germination is high. Solutions containing less than 5 percent of gelatine are liquid and show a sudden decrease in germination. Similar results are shown in the case of the more dilute solutions of tomato juice.

Submergence of Spores.

The behavior of these germinating spores in various dilutions of media, as above described, where other factors

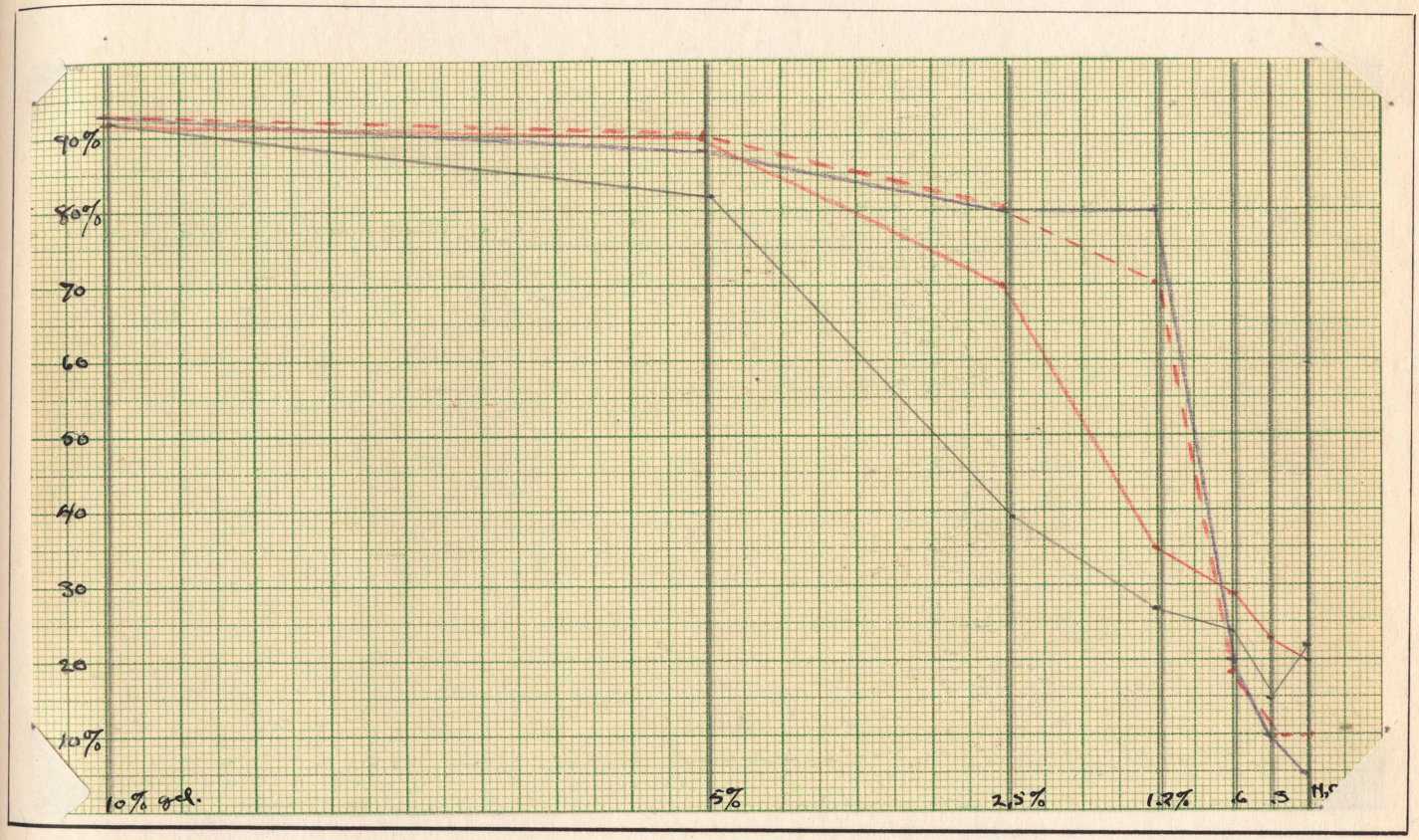

Fig. 7. Graph showing germination of spores in dilutions of gelatine.

Botrytis allii
Cephalothecium roseum
Aspergillus niger
Penicillium expansum.

that might influence germination were maintained constant point to a factor of wetting as playing a part in germination.

In order to test such a hypothesis, spores of Botrytis allii were submerged by shaking and by the use of the centrifuge in different concentrations of tomato juice and then germinated in drops of the same media. The results of this test are shown in the following table.

Table VI. Germination of Submerged and Floating Spores of Botrytis allii.

Dilution of Tomato juice	Percent germination of submerged spores	Percent germination of spores not submerged.
1/3	72	85.7
1/6	60	76
1/12	51	72
1/24	43	54.4
1/48	45	46.7
1/96	37	39
H_2O	30	35

From the above table it is readily observed that spores which are submerged in the more solid media give a lower percentage of germination than those sown on the surface of the medium. On first consideration this would appear to be due to better facilities for respiration; in other words, more favorable oxygen supply.

Effect of Different Supply of Oxygen on Spore Germination

Tests were therefore made as follows: spores of Cephalothecium roseum, Botrytis allii, Aspergillus niger, and Penicillium expansum were placed in water and on agar agar medium. The spores in their respective containers were placed in a Novy jar and the oxygen supply was controlled by exhausting the air from the culture chambers and admitting known amounts (measured with gas burettes) of oxygen from an oxygen tank. The amounts of 21, 31 and 36-percent oxygen and oxygen under pressure (100% plus) were furnished the cultures. Spore cultures were also placed in a container with pyrogallic acid and sodium hydroxide which acted as a reducing agent and withdrew the oxygen. The results of these tests are given in Table VII.

Table VII. Percent Germination of the Different Spores Subjected to Various Amounts of Oxygen.

		Percent germination in				
Spores	Culture Medium	31% oxygen	36% oxygen	Under pressure	In air 21%	No oxygen
Aspergillus	Water	20	15	18	17	0
Penicillium	"	17	14	21	16	0
Cephalothecium	"	21	16	20	18	0
	Agar Agar					
Aspergillus		64	57	59	78	0
Penicillium	"	47	43	46	52	0
Cephalothecium	"	58	62	60	54	0
Botrytis	"	87	85	88	91	0

In the above table little difference in germination is manifest in the various atmospheres of oxygen. Whether in normal air in atmospheres containing 21 percent oxygen, in 31 percent or in 36 per cent oxygen. Even pure oxygen under pressure failed to show appreciable difference. While in total absence of oxygen no germination resulted.

While the reduction of oxygen supply stops germination as shown in the above experiment oxygen in excess of the normal amount in the air on the otherhand fails to increase germination.

It would appear from a consideration of the foregoing germination studies that the amount of oxygen in the air, or even 10 percent or half that amount is ample for germination but lesser amounts are insufficient.

The solubility of oxygen in water is 4 percent at 20°C atmospheric pressure. Submerged spores under this condition therefore might seem not to get sufficient oxygen. In Table VI however, it may be seen that notwithstanding this possibility little difference in germination is manifest between submerged and floating spores in the watery medium. In this same table, however, it may be also seen that in the more watery medium a low percent of germination is obtained while in the more solid media high percent

germination was obtained. This fact is further illustrated with other fungi in Table VII, and increased oxygen supply does not correct the behavior of the spores in a liquid media.

The inference from this data points to "wetting" as a factor in spore germination. In other words, the amount of water furnished the spore or the ease and speed of its imbibition is a limiting factor in spore germination.

Imbibition Rates in Spore Culture Media.

From a general knowledge of the physiology of the cell and the spore in particular, it seems safe to consider the spores of the various fungi used in the present experiments as small packages of colloidal substances. MacDougal (12) and Free (13) have shown the rate of imbibition of colloidal masses by use of gelatine cubes. Using the methods of these workers and gelatine blocks in place of spores, the rates of imbibition of such blocks were tested in tomato juice of the various dilutions used in the foregoing germination tests. The gelatine blocks were cut about 14 m.m. wide and 4 m.m. thick and then dried to constant weight. They were then immersed in tomato juice diluted to 33 percent, 16, 8, 4, 2 and 1 percent for a period of 15 hours after which they were

removed, the excess moisture wiped off and then weighed. The percent of moisture imbibed is summarized in the following table.

Table VIII. Imbibition of Water by Gelatine Blocks in various Concentrations of Tomato Juice.

Culture Media	Number of Disks Used.	Percent of Water Imbibed.
33% tomato juice	36	830
16% " "	"	850
8% " "	"	893
4% " "	"	923
2% " "	"	935
1% " "	"	976
H_2O " "	"	1000

As evidenced in the above table, there is manifestly a greater imbibition by the colloidal blocks in dilute media than in the more concentrated tomato juice. In considering this, the question resolves itself into a tug of war between the colloid ~~and~~ of the block and the colloid of the juice for the water present.

Putting this on a basis of spore imbibition it seems justifiable to consider the spores in the same light as the colloidal blocks and from this data it is evident that the spore in water can imbibe more water than where some competing colloid is present as in agar, gelatine or tomato juice.

Foregoing data on germination in these media show that the spores germinate the highest percent in the colloidal media which suggests that there exists an optimum rate of imbibition approached, not in water alone, where ample water is available, but in the colloidal media where some of the water is held back by the colloid.

That this is the case is further indicated by the fact that spores may be germinated profusely on dry glass plates in a saturated air or on dry celloidin plates in saturated air. Under these conditions the spore only receives water slowly from the saturated air or from a film of condensed water which may be seen surrounding the spore.

Where spores of Puccinia graminis are immersed in water, rapid imbibition takes place. Apparently the spore wall takes in water at a different rate than the cell contents for such immersed spores may be seen to have a wide space between the cell wall and the cell contents after immersion. The cell, under these conditions, imbibes faster than the protoplasm within, and the wall and protoplasm no longer make contact and such immersed spores never germinate. This seems to further

substantiate the theory of imbibition rate as a factor in spore germination where water alone is considered.

The Effect of Oil on Drop Cultures.

From the above discussion, it seems probably that the function of oil on drop cultures Fig. (8) in some way retards the imbibition of water by the spore, approaching thereby the more optimum rate of imbibition and thus increasing percent germination.

Further, tests on the germination of fungal spores were made as follows: Spores of Puccinis graminis tritici, Botrytis allii were germinated in drops of tap water, and in one percent gelatine and in the same media to which oil had been added.

Table IX. will serve to show the reaction in this case.

Table IX. The Reaction of Spores on Media To which Oil was Added.

	No. of cultures	Percent germination. P. graminis tritici.	Botrytis allii
Tap water	20	60	60
" " and oil	20	70	85
Distilled water	40	52	32
Distilled water and oil	40	65	57
1% gelatine	20	45	40
1% " and oil	20	46	30

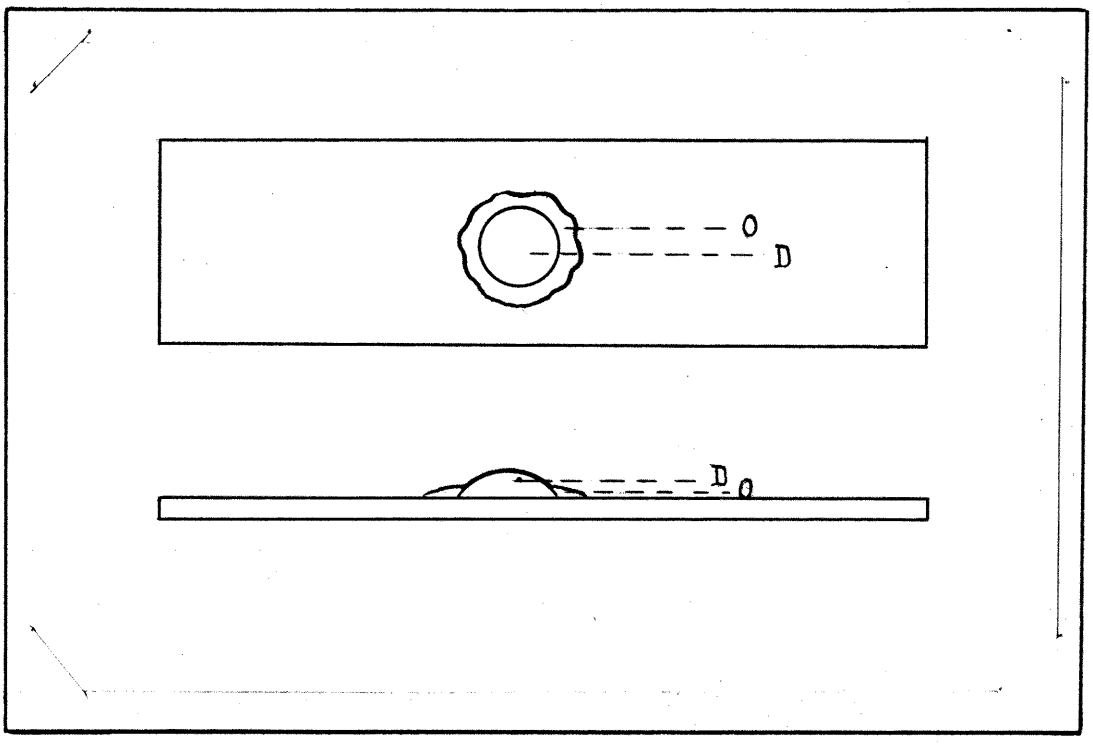

Fig. 8. Diagram of culture drop on slide with oil surrounding it.

O - Neutral paraffin oil
D - Water drop.

From the above table it is evident that small amounts of oil in contact with the culture drop increase spore germination where the medium is liquid. No increase is manifest where oil is added to the colloidal gelatine.

The preceding experiments indicate that excess of water in the culture media is detrimental to spore germination. Evidence further points to a similar retarding action of oil on imbibition.

The question arises as to how this oil acts in its retarding effect. Longmuir (8) and Adam (9) claim that oil in contact with water in such small amounts as used in our germination tests forms a film over the surface one molecule thick. If this be true, such a film would partially insulate the spore from the water drop.

In order to demonstrate that this film exists 1000 readings were made as shown in the following table using the above described method:

Table X. Surface Tension of Culture Media in Dynes.

	In Syracuse Dish			In Drop Culture		
	Medium	Oil	Medium and oil	Medium	Oil	Medium and oil
Water	71.7	39.6	43.5	74.	40.2	44.0
Pfeffer's	70.3	39.6	42.0	69.5	40.2	44.4
1/3 tomato juice	68.	39.6	42.0	69.2	40.2	45.0
1% gelatine	70.7	39.6	62.4	70.3	40.2	61.0

In Table X. the tension in the various media is very nearly alike, in fact within the limits of error. The tension in the drops is slightly different than in the level surface of the dish (this is perhaps due to the curvature of the surface of the drop).

The similarity of results on the different culture media are at variance with the differences obtained in germination tests on the same media as shown in foregoing table. If surface tension, as such, is a factor in germination, this would hardly be the case.

The most striking comparison manifest in Table X is the comparison in tension between oil alone and medium with oil in contact. In the Syracuse dishes oil has a tension of 39.6 dynes while water, Pfeffer's solution and tomato juice in contact with oil have a tension of only 43.5 dynes, in one case and 42 dynes in the other. A similar comparison is apparent in the

drops. This indicates that the tests of tension on media in contact with oil, measure practically the tension of the oil alone and not the underlying media, proving the theory that a film of oil covers the surface of the medium.

Such a film would insulate to a degree at least, the spore floating on the medium. Visual observations on the spore on the culture drop by means of the microscope and light arrangement shown in Fig. (9) indicate that spores dusted on a drop covered by a film of oil do not sink as deep as those dusted on water alone. Less surface of the spore is therefore wetted and the imbibition reduced.

In Fig. (10) is shown a diagram of the behavior of a fungus spore on a drop in presence and absence of oil.

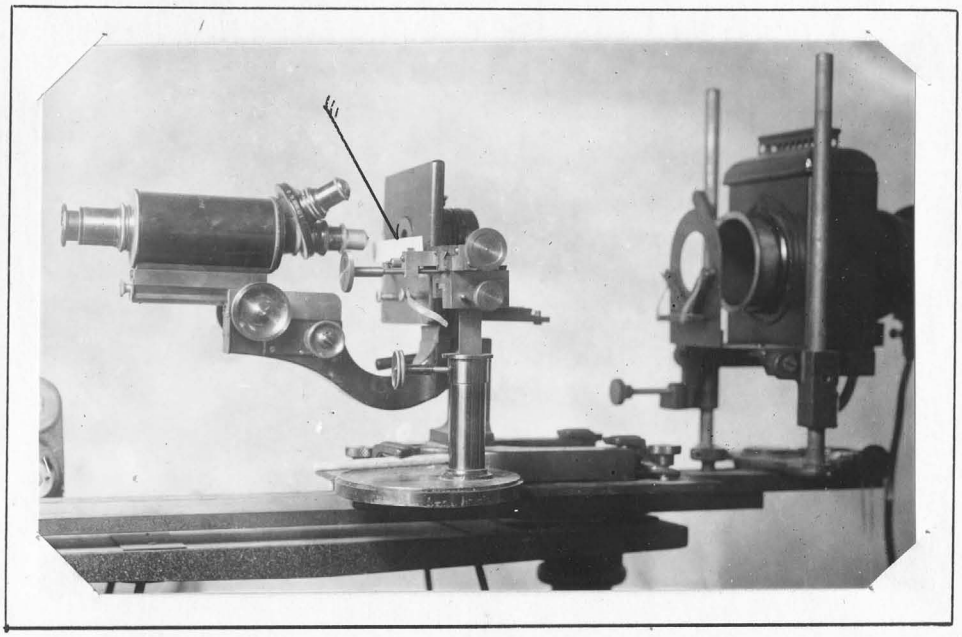

Fig. 9. Arrangement of microscope and light for observing spores floating on drop. Arrow indicates location of drop on edge of glass slide held in Barber apparatus 25 B. & L periplane eyepiece and 1/8 long focus objective.

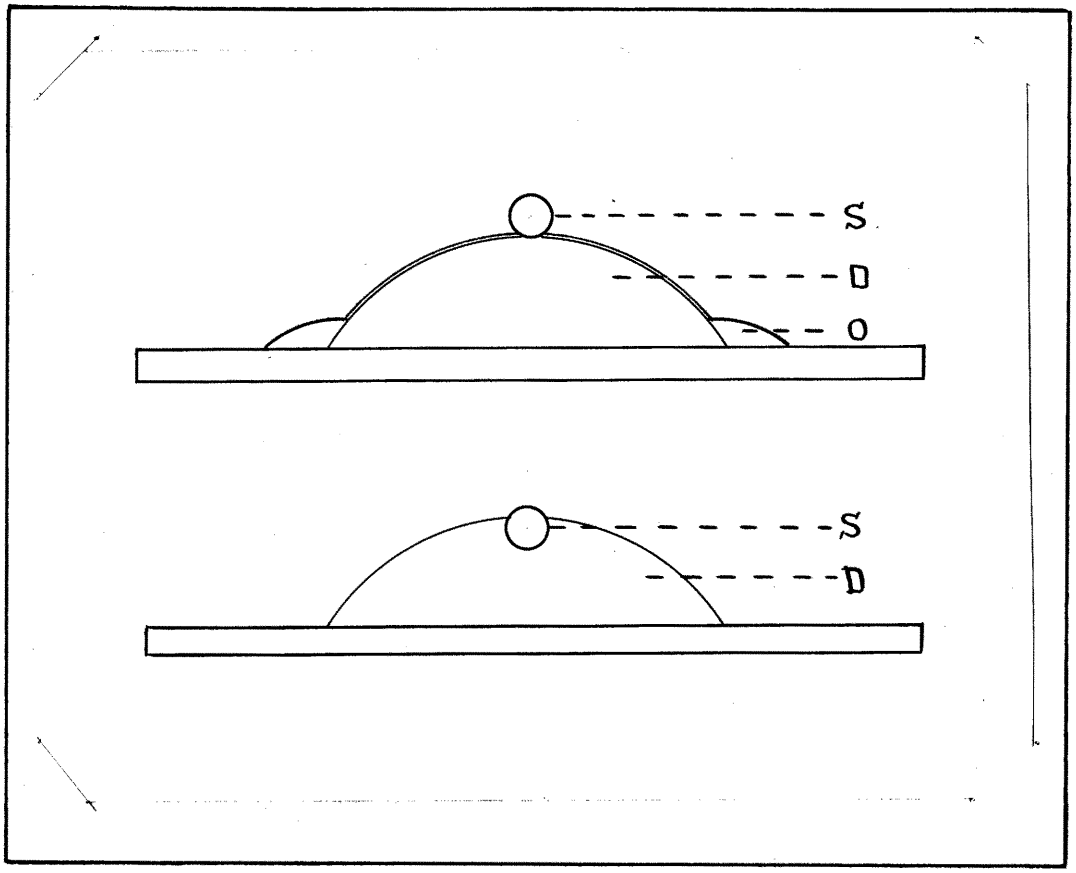

Fig. 10. Diagram showing position of spore on culture drop when dusted on drop and when dusted on oiled drop.

S - Spore
D - Water drop
O - Neutral paraffin oil.

CONCLUSIONS

From the foregoing observations and measurements it seems logical to conclude that the increased germination of certain fungus spores obtained in drop cultures in contact with neutral paraffin oil is due not to change in surface tension as might be expected but to the separation of the spore from the medium.

Experiments on media of different composition indicate that those of a colloidal nature are more favorable to germination than water, suggesting that available water or rate of imbibition influences germination of spores.

The mechanism of the action of oil on drop cultures therefore, appears to be similar to that of the colloidal medium in retarding water intake of the spore to a point of optimum imbibition by insulating it to some degree from the water.

SUMMARY

Preliminary experiments with paraffin oil on drop cultures of fungus spores show not only a reduced surface tension but markedly increased germination of the spores. Several factors suggested themselves as the cause for this difference in percent germination, foremost of which was difference in surface tension.

In order to eliminate other factors and study the possible effect of surface tension, the acidity of the media used, expressed in pH, the viscocity, osmotic pressure and specific gravity were determined and found constant within reasonable limits.

The behavior of the fungus spore studied, on colloidal media and on water, showed higher percent germination in the colloidal media.

Submergence of spores in water were found to be definitely detrimental to their germination. That this was not an oxygen relation was indicated by study of the germination in atmospheres of various oxygen content and by observations on the mechanical behavior of the submerged spore. It was found that the wall of the submerged spores of Puccinia graminis swelled more rapidly than the cell contents causing a pulling away of the wall from the protoplasm within. This suggests wetting or rate of water intake as a factor in spore germination, for floating spores do not exhibit this phenomenon.

By use of dried gelatine blocks the imbibition rate was found to be greater in water and dilute colloidal media than in more concentrated colloidal media. That spores act the same way in the same media is apparent

from germination tests and indicates that water supply is withheld from the spores by the colloidal medium.

Correlation of these studies indicate that a spore can get more water than is favorable for germination and that there exists an optimum point of moisture necessary for the germination of the fungal spores studied.

Tests of the surface tension of media in contact with oil show that a definite film of oil is present over the surface of the media. Such a film insulates the spore from the water supply as may be seen by horizontal microscope observation, of the spore on the culture drop.

Considering that spores are found to be sensitive to water supply and their germination inhibited by excess water and favored with an optimum supply it seems safe to conclude that the oil over the culture drop effects spore germination not by changing the surface tension but by producing optimum moisture conditions.

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