

BOTANICAL GAZETTE

DECEMBER 1898

THE EFFECT OF AQUEOUS SOLUTIONS UPON THE GERMINATION OF FUNGUS SPORES.¹

F. L. STEVENS.

OBJECT.

THE primary object in the work here related was to establish with some degree of accuracy the strengths of various solutions which are necessary to prevent the growth of fungus spores.

The bearing of this question upon the relation of a fungicide to its efficiency is apparent, and from the wide use of these compounds it seems important that all knowledge possible should be gained regarding the principles underlying their action.

Incidentally, new evidence bearing upon the theory of the hydrolytic dissociation of the molecule is adduced; also facts which may throw some light upon the structure of the cell wall

PRELIMINARY.

Before work bearing results could be practically undertaken, it became necessary to select the best manner of working, and the materials to use, and to become familiar with the sources of error most common.

¹ Contributions from the Hull Botanical Laboratory. XI.

After various means of culture had been tried, the van Tieghem hanging drop proved the most satisfactory.

In the selection of material such fungi were chosen as could be obtained in abundance, could be easily kept in stock culture, were not liable to become seriously impure, and, of most importance, such as would uniformly grow in the kind of culture medium used.

Botrytis vulgaris Fr., *Macrosporium* (*sp.* ?) from the fruit of *Datura Tatula*, *Glaeosporium Musarum* C. & M., and *Uromyces caryophyllinus* (Schrank) Schr., were selected. *Penicillium crustaceum* (Linn.) Fries was useful in tube cultures.

Many other fungi were tried but presented disadvantages which prohibited their use.

Several hundred cultures were made to ascertain whether there was any toxic effect from the cells, cement, or oil used to seal the cell, and to note whether or not nourishing materials were useful in the solution.

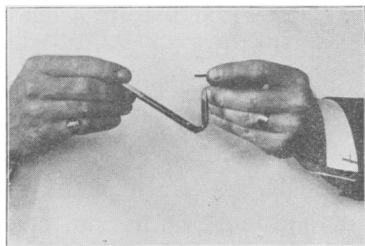
METHODS.

The cultures were made in hanging drop in van Tieghem cells used in the usual manner with vaseline as an adhesive. It was not found necessary to place them in a moist chamber, as they did not dry if carefully sealed. Cultures were uniformly examined about twenty-four hours after their preparation and every time that a series of cultures was made, a series of check cultures in distilled water was prepared. In every case where the checks failed to grow, all negative results were discarded. The cells themselves were frequently thoroughly cleansed and the cover glasses were carefully wiped each time before using washed in alcohol and wiped again.

In order to facilitate the making of cultures a tube was bent as shown in the figure on opposite page. By slightly raising the long end the liquid rises in the short end and a hanging drop of any desired size is readily placed on the cover glass.

This tube was washed, then rinsed with the solution under observation, then filled with that solution. This and all other

tubes and bottles used were, of course, very thoroughly washed before introducing another chemical or one of different strength. The drop being upon the cover glass, a clean teasing needle or platinum needle was touched to the stock culture, and then to the hanging drop. Before using this needle with another chemical it was washed. Sterilization was nowhere necessary, as the culture was to be of such short duration that bacteria or other fungi did not develop.



The cultures of *Penicillium crustaceum* (Linn.) Fries were made otherwise. A small piece of bread $2 \times 1 \times 1$ cm was soaked in the solution under experimentation, then placed in a 15 cm test tube where it was about three quarters immersed in the solution. It was then inoculated with the *Penicillium*, evaporation being prevented by a stopper to the tube.

Readings were made with this fungus when the checks had begun to grow well.

In order to insure that a proper planting had been made, and to be sure that no spores had been germinated previous to being placed in the solution, each slide was microscopically examined before placing away.

The series of cultures being made, the slides bearing them were placed away in a thermostat on a metallic slide holder.

SOLUTIONS.

Inasmuch as the object of the work was to investigate the effect of the salts upon the growth of the fungus, it was deemed advisable to prepare solutions the composition of which should be based upon their molecular weight rather than upon a percentage basis. To do this the molecular weight of the salt, base, or acid was estimated, and this weight taken in grams was dissolved in one liter of water. Such a solution is designated throughout this article as a *normal solution*. Thus, potassium

hydrate has the formula KOH. K, O and H have respectively the atomic weights 38.85, 15.88 and 1. Therefore the molecular weight of KOH is 55.73. Then 55.73^{gm} of potassium hydrate dissolved in one liter of water constitutes a normal solution of potassium hydrate.

It will be readily seen then that, the work being accurately done and the molecular formulæ being correct, a normal solution of any substance will contain as many molecules per cubic centimeter as a normal solution of any other substance.

In a few cases solutions of greater than normal strength were used, but generally, if the substance did not prevent growth when in one-tenth of normal strength, the substance was considered non-poisonous and was not experimented with further.

Starting with a normal solution, dilutions were easily made till such strength was reached that the spores would germinate. The various strengths used are designated as fractions of normal. Thus $\frac{n}{800}$ signifies a solution of one eight-hundredth normal, a strength which could be prepared by taking one cubic centimeter of normal solution and diluting it to 800^{cc}. Ten cubic centimeters of $\frac{n}{800}$ diluted to 80^{cc} would furnish $\frac{n}{640}$, etc.

The atomic weights used are those given by Roscoe and Schorlemmer.²

TABULATION AND DISCUSSION.

In tables I–XVI appears a record of the cultures made, more than 1500 in all, each culture bearing from fifty to a few thousand spores. The fraction shows the strength of solution used, and the figure to the left of the fraction shows the number of times this strength was tried. If the fungus grew, the strength in which it grew appears in the corresponding column. Those strengths in which it failed are to be found in the column headed Failed. Reference to a footnote indicates some peculiarity regarding the culture, which will be found explained in the note. The numbers used in these footnotes are original numbers of the cultures.

² Treatise on Chemistry 1 : 52. 1895 [ed. 3].

TABLE I.
MERCURIC CHLORID.

Botrytis		Macrosporium		Penicillium		Uromyces	
Grew	Failed	Grew	Failed	Grew	Failed	Grew	Failed
1 $\frac{n}{51200}$ *	4 $\frac{n}{12800}$	3 $\frac{n}{819200}$	1 $\frac{n}{819200}$	2 $\frac{n}{25600}$	2 $\frac{n}{3200}$	2 $\frac{n}{204800}$	6 $\frac{n}{6400}$
3 $\frac{n}{102400}$	5 $\frac{n}{25600}$	5 $\frac{n}{409600}$ †	3 $\frac{n}{409600}$	2 $\frac{n}{12800}$		1 $\frac{n}{51200}$	
8 $\frac{n}{204800}$	2 $\frac{n}{51200}$	4 $\frac{n}{204800}$ †	6 $\frac{n}{204800}$	2 $\frac{n}{6400}$		3 $\frac{n}{25600}$	
	1 $\frac{n}{102400}$	1 $\frac{n}{102400}$	3 $\frac{n}{102400}$	6 $\frac{n}{3200}$ §		4 $\frac{n}{12800}$ ¶	
			4 $\frac{n}{51200}$				
			4 $\frac{n}{25600}$				

* No. 436. Growth scattered.

† No. 157. Four cultures normal and one slight but uniform.

‡ No. 156. One culture, slight but uniform.

§ No. 927. Slight growth; was dry.

¶ No. 200. Scattered growth.

Mercuric chlorid.—Thus we see that *Botrytis* grew in $\frac{n}{204800}$ in eight different cultures, and there is no evidence that this strength injures it. In $\frac{n}{102400}$ three cultures grew normally and one failed to grow. The growth here may be considered as normal. In $\frac{n}{51200}$, twice as strong as the last, growth was poor in one culture, two failed utterly, and the strength necessary to prevent germination is evidently reached. Five cultures of twice this strength or $\frac{n}{25600}$ were tried, but all failed to germinate.

So it can be concluded that the strength which prevents most spores from growing is $\frac{n}{51200}$, and that $\frac{n}{25600}$ is a sure preventive. Then the range from sure prevention of growth to a normal growth is from $\frac{n}{25600}$ to $\frac{n}{102400}$.

With *Macrosporium* the growth was normal for $\frac{n}{819200}$, while $\frac{n}{409600}$ and $\frac{n}{204800}$ grew but gave evidence of injury, and $\frac{n}{51200}$ proved a sure preventive. With *Penicillium*, starting with

as weak as $\frac{n}{25600}$, the growth was normal, in as strong as $\frac{n}{3200}$ a killing strength was not reached.

Uromyces, from a generalization made in my notebook, grew normally in $\frac{n}{25600}$, not quite as well in $\frac{n}{12800}$, and is prevented by $\frac{n}{6400}$ except in very rare cases, where a single spore grows.

TABLE II.
POTASSIUM CYANID.

Glœosporium		Macrosporium		Penicillium		Uromyces	
Grew	Failed	Grew	Failed	Grew	Failed	Grew	Failed
2 $\frac{n}{6400}$	I $\frac{n}{800}$	2 $\frac{n}{25600}$	I $\frac{n}{400}$	2 $\frac{n}{12800}$	2 $\frac{n}{100}$	2 $\frac{n}{25600}$	2 $\frac{n}{800}$
2 $\frac{n}{3200}$		2 $\frac{n}{800}$	2 $\frac{n}{50}$	2 $\frac{n}{800}$		2 $\frac{n}{6400}$	3 $\frac{n}{200}$ †
I $\frac{n}{800}$		I $\frac{n}{400}$	I $\frac{n}{50}$ *	2 $\frac{n}{400}$		2 $\frac{n}{3200}$	7 $\frac{n}{100}$
2 $\frac{n}{400}$		2 $\frac{n}{100}$		2 $\frac{n}{200}$		4 $\frac{n}{800}$	
						I $\frac{n}{400}$	
						2 $\frac{n}{100}$	

* No. 1369. Some grew poorly.

† No. 1363. Nearly all grew to about half normal size.

Potassium cyanid.—Potassium cyanid was tried upon Glœosporium and other fungi, and, notwithstanding its great toxic action upon animal organisms, proved comparatively harmless to the spores. Starting with $\frac{n}{6400}$ on Glœosporium, successively stronger solutions were tried till $\frac{n}{400}$ was reached without preventing growth. No stronger solutions were tried.

Macrosporium with potassium cyanid was started at $\frac{n}{25600}$, and successively stronger solutions taken till $\frac{n}{50}$ was reached. In this strength one culture grew poorly and two failed, so that the fatal strength was evidently reached. With Penicillium $\frac{n}{100}$ proved fatal. Uromyces grew normally till a strength of $\frac{n}{3200}$ was reached. In this the growth was much stunted in one culture,

and two failed. In $\frac{n}{100}$ two cultures grew somewhat, while seven failed utterly.

The unexpectedly low toxic action of potassium cyanid is puzzling, especially as other experimenters³ upon spermatophytes find its toxic action about one-half that of mercuric chlorid.

It is evident that the action of this salt upon fungi is not as vigorously toxic as upon higher plants and animals.

TABLE III.
HYDROCHLORIC ACID.

Glœosporium		Macrosporium		Penicillium		Uromyces	
Grew	Failed	Grew	Failed	Grew	Failed	Grew	Failed
3 $\frac{n}{800}$		2 $\frac{n}{6400}$	1 $\frac{n}{200}$	1 $\frac{n}{6400}$	1 $\frac{n}{6400}$	2 $\frac{n}{3200}$	2 $\frac{n}{800}$
2 $\frac{n}{400}$		2 $\frac{n}{400}$	3 $\frac{n}{50}$	2 $\frac{n}{400}$		2 $\frac{n}{800}$	1 $\frac{n}{400}$
1 $\frac{n}{200}$		1 $\frac{n}{200}$		2 $\frac{n}{200}$		1 $\frac{n}{400}$	2 $\frac{n}{200}$
1 $\frac{n}{100}$		4 $\frac{n}{100}$		2 $\frac{n}{100}$		2 $\frac{n}{100}$	2 $\frac{n}{100}$
		2 $\frac{n}{50}$ *		2 $\frac{n}{50}$			4 $\frac{n}{50}$ †

* No. 1376. Not quite normal.

† No. 1358. Stunted.—1359. About 1 in 200 grew.

Hydrochloric and sulfuric acids.—These acids have about an equal status in the results of the work upon Glœosporium. Starting with a solution of $\frac{n}{800}$ the spores grew normally. Gradually stronger solutions were taken till growth was evident in $\frac{n}{100}$ with HCl, but H₂SO₄ of this strength weakened the growth perceptibly. Glœosporium here became irregular in its behavior, and the killing point was not reached. The secondary spores so common in this genus were produced, however, in

³KAHLENBERG and TRUE, On the toxic action of dissolved salts and their electrolytic dissociation. BOT. GAZ. 22:81.

F. D. HEALD, On the toxic effect of dilute solutions of acids and salts upon plants. BOT. GAZ. 22:125.

unusual abundance in these solutions, and may have been an indication of injurious action. The fact may also be significant that abnormal and distorted mycelium more frequently resulted than in ordinary nutrient solution.

TABLE IV.
SULFURIC ACID.

Glœosporium		Macrosporium		Penicillium		Uromyces	
Grew	Failed	Grew	Failed	Grew	Failed	Grew	Failed
3 $\frac{n}{800}$ *	1 $\frac{n}{800}$	3 $\frac{n}{800}$	1 $\frac{n}{800}$	2 $\frac{n}{6400}$		2 $\frac{n}{800}$	1 $\frac{n}{800}$
2 $\frac{n}{400}$		2 $\frac{n}{400}$	2 $\frac{n}{100}$	2 $\frac{n}{400}$		1 $\frac{n}{400}$	1 $\frac{n}{400}$
2 $\frac{n}{200}$		2 $\frac{n}{200}$ †	2 $\frac{n}{50}$	2 $\frac{n}{200}$		1 $\frac{n}{200}$	1 $\frac{n}{200}$
1 $\frac{n}{100}$		2 $\frac{n}{100}$		2 $\frac{n}{100}$		1 $\frac{n}{100}$	6 $\frac{n}{100}$
		2 $\frac{n}{50}$		2 $\frac{n}{50}$			

*No. 1338. Poorly.

†No. 1094. Stunted.

‡No. 1028. Ten in 100 grew poorly.

With Macrosporium and Penicillium neither acid prevented growth at $\frac{n}{50}$. Uromyces was exceedingly variable in its behavior with these acids, and the results, as far as they can be interpreted, seem to show H_2SO_4 to be a trifle more toxic. The killing point of HCl apparently is $\frac{n}{50}$, while for H_2SO_4 it is $\frac{n}{100}$.

Alcohol.—Glœosporium germinated normally in alcohol of semi-normal strength. Macrosporium even grew in five times normal strength, while Penicillium grew in semi-normal, but failed in normal. Uromyces grew in five times normal. Stronger solutions were not tried, owing to the inability to secure hanging drops in stronger solutions. The low toxic power might be sought here in volatility, but this seems not to be the cause, as it was evident that the Uromyces, Botrytis, and Macrosporium

were stimulated by the alcohol. More spores grew, and they grew far more luxuriantly than in water.

TABLE V.
ALCOHOL.

Botrytis		Macrosporium		Penicillium		Uromyces	
Grew	Failed	Grew	Failed	Grew	Failed	Grew	Failed
2 $\frac{n}{10}$		2 $\frac{n}{10}$		2 $\frac{n}{5}$	4 $\frac{2n}{1}$	2 $\frac{n}{10}$	
2 $\frac{n}{1}$		2 $\frac{n}{1}$		2 $\frac{n}{2}$	2 $\frac{n}{1}$	2 $\frac{n}{1}$ *	
		2 $\frac{5n}{1}$				2 $\frac{5n}{1}$	

*No. 480. Much knotted and distorted.

TABLE VI.
COPPER SULFATE.

Botrytis		Macrosporium		Penicillium		Uromyces	
Grew	Failed	Grew	Failed	Grew	Failed	Grew	Failed
2 $\frac{n}{12800}$	1 $\frac{n}{6400}$	1 $\frac{n}{12800}$	6 $\frac{n}{6400}$	2 $\frac{n}{6400}$	1 $\frac{n}{800}$	2 $\frac{n}{12800}$	3 $\frac{n}{6400}$
5 $\frac{n}{6400}$ *	2 $\frac{n}{3200}$	2 $\frac{n}{6400}$ †	2 $\frac{n}{3200}$	2 $\frac{n}{3200}$	2 $\frac{n}{200}$	5 $\frac{n}{6400}$ ‡	2 $\frac{n}{3200}$ §
	2 $\frac{n}{100}$		2 $\frac{n}{100}$	2 $\frac{n}{1600}$			1 $\frac{n}{100}$ ¶
				3 $\frac{n}{800}$			
				2 $\frac{n}{400}$			

*No. 274. Scattered.

†No. 377. Grew slightly.

‡No. 706. One culture stunted.

§No. 700. One in 300 grew.

¶No. 290. A few grew in one culture.

Copper sulfate.—Botrytis failed in $\frac{n}{3200}$, grew poorly in $\frac{n}{6400}$, and normally in $\frac{n}{12800}$. Macrosporium behaved in a similar manner, but was more injured by $\frac{n}{6400}$.

TABLE VII.
COPPER NITRATE.

Botrytis		Macrosporium		Penicillium		Uromyces	
Grew	Failed	Grew	Failed	Grew	Failed	Grew	Failed
$2 \frac{n}{6400}$	$2 \frac{n}{3200}$ $2 \frac{n}{100}$	$I \frac{n}{6400}$ $I \frac{n}{3200}$	$2 \frac{n}{100}$	$2 \frac{n}{6400}$ $2 \frac{n}{3200}$ $2 \frac{n}{800}$ $2 \frac{n}{400}$	$2 \frac{n}{200} *$	$2 \frac{n}{6400}$	$2 \frac{n}{3200}$ $2 \frac{n}{100}$

* No. 956. Grew on dry part only in one culture.

TABLE VIII.
COPPER ACETATE.

Botrytis		Macrosporium		Penicillium		Uromyces	
Grew	Failed	Grew	Failed	Grew	Failed	Grew	Failed
$2 \frac{n}{6400}$	$2 \frac{n}{3200}$	$2 \frac{n}{6400}$ $I \frac{n}{3200}$	$3 \frac{n}{3200}$ $2 \frac{n}{1000}$	$2 \frac{n}{3200}$ $2 \frac{n}{1600}$	$2 \frac{n}{800}$	$2 \frac{n}{6400}$ $4 \frac{n}{3200}$	$6 \frac{n}{1600} *$ $3 \frac{n}{800} \dagger$ $4 \frac{n}{400} \ddagger$ $4 \frac{n}{200}$

* No. 524. Twenty per 100 grew.—No. 525. Eighteen in 150 grew.

† No. 551. Five in 100 grew.—No. 552. A few in a bunch grew.

‡ No. 586. Six in 100 grew.—No. 587. Four in 100 grew.

Copper nitrate.—Botrytis grew in $\frac{n}{6400}$ and failed in $\frac{n}{3200}$. Macrosporium grew in $\frac{n}{3200}$. Penicillium grew in $\frac{n}{400}$, and in $\frac{n}{200}$ one culture became dry and grew at the dry portion, but the culture which remained moist showed no growth. Uromyces grew in $\frac{n}{6400}$, but failed in $\frac{n}{3200}$.

Here it first became evident that *Uromyces*, as the toxic solution becomes stronger, does not diminish in vigor of growth, but does diminish in the number of germinating spores.

Copper acetate.—*Botrytis* grew in $\frac{n}{6400}$ and failed in $\frac{n}{3200}$. *Macrosporium* grew in $\frac{n}{6400}$ and failed in $\frac{n}{3200}$. *Penicillium* grew in $\frac{n}{1600}$ and failed in $\frac{n}{800}$. *Uromyces* was a practical failure in $\frac{n}{800}$ or stronger, but some spores grew poorly in $\frac{n}{400}$, and, on the other hand, were much injured by $\frac{n}{3200}$. There was no growth it $\frac{n}{200}$, which was tried four times.

TABLE IX.
COPPER CHLORID.

Glœosporium		Macrosporium		Penicillium		Uromyces	
Grew	Failed	Grew	Failed	Grew	Failed	Grew	Failed
I $\frac{n}{12800}$	2 $\frac{n}{6400}$		2 $\frac{n}{3200}$	2 $\frac{n}{6400}$	2 $\frac{n}{200}$	I $\frac{n}{6400}$	4 $\frac{n}{3200}$ *
	2 $\frac{n}{3200}$			2 $\frac{n}{3200}$			4 $\frac{n}{1600}$ †
	2 $\frac{n}{1600}$			2 $\frac{n}{1600}$			
				2 $\frac{n}{800}$			
				2 $\frac{n}{400}$			

*No. 758. Four in 100 grew.—No. 759. One half usual number grew.

†No. 757. Three in 200 grew.

Copper chlorid.—This was tried upon *Glœosporium*. In $\frac{n}{12800}$ it grew, while in $\frac{n}{6400}$ it failed. *Macrosporium* was tried with only one strength, $\frac{n}{3200}$. It failed to grow. *Penicillium* grew in $\frac{n}{400}$ and weaker, and failed in $\frac{n}{200}$. *Uromyces* in $\frac{n}{6400}$ grew; in $\frac{n}{3200}$ three grew very poorly and one failed. This is evidently a killing strength.

Potassium permanganate.—The killing strength of this chemical was determined with much accuracy, and in experimenta-

tion presented interesting phenomena. In strong solutions, that is $\frac{n}{100}$ or stronger, it stained the protoplasm and prevented growth. With *Glæosporium* $\frac{n}{800}$ prevented growth and $\frac{n}{1600}$ allowed it. At this strength the protoplasm was barely tinted.

That strong solutions are withstood by *Penicillium* may be partly explained by the decompositions due to the organic matter present; but this can only slightly reduce the amount of the salt present.

Uromyces grew normally in $\frac{n}{400}$, and there was practically no growth in $\frac{n}{200}$ or stronger, although in culture 749 one spore, standing unprotected in the hanging drop and to all appearances in the same conditions as any of the others in the drop, grew normally, although the protoplasmic contents of its germ tube were colored strongly. This apparently perfectly normal growth, while the surrounding spores remained completely undeveloped, may be taken as almost typical of the behavior of the uredospores of *Uromyces caryophyllinus* when in highly poisonous solutions.

TABLE X.
POTASSIUM PERMANGANATE.

Glæosporium		Macrosporium		Penicillium		Uromyces	
Grew	Failed	Grew	Failed	Grew	Failed	Grew	Failed
2 $\frac{n}{1600}$	2 $\frac{n}{800}$			2 $\frac{n}{20}$		4 $\frac{n}{400}$	2 $\frac{n}{200}$
	4 $\frac{n}{400}$			2 $\frac{n}{10}$			2 $\frac{n}{100}$ *
	2 $\frac{n}{200}$						

* No. 749. One spore grew well.

Sodium chlorid.—This proved practically non-toxic. With *Botrytis* a semi-normal solution did not prevent normal germination. *Macrosporium* grew in a normal solution. With these two fungi a killing strength was not obtained.

TABLE XI.
SODIUM CHLORID.

Glucosporium		Macrosporium		Penicillium		Uromyces	
Grew	Failed	Grew	Failed	Grew	Failed	Grew	Failed
4 $\frac{n}{6400}$		4 $\frac{n}{3200}$		3 $\frac{n}{50}$		2 $\frac{n}{3200} \dagger$	5 $\frac{n}{2} \dagger$
2 $\frac{n}{1600} *$		2 $\frac{n}{100}$		2 $\frac{n}{2}$		2 $\frac{n}{100}$	2 $\frac{n}{1}$
4 $\frac{n}{100}$		2 $\frac{n}{2}$		2 $\frac{2n}{1}$			4 $\frac{2n}{1}$
2 $\frac{n}{1}$		1 $\frac{n}{1}$					2 $\frac{4n}{1}$

*No. 25. Apparently stimulated.

† No. 146. Apparently stimulated.

‡ No. 446. In one culture only 3 per cent. grew.

TABLE XII.
POTASSIUM CHROMATE.

Botrytis		Macrosporium		Penicillium		Uromyces	
Grew	Failed	Grew	Failed	Grew	Failed	Grew	Failed
	1 $\frac{n}{640}$	2 $\frac{n}{640} \dagger$	1 $\frac{n}{320}$		2 $\frac{n}{640}$	2 $\frac{n}{320}$	3 $\frac{n}{40} \P$
	2 $\frac{n}{320}$	1 $\frac{n}{320}$	2 $\frac{n}{10}$		2 $\frac{n}{320}$	4 $\frac{n}{160} \dagger$	2 $\frac{n}{10}$
	2 $\frac{n}{40}$	1 $\frac{n}{40} *$			2 $\frac{n}{160}$	3 $\frac{n}{80} \S$	
	2 $\frac{n}{10}$				2 $\frac{n}{80}$		
					2 $\frac{n}{10}$		

*No. 539. Growth slight.

† No. 652. Growth slight.

‡ No. 611. Many failed, but those which grew were normal.

§ Nos. 602, 604. Nearly every spore grew, but the tubes were about half the usual length, and the protoplasm was granular and finally plasmolyzed.

¶ Nos. 640, 641. One in 200 grew.—No. 642. Two in 200 grew.

Potassium chromate.—Uromyces failed in three cultures $\frac{n}{40}$.
One culture in $\frac{n}{40}$ had one spore grow, but this grew with

remarkable vigor, producing a tube fully twice the usual length, while in two other cultures of the same strength respectively one in 100 and two in 200 grew. The fatal strength here is thus evidently $\frac{n}{40}$, while $\frac{n}{160}$ has an appreciable toxic action, and $\frac{n}{80}$ is strongly toxic.

TABLE XIII.
POTASSIUM BICHROMATE.

Botrytis		Macrosporium		Penicillium		Uromyces	
Grew	Failed	Grew	Failed	Grew	Failed	Grew	Failed
	$2 \frac{n}{1280}$	$2 \frac{n}{3200} *$	$2 \frac{n}{1280}$		$3 \frac{n}{1600}$	$2 \frac{n}{3200} \dagger$	$2 \frac{n}{1280}$
	$2 \frac{n}{640}$		$2 \frac{n}{640}$		$2 \frac{n}{800}$	$3 \frac{n}{640} \dagger$	$1 \frac{n}{640}$
	$2 \frac{n}{320}$		$2 \frac{n}{320}$		$2 \frac{n}{160}$		$2 \frac{n}{320}$
	$2 \frac{n}{160}$		$2 \frac{n}{160}$		$2 \frac{n}{10}$		$2 \frac{n}{160}$
	$2 \frac{n}{40}$		$2 \frac{n}{40}$				$2 \frac{n}{40}$
	$2 \frac{n}{10}$		$2 \frac{n}{10}$				$4 \frac{n}{10}$

*No. 715. Grew poorly.

†Nos. 712, 713. One in seventy-five grew normally.

‡No. 634. Two spores grew well.—No. 635. One in eighty grew.—No. 636 Three in seventy-five grew.

Potassium bichromate.—This would not allow the germination of Botrytis spores when of $\frac{n}{1280}$ strength. No weaker was tried. Macrosporium would not grow in $\frac{n}{1280}$, and in the next weaker tried, $\frac{n}{3200}$, it grew poorly. Penicillium failed to grow in $\frac{n}{1600}$, and weaker were not tried. Uromyces spores were nearly all killed by $\frac{n}{640}$, would not grow in stronger, and were injured by even weaker. Penicillium grew in as strong as double normal, and killing strength was not reached. Uromyces was prevented almost completely by $\frac{n}{2}$, and stronger solutions absolutely prohibited germination. Weak solutions, such as $\frac{n}{3200}$, seemed to stimulate the early growth somewhat.

TABLE XIV.
AMMONIUM NITRATE.

Botrytis		Penicillium		Uromyces	
Grew	Failed	Grew	Failed	Grew	Failed
2 $\frac{n}{4}$ *	2 $\frac{n}{2}$	2 $\frac{n}{2}$		1 $\frac{n}{4}$	2 $\frac{n}{2}$

* No. 654. Growth slight.

TABLE XV.
POTASSIUM HYDROXID.

Macrosporium		Penicillium		Uromyces	
Grew	Failed	Grew	Failed	Grew	Failed
4 $\frac{n}{200}$		2 $\frac{n}{50}$	1 $\frac{n}{20}$ *	2 $\frac{n}{200}$	2 $\frac{n}{200}$
2 $\frac{n}{100}$		1 $\frac{n}{20}$ †	2 $\frac{n}{40}$	1 $\frac{n}{100}$	

SODIUM HYDROXID.

2 $\frac{n}{200}$		2 $\frac{n}{50}$	2 $\frac{n}{40}$	1 $\frac{n}{200}$	
2 $\frac{n}{100}$			2 $\frac{n}{20}$	2 $\frac{n}{100}$	

AMMONIUM HYDROXID.

		1 $\frac{n}{28}$	2 $\frac{n}{2.78}$		
			2 $\frac{n}{5.56}$		
			2 $\frac{n}{13.7}$		
			1 $\frac{n}{28}$		

* No. 825. Grew not at all.

† No. 824. Grew luxuriantly.

Ammonium nitrate.—This permitted the growth of *Botrytis* in $\frac{n}{4}$ but not in $\frac{n}{2}$. *Penicillium* grew in $\frac{n}{2}$. *Uromyces* was prevented by $\frac{n}{2}$, but grew in $\frac{n}{4}$.

Potassium, sodium, and ammonium hydroxids.—*Macrosporium* gave identical results with the first two of these chemicals and a killing strength was not reached at $\frac{n}{100}$. Stronger solutions could not be tried in drop culture. With *Penicillium* the fatal strength may be considered as $\frac{n}{40}$ for sodium and potassium hydroxids. There is an apparent anomaly in the fact that in one tube the fungus grew finely in $\text{KOH } \frac{n}{20}$, while its mate failed utterly. This can be explained on the assumption that one spore or more found a lodging place in a fragment of unsaturated bread, and thus attained a sufficient protection and

TABLE XVI.
POTASSIUM IODID.

Botrytis		Macrosporium		Penicillium		Uromyces	
Grew	Failed	Grew	Failed	Grew	Failed	Grew	Failed
I $\frac{n}{20}$		2 $\frac{n}{50}$		2 $\frac{n}{10}$		2 $\frac{n}{50}$	
2 $\frac{n}{50}$		I $\frac{n}{40}$				2 $\frac{n}{20}$	
		I $\frac{n}{20}$					

POTASSIUM BROMID.

2 $\frac{n}{20}$		2 $\frac{n}{20}$		2 $\frac{n}{10}$		2 $\frac{n}{20}$	
------------------	--	------------------	--	------------------	--	------------------	--

SODIUM ACETATE.

2 $\frac{n}{4}$	2 $\frac{n}{2}$	2 $\frac{n}{4}$ *		I $\frac{n}{1}$		2 $\frac{n}{4}$ †	2 $\frac{n}{2}$
-----------------	-----------------	-------------------	--	-----------------	--	-------------------	-----------------

*No. 647. Some injured.

†No. 645. Very few grew.

start to resist the further action of the toxic solution. With *Uromyces* results were somewhat variable, but undeniable proof was obtained that growth is not prevented by $\frac{n}{100}$.

Ammonium hydroxid was tried only with *Penicillium*, and $\frac{n}{14}$ prevented growth.

Potassium iodid and bromid.—These agree in being non-toxic toward *Botrytis*, *Macrosporium*, and *Uromyces* at $\frac{n}{20}$, and to *Penicillium* at $\frac{n}{10}$.

Sodium acetate.—This allowed growth of *Botrytis*, *Macrosporium*, and *Uromyces* at $\frac{n}{4}$, and of *Penicillium* at normal, while $\frac{n}{2}$ prevented *Botrytis* and *Uromyces* from growing.

Magnesium sulfate, barium chlorid, ammonium chlorid, magnesium chlorid.—By two tests each, these all proved harmless to *Penicillium* at $\frac{n}{10}$ strength.

A general tabulation of these results is presented in table XVII which will be readily comprehended. The strength expressed represents the dilution of normal solution required to prevent most of the spores from germinating. If any growth occurred, there would be only a few isolated cases of germination. In table XVIII the strength is indicated in parts per million.

The signs $>$ and $<$ are the common mathematical signs for "greater than" and "less than." For example, the killing strength of KCN for *Glœosporium* is greater than $\frac{n}{400}$, while that of potassium bichromate for *Botrytis* is less than $\frac{n}{640}$. The molecular weight of the salt as used in making up the solutions is given, with formula of each substance.

From the more interesting and important generalizations of this table a few may be indicated.

Mercuric chlorid is by far the most poisonous substance used, while potassium cyanid has low toxic power. This may be partially explained by the evaporation of the potassium cyanid from the drop, but this explanation surely cannot apply in the

TABLE XVII
STRENGTH REQUIRED TO PREVENT GERMINATION
[Expressed as parts of normal solution]

Substance	Botrytis	Macrosporium	Gloeosporium	Penicillium	Uromyces
HgCl ₂ 239.2	$\frac{n}{51200}$	$\frac{n}{102400}$		$> \frac{n}{3200}$	$\frac{n}{6400}$
KCN 64.7		$\frac{n}{50}$	$> \frac{n}{400}$	$\frac{n}{100}$	$\frac{n}{100}$
HCl 36.19		$> \frac{n}{50}$	$> \frac{n}{100}$	$> \frac{n}{50}$	$\frac{n}{50}$
H ₂ SO ₄ 97.34		$> \frac{n}{50}$	$> \frac{n}{100}$	$> \frac{n}{50}$	$\frac{n}{100}$
C ₂ H ₆ O 45.7	$> n$	$> 5 n$		n	$> 5 n$
CuSO ₄ +5H ₂ O 247.54	$\frac{n}{3200}$	$\frac{n}{6400}$		$\frac{n}{200}$	$\frac{n}{3200}$
Cu(NO ₃) ₂ +3 H ₂ O 239.6	$\frac{n}{3200}$	$> \frac{n}{3200}$		$\frac{n}{200}$	$\frac{n}{3200}$
Cu(C ₂ H ₃ O ₂) ₂ 179.96	$\frac{n}{3200}$	$\frac{n}{3200}$		$\frac{n}{800}$	$\frac{n}{800}$
CuCl ₂ +2H ₂ O 166.96		$\frac{n}{3200 \text{ or less}}$	$\frac{n}{6400}$	$\frac{n}{200}$	$\frac{n}{3200}$
K ₂ CrO ₄ 192.92	$< \frac{n}{640}$	$\frac{n}{40}$		$< \frac{n}{640}$	$\frac{n}{40}$
K ₂ Cr ₂ O ₇ 292.2	$< \frac{n}{1280}$	$\frac{n}{1280}$		$< \frac{n}{1600}$	$\frac{n}{640}$
NaCl 58.18	$> \frac{n}{2}$	$> n$		$> 2 n$	$\frac{n}{2}$
KOH 54.74		$> \frac{n}{100}$		$\frac{n}{40}$	$> \frac{n}{100}$
NaOH 38.76		$> \frac{n}{100}$		$\frac{n}{40}$	$> \frac{n}{100}$
NH ₄ NO ₃ 79.52	$\frac{n}{2}$			$\frac{n}{2}$	$\frac{n}{2}$
KI 164.76	$> \frac{n}{20}$	$> \frac{n}{20}$		$> \frac{n}{10}$	$> \frac{n}{20}$
KBr 118.21	$> \frac{n}{20}$	$> \frac{n}{20}$		$> \frac{n}{10}$	$\frac{n}{20}$
Na(C ₂ H ₃ O ₂)+3 H ₂ O 134.71	$\frac{n}{2}$	$> \frac{n}{4}$		$> n$	$\frac{n}{2}$
MgSO ₄ +7 H ₂ O 245.7				$> \frac{n}{10}$	
BaCl ₂ +2 H ₂ O 242.13				$> \frac{n}{10}$	
NH ₄ Cl 53.13				$> \frac{n}{10}$	
MgCl ₂ +6 H ₂ O 201.88				$> \frac{n}{10}$	
K ₂ Mn ₂ O ₈ 313.94 ...				$> \frac{n}{10}$	$\frac{n}{200}$
NH ₄ OH 34.82				$> \frac{n}{25}$	

case of Penicillium, which uniformly grew in $\frac{n}{200}$. So the fact must be accepted that KCN has a low toxic power for these fungi.

TABLE XVIII
STRENGTH REQUIRED TO PREVENT GERMINATION
[Expressed as parts per million]

Substance	Botrytis	Macrosporium	Gloeosporium	Penicillium	Uromyces
HgCl ₂ 239.2	4.6	2.3		74	37
KCN 64.7		1294	> 162	647	647
HCl 36.19		> 724	> 362	> 724	724
H ₂ SO ₄ 97.34		> 1947	> 973	> 1947	973
C ₂ H ₆ O 45.7	> 45700	> 228500		45700	> 228500
CuSO ₄ +5H ₂ O 247.54	49—77	25—38		791—1237	49—77
Cu(NO ₃) ₂ +3 H ₂ O 239.6	58	> 58		930	58
Cu(C ₂ H ₃ O ₂) ₂ 179.96	56	56		225	225
CuCl ₂ +2 H ₂ O 166.96		20 or less	20	656	41
K ₂ CrO ₄ 192.92	< 301	4823		< 301	4823
K ₂ Cr ₂ O ₇ 292.2	< 228	228		< 183	456
NaCl 58.18	> 29090	> 59190		< 119380	29090
KOH 54.74		> 547		1368	> 547
NaOH 38.76		> 388		1938	> 388
NH ₄ NO ₃ 79.52	39760			39760	39760
KI 164.76	> 8238	> 8238		> 16476	> 8238
KBr 118.21	> 5910	> 5910		> 11820	> 5910
Na(C ₂ H ₃ O ₂)+3 H ₂ O 134.71	45500	> 20250		> 81000	40500
MgSO ₄ +7 H ₂ O 245.7				> 12554	
BaCl ₂ +2 H ₂ O 242.13				> 20637	
NH ₄ Cl 53.13				> 5313	
MgCl ₂ +6 H ₂ O 201.88				> 9460	
K ₂ Mn ₂ O ₈ 313.94 ...	392			> 31394	1570
NH ₄ OH 34.82					

H₂SO₄ and HCl agree closely in toxic effect upon these fungi and are of also about the same toxic action as KCN. The fatal strength for H₂SO₄ is nearly a one per cent. solution.

All of the copper salts agree closely in toxic action.

Between potassium chromate and bichromate there is a remarkable variance; the bichromate has twice the effect upon Botrytis, thirty-two times the effect upon Macrosporium and sixteen times upon Uromyces that the chromate does.

In all the cases considered so far, it will be noticed that with the exception of *Penicillium*, *Uromyces* will withstand as great or greater strength than any of the other fungi tried. With NaCl we have a case, however, in which the *Macrosporium* survives even a greater strength than the *Uromyces*. The same anomaly is observed with HCl and H_2SO_4 upon these fungi. So it seems that although a fungus may, generally speaking, be more resistant to the action of salts or acids than other fungi are, there may be some particular substances which will affect this fungus at less strength than is required for the other and usually weaker fungus. This fact is especially important in the application of fungicides, in that a fungicide which is most effective for one fungus is not necessarily so for all fungi. It may also be noticed that although *Uromyces* is generally more resistant than *Botrytis* and *Macrosporium*, *Uromyces* is the most susceptible to the action of NaCl.

The hydroxids KOH, NaOH, and NH_4OH gave quite uniform results, and show a low toxic power. In the case of the hanging drop this might partially be explained by the neutralization by CO_2 ; but, substantiated as it is by parallel experiments in gross culture with a *Penicillium*, the fact must be accepted that the hydroxids are of low toxic action on these fungi.

Potassium permanganate is of very low toxic power, but is of peculiar interest in that it has the power of coloring the uredospores a dense black, while the teleutospores were but slightly if at all darkened. The other records made in table XVII show that the solution is practically non-toxic.

An inspection of this table will show that, of the five fungi tried, *Penicillium* is usually more resistant than any of the others. This is rather to be explained by the means of culture than by any structural or selective difference in the fungi themselves.

Single spores might easily have been protected from the action of the fungicide by the nutrient medium. This idea is further supported by the fact, frequently noted in drop cultures of *Uromyces*, that often a spore in the midst of a close bunch would grow when others near it and unprotected would fail. This was of such frequent occurrence as practically to prove that

TABLE XIX.
SHOWING LIMIT PREVENTING SPORADIC GERMINATION.

Substance	Botrytis	Glœosporium	Macrosporium	Penicillium	Uromyces
HgCl ₂	$\frac{n}{25600}$		$\frac{n}{51200}$	$> \frac{n}{3200}$	$\frac{n}{6400}$
KCN		$> \frac{n}{400}$	$> \frac{n}{50}$	$\frac{n}{100}$	$> \frac{n}{100}$
HCl		$> \frac{n}{100}$	$> \frac{n}{50}$	$> \frac{n}{50}$	$> \frac{n}{50}$
H ₂ SO ₄		$> \frac{n}{100}$	$> \frac{n}{50}$	$> \frac{n}{50}$	$> \frac{n}{100}$
C ₂ H ₆ O	$> n$		$> 5n$	$\frac{n}{2}$	$> \frac{n}{5}$
CuSO ₄	$\frac{n}{3200}$		$\frac{n}{3200}$	$\frac{n}{200}$	$> \frac{n}{100}$
Cu(NO ₃) ₂	$\frac{n}{3200}$			$\frac{n}{200}$	$\frac{n}{3200}$
Cu(C ₂ H ₃ O ₂) ₂ ..	$\frac{n}{3200}$		$\frac{n}{1600}$	$\frac{n}{800}$	$\frac{n}{200}$
CuCl ₂		$\frac{n}{6400}$	$\frac{n}{6400}$	$\frac{n}{200}$	$> \frac{n}{1600}$
K ₂ Mn ₂ O ₈		$\frac{n}{800}$		$\frac{n}{400}$	$> \frac{n}{100}$
K ₂ CrO ₄	$< \frac{n}{640}$		$\frac{n}{40}$	$< \frac{n}{640}$	$\frac{n}{10}$
K ₂ Cr ₂ O ₇	$< \frac{n}{1280}$		$\frac{n}{1280}$	$< \frac{n}{1600}$	$\frac{n}{320}$
NaCl	$> \frac{n}{2}$		$> n$	$> \frac{2n}{1}$	n
KOH			$> \frac{n}{100}$	$> \frac{n}{20}$	$> \frac{n}{100}$
NaOH			$> \frac{n}{100}$	$\frac{n}{20}$	$> \frac{n}{100}$
NH ₄ OH				$> \frac{n}{28}$	

close contact of the spores could prevent the toxic effect of the chemical.

Generally speaking, *Botrytis* required a stronger solution to kill than did the *Macrosporium*, while *Uromyces* required much greater strength than either of them. This might have been expected from the relative thickness of their walls, and is further illustrated by table XIX, which gives the strength of solution required to prevent completely the growth of spores. Exceptions to this generalization occur, however, as has already been pointed out, which indicate a selective difference. Some fungi on experimentation gave with some chemicals a sharp and definite killing point. Others were gradually weakened or the number of germinating spores decreased as the strength of the solution increased. This second phase is particularly well illustrated in the cultures of *Uromyces*. With each chemical a strength could be found which just perceptibly injured the fungus, and another which prevented most spores from germinating. This is what has previously been given as the killing strength. At this strength one of two things occurred: either about the usual number grew, but grew only slightly and in stunted or often distorted manner; or, in each culture, nearly all the spores failed utterly to grow, while one or a half dozen in the hundreds of a culture would grow and grow usually vigorously and apparently uninjured. In order to kill these few persistent spores much greater strength was required. Thus, in potassium chromate, for *Uromyces*, $\frac{n}{40}$ killed or prevented most growth. But some spores grew even in $\frac{n}{20}$, and $\frac{n}{10}$ was necessary to inhibit growth completely. On the other hand $\frac{n}{80}$ did weaken growth somewhat. So there is established a wide range between the weakest solution injuring the fungus and the weakest solution surely preventing growth.

Table XX shows the variation in range of susceptibility. In this table two concentrations are named for each fungus. At the weaker normal growth took place, while the stronger completely inhibited growth.

It is here to be noticed that *Uromyces* gives a remarkable

TABLE XX.
SHOWING RANGE OF SUSCEPTIBILITY.

Substance	Botrytis	Gloeosporium	Macrosporium	Penicillium	Uromyces
HgCl_2	$\frac{n}{102400}$ $\frac{n}{25600}$		$\frac{n}{819200}$ $\frac{n}{51200}$		$\frac{n}{25600}$ $\frac{n}{6400}$
KCN			$\frac{n}{100}$ $\frac{n}{50}$ $> \frac{n}{50}$	$\frac{n}{200}$ $\frac{n}{100}$	$< \frac{n}{200}$ $> \frac{n}{100}$
HCl			$\frac{n}{100}$ $> \frac{n}{50}$		$\frac{n}{800}$ $> \frac{n}{50}$
H_2SO_4		$\frac{n}{200}$ $> \frac{n}{100}$			
$\text{C}_2\text{H}_6\text{O}$				$\frac{n}{1}$ $\frac{n}{2}$	
CuSO_4	$\frac{n}{12800}$ $\frac{n}{3200}$		$\frac{n}{12800}$ $\frac{n}{3200}$	$\frac{n}{400}$ $\frac{n}{200}$	$\frac{n}{12800}$ $> \frac{n}{100}$
$\text{Cu}(\text{NO}_3)_2$	$\frac{n}{6400}$ $\frac{n}{3200}$			$\frac{n}{400}$ $\frac{n}{200}$	$\frac{n}{6400}$ $\frac{n}{3200}$
$\text{Cu}(\text{C}_2\text{H}_3\text{O}_2)_2$	$\frac{n}{3200}$ $\frac{n}{6400}$		$\frac{n}{6400}$ $\frac{n}{1600}$	$\frac{n}{1600}$ $\frac{n}{800}$	$\frac{n}{6400}$ $\frac{n}{200}$
CuCl_2		$\frac{n}{12800}$ $\frac{n}{6400}$		$\frac{n}{400}$ $\frac{n}{200}$	$\frac{n}{6400}$ $> \frac{n}{1600}$
$\text{K}_2\text{Mn}_2\text{O}_8$		$\frac{n}{1600}$ $\frac{n}{800}$		$\frac{n}{400}$	$\frac{n}{400}$ $> \frac{n}{100}$
K_2CrO_4					$\frac{n}{160}$ $\frac{n}{10}$
K_2CrO_7			$< \frac{n}{3200}$ $\frac{n}{1280}$		$< \frac{n}{3200}$ $\frac{n}{320}$
KOH				$\frac{n}{50}$ $> \frac{n}{20}$	
NaOH				$\frac{n}{50}$ $\frac{n}{20}$	

range, particularly with copper sulfate, in which most spores were killed at $\frac{n}{3200}$. Some injury was done by $\frac{n}{12800}$, but some spores grew in even so strong a solution as $\frac{n}{100}$.

A general survey of the tables will show that the following list of chemicals may be selected as having practically no toxic action unless in great strength, as they were tried at $\frac{n}{10}$ with no toxic action, and this was in every case greater than a $\frac{1}{2}$ per cent. solution: $K_2Mn_2O_8$, $MgCl_2$, NH_4Cl , $BaCl_2$, $MgSO_4$, $Na(C_2H_3O_2)$, KBr , KI , NH_4NO_3 , $NaCl$, C_2H_6O .

HYDROLYTIC DISSOCIATION.

Salts in aqueous solution present certain deviations regarding the changes of freezing and boiling point (and also other optical physical, and chemical deviations) which are not in accord with the general laws for solutions containing the number of molecules theoretically supposed to be present. "Thus a solution of $KCl + 100 H_2O$, instead of showing a lowering of vapor pressure of .01 as required by the law, shows a lowering of about double this. Solutions in alcohol behave like other substances and give normal diminution of vapor pressure."⁴ In general the behavior of such aqueous solutions of salts, bases, or acids is such as might be expected if they contained more molecules than their formula indicates.

These phenomena led Arrhenius⁵ to the conclusion that each molecule or some of the molecules are separated into part molecules or ions, a term long used by the physicist.

According to this theory of hydrolytic dissociation a solution of mercuric chlorid does not consist of water and molecules of mercuric chlorid, but does consist of water containing ions of mercury and ions of chlorin, designated as Hg^+ and Cl^- , according as the element is electro-positive or electro-negative in electrolytic dissociation.

Careful distinction should be drawn between an element in the condition of dissociation and in a molecular condition. Thus

⁴ OSTWALD: Solutions, trans. by Muir, p. 187.

⁵ Zeits. f. phys. Chem. 1:631. 1887.

ions of sodium are very different in their chemical, physical, and physiological properties from molecules of sodium.

Arrhenius, as quoted by Ostwald (*l. c.*), was thus led to the conclusion that "the properties of salt solutions must be capable of representation as the binary sums of the properties of the ions." This generalization has later been made more particular and has been qualified slightly by Kahlenberg and True (*l. c.*), who extend their reasoning to the domain of physiological effects. They say: "Now, if, in the case of the solutions in question, all the chemical and physical properties are due to the properties of the ions plus those of the undissociated molecules it contains, it seems very probable that the physiological effects produced by such solutions are due to these."

The above proposition is then amply substantiated by these authors and by F. D. Heald (*l. c.*) in a series of experiments upon seedlings of flowering plants.

TABLE XXI.

Non-poisonous			Poisonous		
Substance	Cation	Anion	Substance	Cation	Anion
MgSO ₄	Mg +	SO ₄ —	HgCl ₂	Hg +	CN —
BaCl ₂	Ba +	Cl —	HCl	H +	
NaCl	Na +	Cl —	H ₂ SO ₄	H +	
MgCl	Mg +	Cl —	KCN		
NaC ₂ H ₃ O ₂	Na +	C ₂ H ₃ O ₂ —	CuSO ₄	Cu +	
KBr	K +	Br —	Cu(NO ₃) ₂	Cu +	CrO ₄ — Cr ₂ O ₇ — OH — OH — OH —
KI	K +	I —	Cu(C ₂ H ₃ O ₂) ₂	Cu +	
K ₂ Mn ₂ O ₈	K +	MnO ₄	CuCl ₂	Cu +	
NH ₄ NO ₃			K ₂ CrO ₄		
NH ₄ Cl			K ₂ Cr ₂ O ₇		
C ₂ H ₆ O			KOH		
			NaOH		
			NH ₄ OH		

In the first column of table XXI is given a list of salts which have been proven non-poisonous. The strengths were such that there were in the solution many undissociated molecules; many also of the molecules were dissociated; hence, as these solutions proved non-toxic at the strength used, it may be

concluded that both the molecules and the ions, both negative and positive, are devoid of toxic action.

The two next columns of this table show the ions into which the salts are separated. Here then is proof that to the fungi under investigation the cations Mg, Ba, Na, and K are non-toxic. The anions SO_4 , Cl, Br, I, MnO_4 , and $\text{C}_2\text{H}_3\text{O}_2$ are non-toxic at the strength used. The second half of table XXI shows those salts which were found to have toxic action.

As Cl^- ions have previously been proven non-toxic the effect must rest with the molecule of HgCl_2 or with the ion of Hg^+ . The salt at the strength used was almost completely dissociated, hence the effect is due to the Hg^+ ion, which was the most powerful one experimented with.

With the two acids, HCl and H_2SO_4 , the anions had proven non-toxic; so, by similar reasoning, the poisonous property rests with the H^+ ion. As H_2SO_4 contains twice as much H^+ as does HCl it should have twice its toxic power in equi-molecular solutions. This statement has met no adverse results in experiment, but there has been slight, though by no means positive evidence, to sustain it.

With KCN the cation is non-toxic. The CN^- then must be poisonous, and it has about the same toxic action as does H^+ .

With all the copper salts the anions are non-toxic, hence the poison is in the molecule or in the copper, and in the more dilute solutions it undeniably rests with the copper. As each copper compound has as many atoms of copper per molecule as the others it would be equally toxic. This expectation is almost fully met, with the exception of copper acetate for *Penicillium* and *Uromyces*, and of copper sulfate for *Macrosporium*. These three slight deviations from the theory stand against thirteen observations with the copper salts tending to support it.

In the potassium chromate and bichromate solutions the poisonous action rests evidently with the anions. The bichromate is in every case more poisonous and in most cases far more poisonous than the chromate, as may be seen by tables XII and XIII.

With the hydroxids the toxic effect rests with the anion, and as far as positive results were secured, they agree precisely. It may be noted, in passing, that the Bordeaux mixture, 22-gallon formula, estimated on basis of copper content, would be of about $\frac{n}{7}$ strength, whereas these experiments show $\frac{n}{200}$ for copper is sufficient to prevent growth, except in extremely rare cases, while $\frac{n}{3200}$ is usually fatal to the spores with which it comes in contact. It is possible, however, that in Bordeaux mixture the copper may enter into the formation of a complex molecule, producing in dissociation a complex ion containing copper which is inferior to the copper ion itself in toxic action. This subject, however, is now under investigation and will be treated of in a separate paper.

TABLE XXII.

	Pisum	Zea	Lupinus	Penicillium	Uromyces
HCl	$\frac{n}{12800}$	$\frac{n}{3200}$	$\frac{n}{6400}$	$> \frac{n}{50}$	$\frac{n}{50}$
H ₂ SO ₄	$\frac{n}{12800}$	$\frac{n}{3200}$	$\frac{n}{6400}$	$> \frac{n}{50}$	$\frac{n}{100}$
CuCl ₂	$\frac{n}{51200}$	$\frac{n}{102400}$	$\frac{n}{25600}$	$\frac{n}{100}$	$\frac{n}{3200}$
CuSO ₄	$\frac{n}{51200}$	$\frac{n}{102400}$	$\frac{n}{25600}$	$\frac{n}{200}$	$\frac{n}{3200}$
Cu (C ₂ H ₃ O ₂) ₂	$\frac{n}{51200}$	$\frac{n}{102400}$	$\frac{n}{25600}$	$\frac{n}{800}$	$\frac{n}{800}$
HgCl ₂	$\frac{n}{204800}$	$\frac{n}{51200}$	$\frac{n}{12800}$	$> \frac{n}{3200}$	$\frac{n}{6400}$
KCN	$\frac{n}{12800}$	$\frac{n}{6400}$	$\frac{n}{6400}$	$\frac{n}{100}$	$\frac{n}{100}$

It may be of interest to compare the results of this work on fungi with that of Kahlenberg and True on phanerogams. Table XXII brings together the results upon such substances as we have used in common, and also renders it possible to see at a glance the relative susceptibility of the fungi and phanerogams.

The first two columns are the result of work by Heald, the third that of Kahlenberg and True, and the last two are recorded

for the first time in this paper. An apparent but not real discrepancy arises from the fact that, in considering the two acids, the other authors have estimated the normal solution upon the basis of hydrogen present, whereas in mine, it is on the basis of the whole molecule. In view of this it will be seen that our results agree in making H ions as toxic in one acid as in the other and that about equal to the action of CN ions. It should be also noticed that their results represent strengths in which the plants would just grow, and mine a strength in which they would just not grow.

In general the fungi are far more resistant to action of these salts than are the higher plants. The authors quoted find copper approximately thirty-two times as toxic as hydrogen. My results show it sixty-four times as toxic for the fungi. The results with *Pisum* show Hg to be four times as toxic as copper ions, while with *Zea* and *Lupinus* the copper is twice as toxic as the Hg ion. The fungi show Hg twice as toxic as Cu. They show Cu to be about four times as toxic on *Lupinus* and *Zea* as are H or Cu ions. It would be interesting to know whether the HCN dissociated would be one half as strong as Cu, as would seem probable from theory.

TABLE XXIII.
SHOWING RATIO OF TOXIC ACTION.

	<i>Pisum</i>	<i>Zea</i>	<i>Lupinus</i>	<i>Uromyces</i>
H:CN	1:1	1:2	1:1	1:2
H:Cu	1:4	1:32	1:4	1:64
H:Hg	1:16	1:16	1:2	1:128

These results, and others along this line, are collated in table XXIII, in which hydrogen, both on account of its low atomic weight and its low toxic effect, is taken as a standard and the toxic action of other ions with various plants are represented as ratios.

It has been suggested that the toxic action may be some

function of the atomic weight of the element. To say the least, such a supposition is not strongly supported by this table.

It may be well to add that where the words killed, dead, fatal, etc., have been used it is not intended to imply that life was really extinct, but merely that it was not evident. Experiments are now under way to determine, if possible, whether spores which have been prevented from growth by a toxic solution can grow on being placed in one not toxic.

SUMMARY.

1. Mercuric chlorid is the strongest chemical used in its toxic effect upon the fungi.

2. Potassium cyanid is remarkably weak considering its great toxic action on animals.

3. Various fungi offer different resistance to poisons.

4. The limits of resistance vary in the same species.

5. Alcohol and sodium chloride have a stimulating effect.

6. In general the results are in accord with the theory of hydrolytic dissociation.

7. A chemical may be twice as powerful as another against one fungus, but acting upon another fungus an entirely different ratio may be sustained.

8. The spores of fungi are less susceptible than the roots of seedlings.

9. The Bordeaux mixture holds far more copper than would be needed if it dissociated into simple copper ions.

10. The cations Hg, H, and Cu are poisonous.

11. The anions CN, CrO_4 , Cr_2O_7 and OH are poisonous.

12. The halogen anions are not poisonous.

13. *Uromyces* offers the greatest range in its susceptibility to poisons.

14. The secondary spores of anthracnoses increase in abundance under the adverse conditions of a toxic solution.

15. Spores protected by actual contact with others may germinate and the tube may grow through a solution which in itself

would have prevented the germination of the spore had it been in contact with it.

16. Peculiar knotted or twisted hyphæ frequently result from the attempt to grow in a poisonous solution.

17. A spore may be able to germinate and grow slightly in a solution but still be unable to attain full development.

18. Potassium permanganate at certain strengths acts as a selective stain, differentiating uredo- from teleutospores of *Uromyces caryophyllinus*.

19. Bread may be moistened with a solution which prevents germination of spores. This solution may evaporate and the spores can then grow.

20. An occasional spore may germinate and grow *perfectly normally* in a solution which prevents hundreds of normal spores around it from germinating.

21. Penicillium in a nutrient medium offers greater resistance to poisons than do any of the other fungi worked upon.

22. *Uromyces* does not diminish in vigor of growth with the increased strength of the poison, but it does diminish in the percentage of spores which germinate.

In conclusion I offer my most grateful thanks to Dr. B. D. Halsted for much kind assistance and advice as well as for preliminary training in former years; to Dr. W. A. Kellerman for his courtesy in extending to me the privileges of his physiological laboratory and also for material and advice; to Mr. E. M. Wilcox for many courtesies in the laboratory.

THE UNIVERSITY OF CHICAGO.