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ON THE TOXIC EFFECT OF DELETERIOUS AGENTS ON THE GERMINATION AND DEVELOPMENT OF CERTAIN FILAMENTOUS FUNGI.[1]

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INTRODUCTORY.

THE object of the writer in undertaking the investigation, the results of which are presented in this paper, was to determine approximately the relative and absolute toxic properties of a few deleterious agents as shown by their influence on the spores, the mycelium, and the fructification of certain of the mold fungi.

In recent years the study of plant pathology has come to be one of the most important in the whole range of botanical research. It seems desirable, therefore, that all possible light should be thrown upon the toxic properties of the various agents used in combating fungus pests. It is also very desirable, from a scientific point of view, to throw all light possible upon the problem as to the *element* or *group of elements* to the influence of which the toxic properties of the compound are to be attributed.

Thanks to the progress of modern physical chemistry, plant physiologists are now enabled to make up solutions of all chemi-

[1] A thesis presented to the Faculty of Cornell University for the degree Master of Arts.

cal agents having the same number of molecules present in equal volumes of the various solutions. This is a decided step in advance, inasmuch as it enables investigators to compare the properties of a molecule of any substance with those of any other molecule, a comparison obviously impossible under the old method of making up solutions of a certain per cent. by weight. The vital error in making a comparison between percentage solutions is readily seen when we recall that a 1 per cent. solution of formaldehyde contains over eight times as many molecules per cc. as a 1 per cent. solution of mercuric chloride.

These equi-molecular solutions are made by dissolving as many grams of the compound as there are units in its molecular weight in 1000 grams of the solvent. Such a solution is termed a "*normal solution*" of that compound, and it is represented conveniently by the formula $\frac{n}{1}$. Diluted to half this strength we get $\frac{n}{2}$, or one half normal solution, and so on. Solutions stronger than normal may be made by using double or quadruple the number of grams specified to the 1000^{cc} of the solvent, giving $\frac{2n}{1}$ and $\frac{4n}{1}$, respectively, of the substance.

Still more recent chemical and physical research has shown that in the case of very many substances in solution, many of the molecules of the dissolved substance are no longer present as such, but have become divided into two or more parts. These part-molecules have been termed *ions*. To illustrate, let us suppose that 36.37 grams of pure HCl have been added to 1000 cc. of water; we know that the HCl is no longer all present as whole molecules. It fact, we have every reason to believe that about 80 per cent. of it has become *ionized* into $\overset{+}{H}$ and $\overset{-}{Cl}$ ions, the $\overset{+}{H}$ ions being charged with positive electricity, the $\overset{-}{Cl}$ ions having a corresponding negative charge. The ions of all substances capable of ionization are similarly charged, and have been named *cathions* and *anions*, respectively, from the fact that those charged with positive electricity (cathions) migrate

towards the cathode when a current of electricity is passed through the solution, while those charged with negative electricity migrate in the opposite direction towards the anode. As we have already said, a $\frac{n}{1}$ solution of HCl is about 80 per cent. ionized. This percentage of ionization increases with each increase of dilution, becoming practically complete at $\frac{n}{1000}$ in the case of HCl. Limited space forbids further discussion of this interesting and important phenomenon. For further information the reader is referred to the excellent papers by Drs. Kahlenberg and True ('96) and Mr. F. D. Heald ('96), or to the more recent text-books on physical chemistry. Suffice to say in this place that the ionization of the molecule has enabled chemists and physiologists to determine in many cases the *toxic element* or *group* in poisonous compounds. We shall have occasion to refer frequently to this matter in discussing the experimental data presented in this paper.

It has been the writer's aim to supplement the work of Drs. Kahlenberg and True, and Heald on phanerogams, and Drs. Krönig and Paul ('97) on bacteria, by applying the theory of the ionization of the molecule to the study of the physiology of fungicides.

METHODS.

Selection of forms.—In the selection of forms the following points were given particular consideration: (*a*) *regularity of germination*, (*b*) ability to grow normally in liquid media, (*c*) ability to fruit normally in a saturated atmosphere. After experimentation with a large number of forms the following were chosen as being well suited for the work: *Aspergillus flavus* (?) Link, *Sterigmatocystis nigra* v. Tieghem, *Œdocephalum albidum* Saccardo, and *Penicillium glaucum* Link. *Botrytis vulgaris* Fr. was afterwards chosen as a fifth form, it being entirely satisfactory in regard to germination and mycelial development, and especially desirable because of its semi-parasitic habit; it, however, failed to fruit in cell cultures. Pure cultures of these

molds were obtained and renewed from week to week. The spores used in inoculating the cultures in the experimental work were taken from fresh tubes in which the fungus had been growing seven to fourteen days. A solid medium made by adding 12 gm of agar to a liter of sugar beet infusion was found to be very satisfactory for stock cultures.

Selection of medium.—The object being to test the effect of the deleterious agents on the fungi under as nearly normal conditions of development as possible, the selection of a suitable medium was of primary importance. Many preliminary cultures were made with various media, including distilled water, infusions of potato, celery, sugar beet, prune, and bean (stems, pods, and mature seeds), besides various others compounded from inorganic salts, sugars, asparagin, etc. The results in germination and development were very varied. In distilled water Sterigmatocystis and Penicillium failed to germinate in 24 hours at 28° C., and of Botrytis, which did the best of the five forms, but 40 per cent. germinated in that time. Mycelial development in all was meager, and fruiting generally *nil*. Very minute quantities of the deleterious agents were found to inhibit germination, but the *death-point* of the spore was found in the case of dichloracetic acid, potassium hydroxid, and cobaltous sulfate, to be the same as in the medium finally selected. The media compounded from salts, sugars, etc., were more satisfactory; the vegetable infusions, however, were superior to all others. An infusion of sugar beet was ultimately chosen as being on the whole the medium best suited for the forms used. The fact that, in the case of the three typical poisons mentioned above, the concentration causing the death of the spore proved to be the same, whether the agent was dissolved in distilled water or the beet infusion, is very important in that it shows that this medium, which was used throughout the study, does not perceptibly change the toxic properties of these agents towards the fungus spores. The infusion of sugar beet was prepared by steeping 450 grams of the root in a liter of water for 3 hours at 100°C. It was then strained, cooled, and stirred up with the whites of two

eggs, after which it was boiled, strained, filtered, and poured into flasks. After thorough sterilization the infusion was ready for use. In order to get the greatest possible uniformity it was found desirable to make up four or five liters at a time. In this medium the spores of all the forms used germinated quite uniformly in from 3 to 8 hours (according to the species), grew rapidly, and fruited normally (except Botrytis) in from 18 to 44 hours at a temperature of 28°C.

Method of culture.—The van Tieghem hanging-drop culture was found to be entirely satisfactory. The cylinder part, which was of glass, had an internal diameter of 17.5^{mm}, and a height of 10.7^{mm}. This cell, with a capacity of over 2.5 cubic centimeters, provided an abundance of oxygen for the normal development of the fungi. The cylinders were cemented to the slip by means of beeswax, and the cell was completed by sealing the cover to the top of the cylinder by means of a ring of vaseline, applied by inverting it on a glass slip covered by a thin layer of melted vaseline. A small nick was made in this ring so that when the cover was applied a minute opening might be left through which the expanding air could pass when the cultures were placed in the thermostat. An hour later they were carefully examined, and such as had not already become hermetically sealed were now made so by tapping with a pencil over the tiny opening referred to above. This precaution prevents much trouble and loss when culturing with volatile substances. For convenience in handling and examining under the microscope two cells were placed on each slip. The cells were permanently labeled by gumming lettered and numbered labels to the ends of the slips. As a precaution against accidents duplicate cultures were not placed on the same slip.

The various dilutions in beet infusion of the compound to be tested were made up in a dozen or more bottles of about 30^{cc} capacity. Each bottle was provided with a glass rod, drawn to a blunt point, by means of which the culture drop was transferred to the cover. *Four or five drops of the same solution were then placed in the bottom of the cell.* The spores of the fungus to

be tested were transferred from a pure culture to the hanging-drop by means of a sterile platinum needle, the utmost care being taken to *prevent the adherence of the spores in bunches* in making the inoculation. The cover bearing the culture was then inverted on the cell and gently pressed until completely closed, except for the minute opening already fully described. When a set of cultures was complete all were placed in a thermostat which was kept at a constant temperature of 28°C.

Care of cells, covers, pipettes, etc.—After completion of a series of cultures, all bottles, rods, etc., were thoroughly washed and placed in running water for several hours, then dried and placed in a dry oven at 160°C. When the cultures in the cells matured, the covers were removed and the cells were thoroughly washed under the water tap, wiped, air-dried, and finally placed in the dry oven at 110°–120°C. for an hour. This insured thorough sterilization, and at the same time drove off the last trace of any volatile substance that might have escaped the washing. The covers were first boiled in strong KOH, then in several changes of water; this was followed by boiling in strong $H_2SO_4 + K_2Cr_2O_7$. They were again thoroughly rinsed and again boiled in four changes of clear water, rinsed in 95 per cent. alcohol, wiped, and sterilized at 160°C. The pipettes were cleaned by forcing water through them for an hour by attaching to the water tap. They were then sterilized in a steam sterilizer.

An occasional culture was found to be contaminated with bacteria, due no doubt to dust particles bearing spores coming in contact with the cultures in the making. Such contamination, however, by bacteria or fungi amounted to less than 1 per cent. of the cultures made after the completion of preliminary experimentation.

Vapor pressures in the cell.—It has long been known to physical chemists that every liquid has a certain vapor pressure. Pure water at standard atmospheric pressure and 28°C., has a vapor pressure of 28^{mm} of mercury. Any addition of a substance or substances to this water will lower its vapor pressure. If the substance added be hygroscopic the lowering of the vapor

pressure may be very great, as is the case with KOH and H_2SO_4. On the other hand, should the substance be quite volatile the increased vapor pressure of the substance added may more than counterbalance the lowering of the vapor pressure of the water, as is the case when ammonia or alcohol is mixed with water. To recapitulate in brief, the addition of any substance or substances to water gives a mixture with a vapor pressure at variance with that of pure water. This vapor pressure may be greater or less than that of pure water, depending on the physical properties of the substance or substances added. Thus, a hanging-drop containing KOH in a cell in which water has been placed below (as is the usual method), absorbs moisture by reason of its low vapor pressure. Indeed, there will be a constant distillation of water vapor from the water below to the culture drop, until all the water has passed up or the drop, becoming too large to "hang," falls to the bottom of the cell. With alcohol the reverse takes place. No sooner is such a culture made up than the alcohol begins to distill from the hanging-drop, and it has not, even for an hour, the concentration supposed to be present. Such a culture as a test of toxicity is valueless.

Were it possible to have all hanging-drops of exactly the same size, and exactly the same quantity of water below, we should expect uniformity in results. Such uniformity, however, would be useless, perhaps worse than useless, as it might prevent attention being called to the fundamental error of the method, viz., the use of solutions of varying vapor pressures in the same cell. As a matter of practical experience, however, it is impossible to have all hanging-drops of exactly the same size, and erratic germinations are consequently the inevitable result. To quote from one of the more recent studies, Stevens ('98) found that of four cultures of Macrosporium in a $\frac{n}{819200}$ solution of mercuric chlorid, three grew and one failed; of eight cultures in $\frac{n}{409600}$ five grew and three failed; of ten cultures in $\frac{n}{204800}$ four grew and six failed; of four cultures in

$\frac{n}{102400}$ one grew and three failed! With a strictly non-volatile substance we should expect less variation, because with less difference between the vapor pressures of the hanging-drop and that of the water below, the changes in the concentration of the deleterious agent in the hanging-drop would necessarily take place more slowly. Of the volatile properties of $HgCl_2$ we shall speak later.

In looking for a method that would meet every requirement of the case, many preliminary tests were made with all five molds and with different deleterious agents. Two such tests with potassium cyanid are here presented in detail. It is of interest to note that while KCN is not in itself a volatile compound, in aqueous solution more or less hydrolysis takes place, resulting in the formation of a corresponding amount of HCN (Shields '93), which is quite volatile. Hence, aqueous solutions of KCN are in their behavior quite typical of volatile compounds.

Column 1 gives the culture label by means of which the cultures were identified; column 2 the concentrations of KCN in the hanging-drops of the various cultures; column 3 the solution—if any—used in the bottom of the cell. "Dry" implies that no water or other solution was placed in the bottom of the cell. Under the head "germination" the percentage of spores germinated is given for three observations, at 12, 24, and 36 hours, respectively. Under "development" the length of the germ tubes of spores showing an average development is given in micromillimeters.

These data not only show that cultures having *water* in the bottoms of the cells are unreliable and vary according to the *amount* of water present, but that "dry" cells are equally unsatisfactory. Especial attention is called to the record of the cultures labeled "A10." In these the culture drop contains but one fourth of the KCN present in cultures labeled "A9," the former, however, had several drops of the solution from which the hanging-drop was made placed in the bottom of the cell instead of the customary drop of water. The striking difference in the results

ASPERGILLUS IN BEET INFUSION + KCN

Temperature 28° C.

Culture label	Str'ngth of solution	Conditions of cell	Germination			Development			Notes
			12 hrs.	24 hrs.	36 hrs.	12 hrs.	24 hrs.	36 hrs.	
			%	%	%	μ	μ	μ	
A1	−KCN	(Check culture)	100			250	∞	∞	Fruited in 20 hrs.
A7	$\frac{n}{32}$	Dry	25	100		barely germ.	45	200	
A7	$\frac{n}{32}$	"	10	100		"	40	100	
A8	$\frac{n}{32}$	One drop of water	7	100		"	120	500	
A8	$\frac{n}{32}$	" "	5	100		"	100	800	
A9	$\frac{n}{32}$	Cell half filled with water	45	100		140	∞	∞	Well fruited at 38 hrs.
A9	$\frac{n}{32}$	" " " "	70	100		150	∞	∞	" "
A10	$\frac{n}{128}$	$\frac{n}{128}$ KCN solution	0	0	75	..	..	100	
A10	$\frac{n}{128}$	" "	0	0	10	..	..	80	

PENICILLIUM IN BEET INFUSION + KCN

Temperature 28° C.

Culture label	Str'ngth of solution	Conditions of cells	Germination			Development			Notes
			12 hrs.	24 hrs.	36 hrs.	12 hrs.	24 hrs.	36 hrs.	
			%	%	%	μ	μ	μ	
E1	−KCN	(Check culture)	100			110	∞		Fruited at 24 hrs.
E7	$\frac{n}{32}$	Dry	0	5	10	...	barely germ.		No development
E7	$\frac{n}{32}$	"	0	3	10	...	"	70	
E8	$\frac{n}{32}$	One drop of water	.1	10	25	barely germ.	20	150	
E8	$\frac{n}{32}$	" "	.01	10	20	"	20	130	
E9	$\frac{n}{32}$	Cell half filled with water	2	70		8	80	∞	Fruited at 36 hrs.
E9	$\frac{n}{32}$	" " " "	10	75		15	100	∞	" "
E10	$\frac{n}{64}$	$\frac{n}{64}$ KCN solution	0	0	0	...	...	...	
E10	$\frac{n}{64}$	" "	0	0	0	...	...	..	

needs no further comment. The results with Penicillium and other forms abundantly confirmed the test, and lead to the conclusion that *when water is used at the bottom of cells containing hanging-drop cultures and solutions of substances having very high vapor pressures are being tested, erratic germinations are to be expected, and in the case of a test of any substance—particularly those of high or low vapor pressure—the toxic properties shown will be less than a correct method would indicate.*

In the regular work reported in this paper, a few drops of the same solution as that used in the hanging-drop of any particular cell was in every case placed in the bottom of that cell, thus establishing complete equilibrium of vapor pressures in the cells, and thereby preventing changes in the concentration of the solutions under test as must take place when this precaution is not observed. By this method it was found quite feasible to make hanging-drop cultures of any composition whatever, ranging from a $\frac{16n}{1}$ (70 per cent.) solution of alcohol to a $\frac{4n}{1}$ (21 per cent. solution of potassium hydroxid.

Stock solutions of chemicals.—These were prepared in all cases by a responsible chemist from the purest chemicals obtainable. It was found desirable to have stock solutions in highly concentrated form so that all necessary diluting could be made by adding beet infusion. The stock solution of HCl, for example, contained 25 grams pure HCl per 100^{cc}. $\frac{n}{1}$ HCl contains 3.58 per cent. HCl by weight. Such a solution was gotten by taking 5^{cc} of the stock solution and diluting, by adding beet infusion, to 34.9^{cc}. $\frac{n}{2}$ was gotten by diluting 10^{cc} $\frac{n}{1}$ to 20^{cc}, and so on. Chemical agents liable to deteriorate in quality (*e. g.*, KCN) were titrated by the chemist on the morning of the day on which cultures with it were made up.

Trial and regular cultures.—Inasmuch as the work was in most cases of a pioneer character, it was found desirable to make up a large number of trial cultures preliminary to the regular work in order to get some definite idea as to the toxicity of the

various chemicals to this group of plants; for it was of course early learned that the data worked out for phanerogams by Kahlenberg and True ('96) and Heald ('96) were a contrast rather than a comparison when placed beside the facts learned by experiment from the fungi. Heald ('96) called attention to this fact on finding a fungus growing vigorously in a solution of HCl that had killed the root of Pisum. The data obtained from the trial cultures proved invaluable in making up the regular cultures, as it was then possible in most cases to make up a series of twelve dilutions in no. 1 of which the spores would certainly be killed, while in no. 12 the fungus would be practically unharmed.

In the regular work all cultures were made up in duplicate, including duplicate checks in pure beet infusion. Important points were quite frequently checked over in duplicate and sometimes in quadruplicate. This was also done in the case of *unexpected* developments; for instance, the writer was surprised to find the spores of Penicillium showing so great a specific resistance to acetic acid. Repeated checking, however, proved the correctness of the first observation.

One of the most marked features of the entire work was the *regularity in results*. It is true that the cultures nearest the inhibiting point in a few cases varied to the extent that germination of a number of spores took place in one culture while the duplicate failed. Such were not considered *erratic*. If, however, both cultures of a certain strength grew and one of some weaker concentration failed, the latter would be considered erratic. Such erratic cultures, however, did not exceed a dozen in a work requiring upwards of forty-five hundred regular cultures aside from preliminary work. Such as did occur were doubtless due to oversight in cleaning the cells or other accident.

Examination of cultures, noting results, etc.—Cultures were made up in the early part of the day, and were examined at intervals of from three to six hours for the fourteen hours following, and at longer intervals until the fungus had matured, or the spores in the cultures which had failed to germinate were transferred to pure beet infusion to test their vitality. The percentage of

spores germinated in the various cultures was noted on two occasions, first when from 30 per cent. to 70 per cent. of those in the checks had germinated, and again a few hours later. A similar method was adopted to indicate the early mycelial development. At some time after germination the germ tubes showing an average development were measured and noted; this was repeated a few hours later, and sometimes a third measurement was made. The first appearance of conidia was also noted, but as this very frequently occurred at night (between 15 and 22 hours after inoculation) the point was not so well noted.

In the earlier work, the cultures in which the spores *failed to germinate* were opened at 72 hours and a number of the spores were transferred by means of a sterile platinum needle to a hanging-drop of pure beet infusion in a clean cell, in order to test whether they were killed by the agent or merely inhibited. If 1 per cent. or more survived, the culture was classed as *inhibited;* if none at all or less than 1 per cent. survived, they were classed as *killed.* In most of the work, however, the transfers were made at 48 hours, it being found that all spores that could germinate did so in less than 36 hours and usually much less.[1]

The cultures which germinated in the presence of the deleterious agent were likewise divided into two classes: (1) those which although they may have been retarded or stimulated in mycelial development by the agent, finally matured a fair crop of conidia in about the normal time; (2) those, which, although they germinated and continued to grow, presented a markedly irregular or retarded mycelial development, and generally failed to fruit. Between these two classes came—as might be supposed—a number of cultures which were very difficult to classify. In some cases there would be an apparently normal mycelial development but almost total supression of fruiting. In other cases an irregular, meager, and even yeast-like mycelium would cause surprise by finally developing a number of apparently

[1] Cultures in alcohol, formaldehyde, H_2O_2, and KCN were transferred at 72 hours, all others at 48 hours.

normal fruits. In general, a fungus was said to be "injured" by that concentration of the deleterious agent which prevented its classification in class I as above.

In discriminating between class III (inhibited spores) and class IV (killed spores) attention is again called to the importance of avoiding *bunches* of spores in making the inoculation. In many solutions such bunches — doubtless containing air — float on the surface of the drop and fail to receive the full influence of the agent. When spores from such a culture are transferred to pure beet infusion to test their vitality the bunch may be broken up in the process and the spores germinate readily while all others are dead. Much can be done with care in making the inoculation, but at best it is a serious source of error, and it has been on this account, and on account of the impossibility of transferring the spores without taking with them small quantities of the agent, that the *inhibiting concentration* rather than that causing death has been adopted as the *chief critical point* in discussing the experimental data.

To illustrate what has been said regarding the taking of notes on cultures, a typical left-hand page of the culture notebook is here reproduced. This will also aid the reader to understand the classification of the experimental data presented later. The opposite page was always reserved for more extended notes on points observed from time to time in the progress of the experiment.

Sources of error.—Before proceeding to a discussion of the experimental data it might be well to mention briefly the sources of error observed during the preliminary study and guarded against in the progress of the work.

1. Xylonite cells were found to have an injurious influence on some fungi when used for hanging-drop cultures at 28°C.

2. Bacterial contamination.

3. Lack of equilibrium in vapor pressures in the cells.

4. Deterioration of stock solutions (see details of experiment with KCN).

5. Use of impure vaseline for sealing cells.

ASPERGILLUS IN BEET INFUSION + $CoSO_4$.

Cultured at 9 a. m., Feb. 21, 1899. Temperature, 28° C.

Chart	No. of culture	Culture label	Strength of solution	Germination				Mycelial development						Fruit hrs.	Notes
				hrs.	%	hrs.	%	hrs.	μ	hrs.	μ	hrs.	μ		
I.	2479	A1	$-CoSO_4$	6	85			6	30	12	200	22	∞	22	Record of duplicates omitted to save space
	2481	A2	$\frac{n}{2048}$	6	80			6	30	12	210	22	∞	22	
	2483	A3	$\frac{n}{1024}$	6	85			6	30	12	200	22	∞	22	
	2485	A4	$\frac{n}{512}$	6	80			6	30	12	180	22	∞	27	
	2487	A5	$\frac{n}{256}$	6	75	8	100	6	25	12	170	22	∞	27	
	2489	A6	$\frac{n}{128}$	8	50	12	100			12	60	22	∞	36	
II. Intermediate	2491	A7	$\frac{n}{64}$	12	1	22	100					22	170	48	Well fruited at 72 hrs.
	2493	A8	$\frac{n}{32}$	22	1	36	2					22	15	72	Few fruits only
III.	2495	A9	$\frac{n}{16}$	[Trans	ferred	at 48	hrs. 12	hrs. 5%	germ.;	24 hrs.	many	more.]			
	2497	A10	$\frac{n}{8}$	[Trans	ferred	at 48	hrs.	Failed.]							
Class IV.	2499	A11	$\frac{n}{4}$	"	"	"	"	"							
	2501	A12	$\frac{n}{2}$	"	"	"	"	"							

6. Use of spores of uncertain age and vitality.

7. Use of culture media unsuited for the normal development of the fungi tested.

8. Imperfect sealing of the cells, due generally to the raising of the cover by expansion of contained air when the cultures were placed in the thermostat.

9. Transference of bunches of spores in making inoculations.

EXPERIMENTAL DATA AND DISCUSSION.

Details regarding the critical points (*i. e.*, concentrations causing injury, inhibition, and death) of the various fungi in the more important of the thirty-seven compounds tested may be found by referring to the diagrams prepared to accompany this paper. This device, primarily intended to conserve space by giving in a condensed form the various specific resistances of the different molds will also be useful, it is hoped, in conveying to the reader, by means of the eye, a general impression as to the relative toxic properties of the agents tested. It should be distinctly borne in mind in consulting these diagrams that each vertical line represents a *doubling* of the number of molecules present in the solutions in passing toward the right, the whole space between two vertical lines representing one concentration. The relative *average* toxic properties of the more poisonous agents are further graphically depicted by means of the two charts in the final installment of this paper.

ACIDS.

In the case of acids, diagrams have been prepared, giving the resistances of the individual molds in the eight acids. These diagrams will be found to accompany the diagrams for the various acids on pages 307–308. In these diagrams an attempt has been made to emphasize the fact that the solutions double in concentration in passing to the right, by placing at the head of the columns the proportions of molecules present in each solution in terms of x; x being in every case the number of molecules present in a $\frac{n}{262144}$ solution. Thus a normal solution

always contains $262144x$ molecules, $\frac{2n}{1}$ contains $524288x$ molecules, and so on.

Hydrochloric acid, HCl; 70, *230*, 614. This acid, on account of its very high ionization at the critical points, and because of its very wide use in physiological and chemical investigations by other workers, has been taken as a *standard* by which we shall compare the others. In making these comparisons, HCl, being the most highly ionized of all acids, is *assumed* to represent the value of ionic $\overset{+}{H}$. This is a purely arbitrary assumption, and the reader will bear in mind that when we speak of "the toxic value of ionic $\overset{+}{H}$" we mean the nearest approach we could make to determining its toxic value for the molds, viz., *that of 91% ionic* $\overset{+}{H}$ *+9% HCl.*

The following "coefficients" have been worked out for HCl with these five molds: coefficient of injury, 70; coefficient of inhibition, *230;* coefficient of death-point, 614.

These coefficients mean that *on an average for the five forms used* $\frac{70}{2048}$ of a normal solution caused distinct injury to the cultures, $\frac{230}{2048}$ of a normal solution inhibited the germination of the spores, and $\frac{614}{2048}$ of a normal solution killed the spores. The denominator of the fractions, 2048—the eleventh power of 2—has been used throughout this paper in determining coefficients. As a matter of convenience the numerator only has been expressed in discussing the three critical points of the various agents, giving as it does at a glance the correct *relative* toxicity of the agents. The *absolute* value of any coefficient may (in either mold or agent) be determined by simply supplying the omitted denominator, 2048, the resulting fraction being in every case that proportion of a normal solution. In discussing the various agents these coefficients will be placed immediately after the chemical formulæ in the order given. That expressing the inhibiting value is *italicized* to emphasize the fact that it is regarded as the most significant.

From time to time attention will be called to the wonderful resistance of the fungi to many deleterious agents as compared with that of the higher plants. The comparison (or contrast?) in resistance to ionic $\overset{+}{H}$ is as 800 : 1, the average death-point for the five species of molds being $\frac{n}{3.3}$ HCl, while that of three species of phanerogams is $\frac{n}{2740}$ HCl. (Heald, '96, p. 152.)

On the whole, HCl was at the same time the most completely ionized and the least toxic of the acids tested. Sterigmatocystis proved most resistant, for although germination and early mycelial development were distinctly retarded by $\frac{n}{1024}$, $\frac{n}{4}$ was required to inhibit all the spores, and $\frac{n}{2}$ for forty-eight hours to kill them. Cultures in $\frac{n}{16}$, although much retarded in early development, had at forty-eight hours quite overtaken the checks, and at seventy-two hours far surpassed them in amount of mycelium produced. Distinct retardation of fruiting was first noticed in $\frac{n}{128}$; $\frac{n}{16}$ required nearly double the time to mature its fruit as

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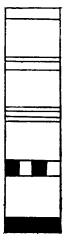
EXPLANATION OF DIAGRAMS.

The initials A., S., Œ., B., P. stand for the respective generic names of the fungi used.

The fraction of a normal solution placed at the head of a column refers to the *space between the vertical lines* over which it stands. In this space is depicted the result of culturing the various fungi in this concentration of the different agents by the symbols described below:



Two lines indicate normal or almost normal development.

Three lines indicate distinct injury.

Four lines indicate very great injury.

Alternate blocks indicate total inhibition of germination.

Solid black indicates death of spore when tested for vitality at 48 hours.

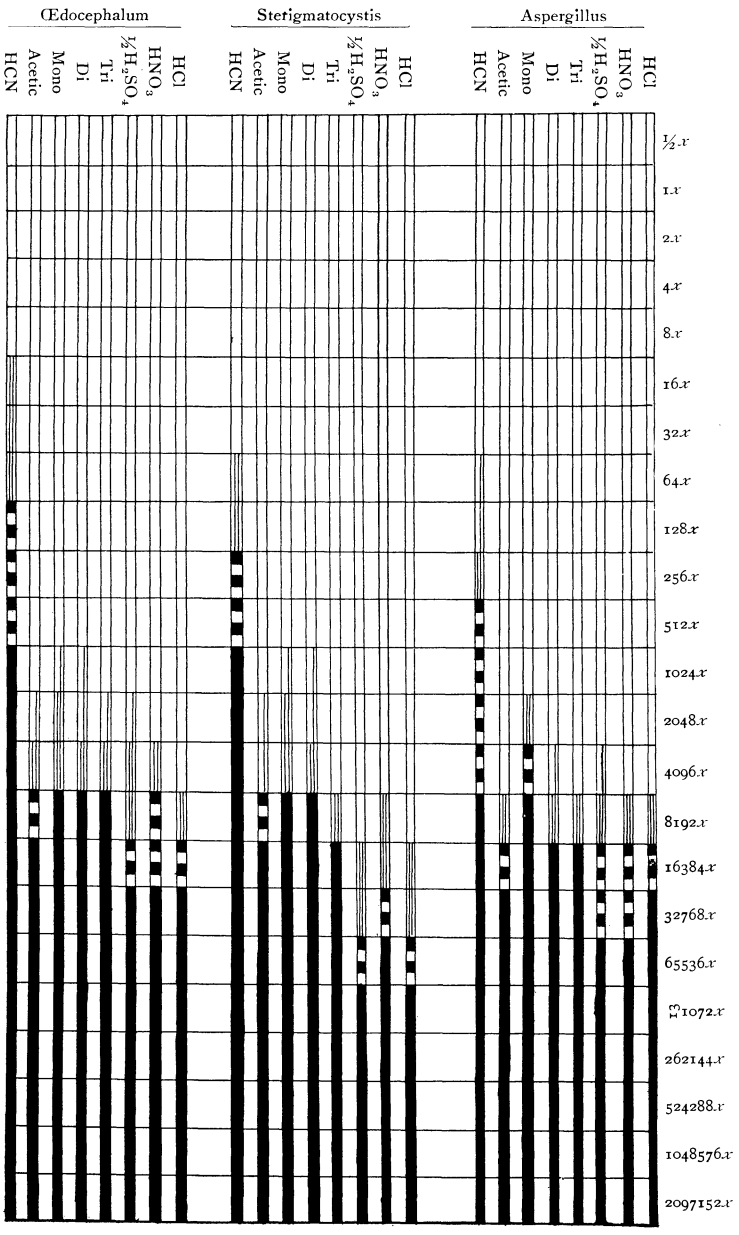
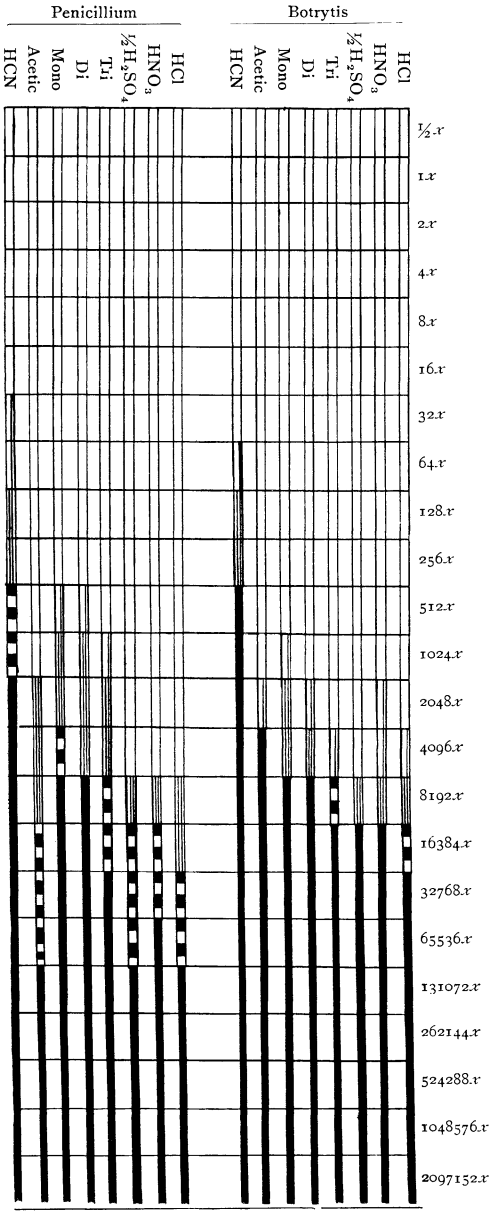


DIAGRAM I



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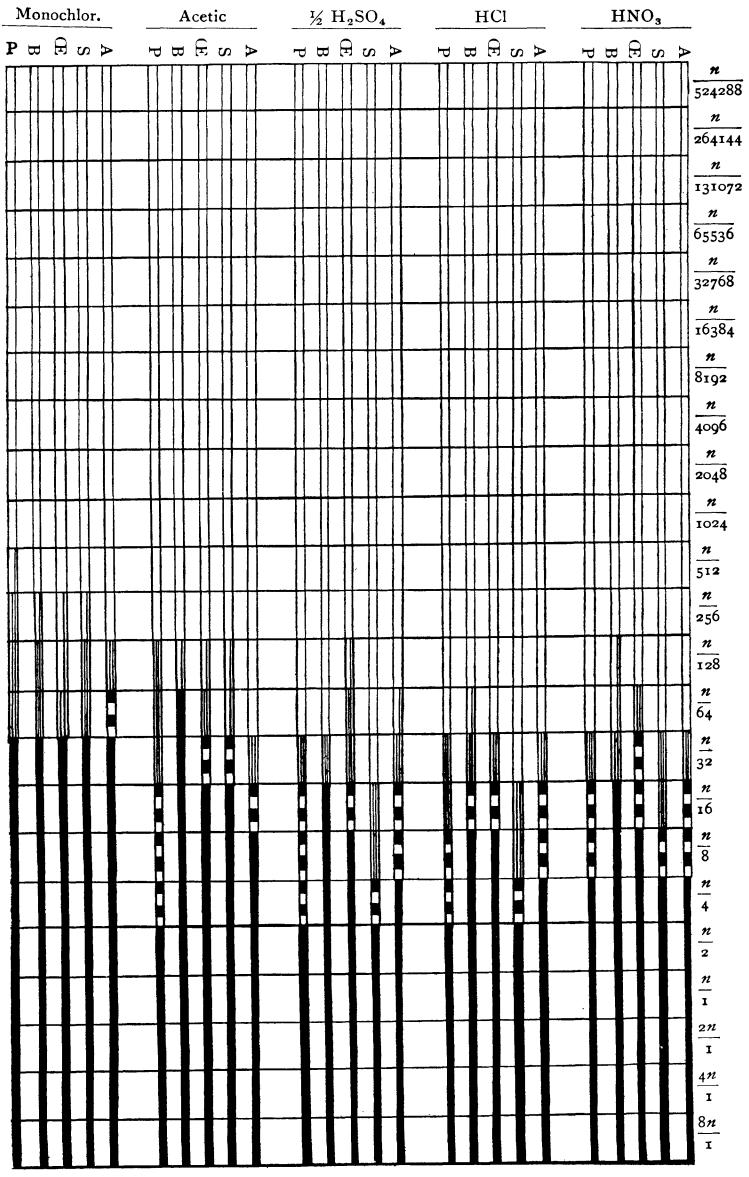

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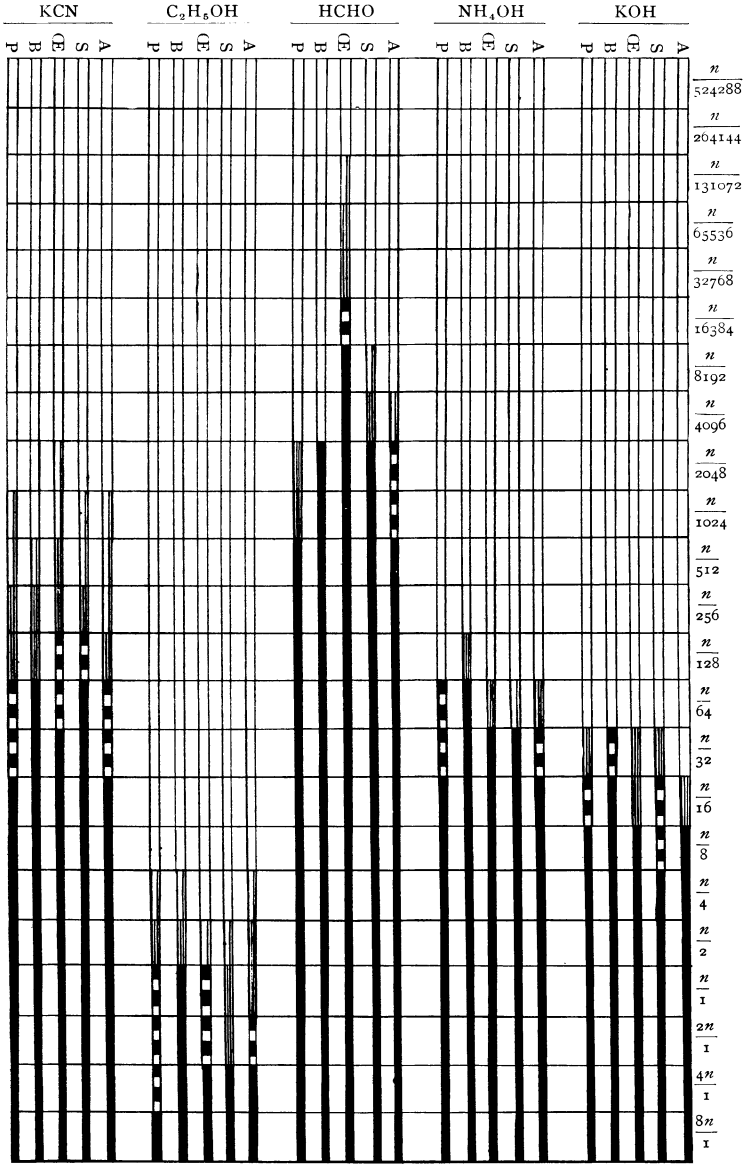

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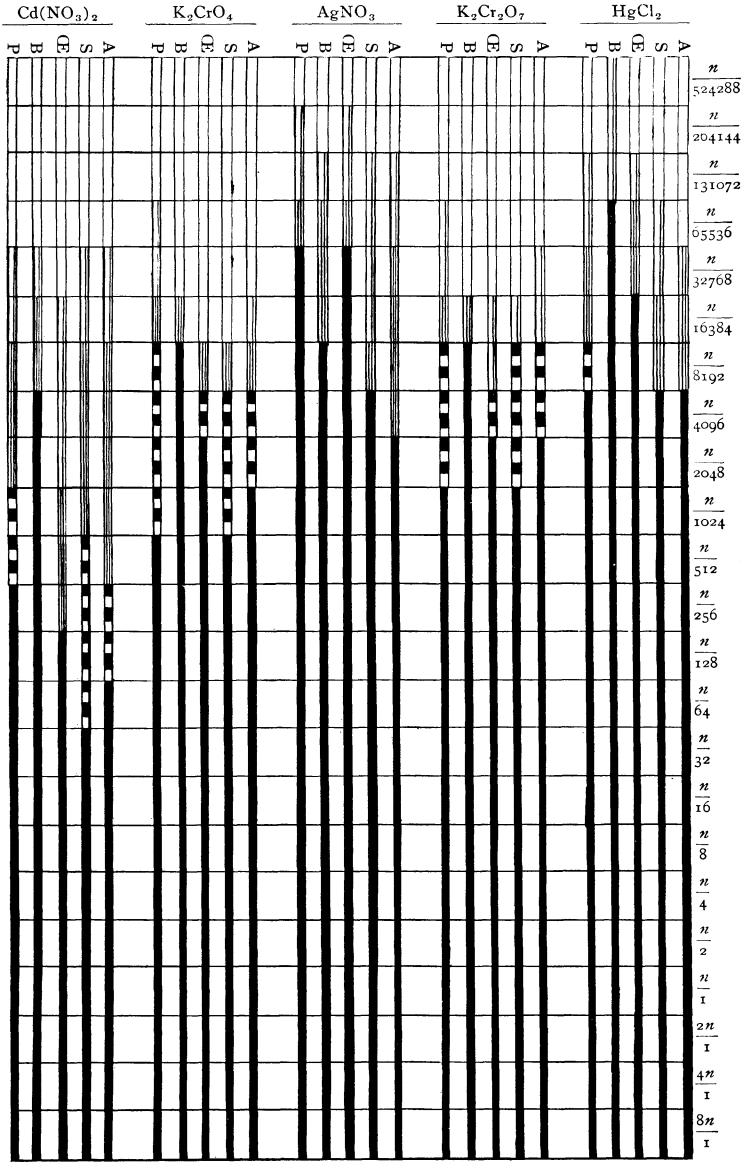


DIAGRAM V

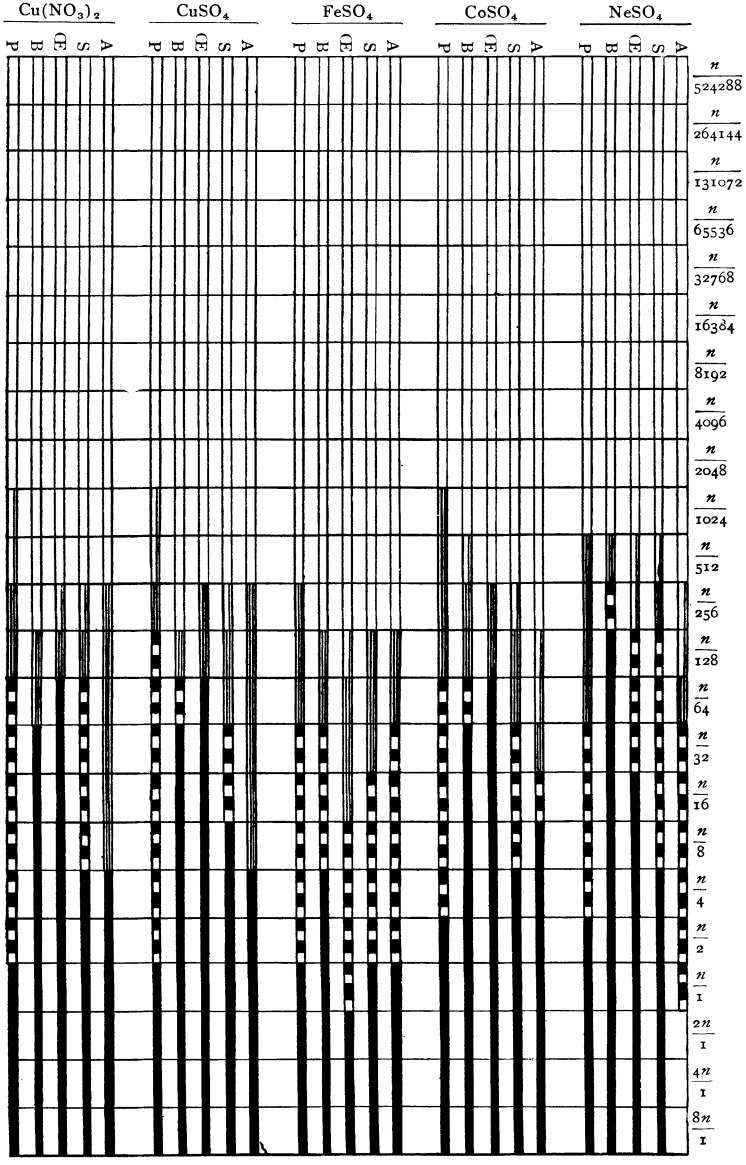


DIAGRAM VI

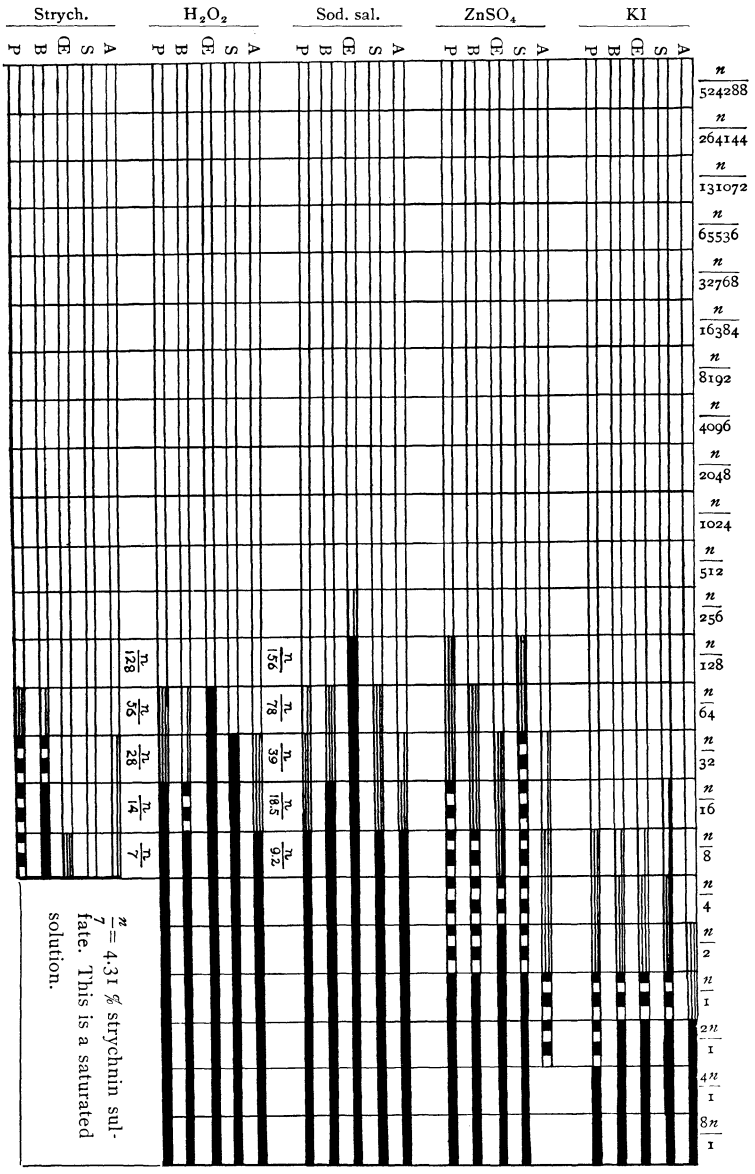


DIAGRAM VII

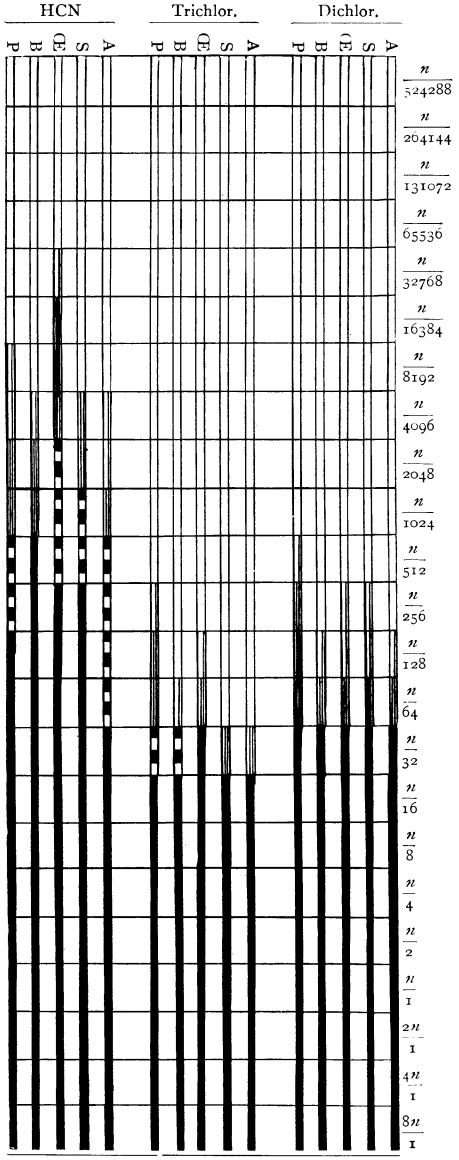


DIAGRAM VIII

compared with the checks; while $\frac{n}{8}$, the limiting culture, failed to fruit in six days.

With HCl a sixth form, *Rhizopus nigricans*, was tested. Like its near relatives the mucors, it makes very unsatisfactory growth in a liquid medium, and was therefore discarded. Its resistance to HCl proved to be practically the same as that of Œdocephalum. The limiting culture of Aspergillus, $\frac{n}{32}$, developed a number of abnormal conidiophores, each bearing 1–4 sterigmata, on the sixth day.

In the case of HCl, as in the case of all other acids tested, an attempt was made to determine the relative toxic value of the anion. The potassium salt was used. This salt becomes highly ionized into $\overset{+}{K}$ and $\overline{Cl}$ ions in moderately concentrated solutions. In a $\frac{n}{4}$ KCl solution 84 per cent. of the salt is thus ionized. In a similar concentration of HCl 89 per cent. is ionized into $\overset{+}{H}$ and $\overline{Cl}$ ions. (Kohlrausch, '85.) In the former we have $\overset{+}{K}$ and $\overline{Cl}$ ions and some KCl; in the latter, $\overset{+}{H}$ and $\overline{Cl}$ ions and some HCl. It is evident therefore that the essential difference between these solutions is the replacing of $\overset{+}{H}$ in the latter by $\overset{+}{K}$, and the HCl by KCl in the un-ionized portion. The significant point is that the *concentration of $\overline{Cl}$ ions is practically the same in both.* The $\frac{n}{4}$ HCl solution is fatal to the spores of Aspergillus, while $\frac{n}{4}$ KCl solution has practically *no injurious influence.* Indeed, this fungus germinates, grows, and fruits normally in a solution of double this concentration of KCl. Hence we know that the $\overline{Cl}$ ion is *relatively harmless* to this mold. Similar tests proved its low toxic value to all the molds used.

The absolute toxic value of ionic $\overline{Cl}$, and other weakly toxic anions, is, however, very difficult to determine. In this connection it should be remembered that the potassium salts of the various acids are, although highly, far from being completely ionized at the concentrations permitting germination of mold

spores. Then, too, we are, perhaps, not justified in assuming that ionic $\overset{+}{K}$ is entirely non-toxic; for although potassium is a necessary food element for all plants, may it not be that great concentrations of even so good a thing as ionic $\overset{+}{K}$ may be bad for fungi? Later, this will be shown to be the case with iron, an element which, while absolutely necessary in small quantities for all plants (Molisch, '94), is quite toxic in excess for both fungi and higher plants.

Experiments with Aspergillus and Œdocephalum in KCl solutions, however, enable us to say quite positively that for these forms the anion $\bar{Cl}$ has at most *less* than one thirty-second the toxic value of ionic $\overset{+}{H}$, and may therefore be disregarded in a discussion of the toxic properties of HCl.

Nitric acid, HNO_3; 48, *141*, 384. Ionized in almost the same proportion as HCl (Kohlrausch, '85), HNO_3 proved much more toxic. Inasmuch as the concentration of $\overset{+}{H}$ ions is practically the same as in HCl, and the $\bar{N}O_3$ ion is practically non-toxic, having a toxic value of less than one thirty-second that of ionic $\overset{+}{H}$, we must look for an explanation in the toxic value of the un-ionized molecule, HNO_3. This was found to be approximately 7.7 times that of ionic $\overset{+}{H}$[2]. In other words a molecule of HNO_3 loses nearly seven eighths of its toxic properties for molds on becoming ionized. Krönig and Paul ('97) found that anthrax spores immersed in a $\frac{n}{1}$ solution of HNO_3 for two hours were entirely destroyed. A similar immersion of a similar preparation of spores in the same concentration of HCl permitted the survival of 385 colonies. Preparations of spores immersed in $\frac{n}{16}$ solutions of these acids, however, showed far *less* variation in toxic properties, although the HNO_3 was still distinctly more toxic. This was evidently due to the fact that at the latter concentration the acids were both much more highly ionized,

[2] See table I, p. 325.

hence both more nearly approximated the toxic value of ionic $\overset{+}{H}$. This toxic power of the un-ionized molecule these workers termed its "specific poisonous effect."

According to this reasoning, then, were HCl and HNO_3 both completely ionized we should expect to find them equally toxic. This matter has been fully tested by Kahlenberg and True ('96). They found that toward Lupinus which is killed by a $\frac{n}{3200}$ concentration of ionic $\overset{+}{H}$, they and all other completely ionized acids with non-toxic anions had *exactly the same toxic value.*

The influence of HNO_3 on germination was much the same as that of HCl, $\frac{n}{1024}$ causing distinct retardation of both germination and early growth. Later, however, a marked difference in the appearance of the cultures manifested itself. Cultures containing the HNO_3 in $\frac{n}{32}$ to $\frac{n}{256}$ concentration produced in nearly every case a much heavier mycelium than in the corresponding cultures in HCl. Fruiting was retarded, but not so greatly retarded as was usually the case where mycelial development was so strongly stimulated.

The stimulation of mycelial development was possibly due to the non-toxic nitrogenous $\overline{N}O_3$ ion. Cultures of Aspergillus and Œdocephalum in $\frac{n}{1}$ solutions of KNO_3, however, did not establish this view, as they did not greatly differ from those in similar concentrations of KCl and K_2SO_4, although the concentration of $\overline{N}O_3$ ions would in this case be some forty times as great as in the cultures of HNO_3 showing the most stimulation. A more likely proposition is that it was due to the same factor as the increased toxicity, viz., the un-ionized molecules. The fact that the general appearance of the cultures resembled that of cultures injured by the oxidizing poisons considered later would suggest that power of the nitric acid molecule as being the active influence. The fact that fruiting was not greatly retarded, considering the abnormal mycelial development, is in harmony with this suggestion.

Sulfuric acid, ½ H_2SO_4; 61, *205*, 589. As will be observed, $\frac{n}{1}$ of this agent is based on the half molecule in order to make it strictly comparable with the other acids, the others being all monobasic.

H_2SO_4 becomes ionized first into $\overset{+}{H}$ and $\bar{H}SO_4$ ions, but as dilution increases the $\bar{H}SO_4$ ion further breaks up into $\overset{+}{H}$ and $\bar{\bar{S}}O_4$. The ionization of H_2SO_4 at the average inhibiting point is about 62 per cent. only (Kohlrausch '85). Each 100 molecules, then at this concentration breaks up into approximately 124 $\overset{+}{H}$, 76 $\bar{H}SO_4$ and 24 $\bar{\bar{S}}O_4$ ions. This solution having a greater toxic value than a similar concentration of ionic $\overset{+}{H}$ and the anion being practically non-toxic, the excess of toxic properties must be due to the partially ionized group $\bar{H}SO_4$. By referring to table I it will be seen that the toxic value of this anion is approximately 1.3 in terms of ionic $\overset{+}{H}$.

In this as in the other mineral acids, Sterigmatocystis proved the most resistant, $\frac{n}{2}$ being necessary to kill. Botrytis was the most easily killed, $\frac{n}{16}$ being fatal. Œdocephalum, although requiring $\frac{n}{8}$ concentration to kill the spores, was considerably injured by $\frac{n}{128}$, greatly injured by $\frac{n}{64}$, and produced a very light yeast-like mycelium in $\frac{n}{32}$, which on the third day practically ceased growing. On the whole, H_2SO_4 retarded germination less than HCl and HNO_3.

Acetic acid, CH_3COOH; 25.6, *83*, 314. This acid at the inhibiting point, $\frac{n}{24}$ is but 2 per cent. ionized (Kohlrausch '85). The toxic value of the anion was found to be about $\frac{1}{13}$ $\overset{+}{H}$, but as so small a proportion of the acid is ionized the influence of the anion may be disregarded. The toxic properties of this acid are

therefore to be attributed almost wholly to the un-ionized molecule, CH_3COOH, which proves to have a toxic value of 2.8.$\overset{+}{H}$.

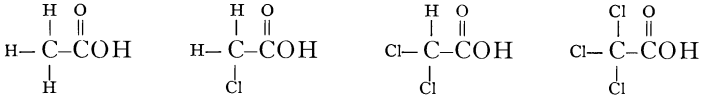
Penicillium showed a marked specific resistance to this acid, requiring $\frac{n}{2}$ for 48 hours to kill. This observation was accepted only after repeated trials. Sterigmatocystis, so resistant to the mineral acids, succumbed to $\frac{n}{16}$, and $\frac{n}{32}$ inhibited germination. Botrytis was particularly susceptible, being killed by $\frac{n}{64}$, which is but one eighth the strength of HCl required for the same result. Although so much more fatal to Sterigmatocystis and Botrytis, germination and early mycelial development was much less retarded than with the mineral acids; $\frac{n}{256}$ retarded the germination of Œdocephalum, but in the other forms this concentration had little effect.

That acetic acid should prove so much more toxic to fungi than the mineral acids was not anticipated (Migula '90). Heald ('96) found that it had but one eighth the toxic value of $\overset{+}{H}$ on Zea, and one fourth on Pisum. Kahlenberg and True ('96) found about the same relation with Lupinus. The great variation in protoplasmic resistance to this acid is well shown by the following data: the vinegar eel, *Rhabditis aceti*, thrives in a $\frac{n}{2}$ solution, which is the fatal concentration for Penicillium. Aspergillus spores are killed by $\frac{n}{8}$, those of Sterigmatocystis and Œdocephalum by $\frac{n}{16}$, and those of Botrytis by $\frac{n}{64}$. $\frac{n}{200}$ is fatal to Zea, and $\frac{n}{1600}$ to Pisum.

It is of interest to note in this connection that not only are great differences to be expected between different organisms in their resistance to deleterious agents, but different individuals of the same genus and even of the same species may have very different powers of resistance, depending largely no doubt on previous environment. Pfeffer ('95) grew Aspergillus on a nutritive medium containing 8 per cent. dextrose and 1 per cent. acetic acid, and found that the fungus assimilated a far larger amount

of acid than the dextrose. It will be noted that $\frac{n}{8}$ (0.7 per cent.) acetic acid proved fatal to the spores of Aspergillus used in this study, and in a 0.17 per cent. solution less than 1 per cent. germinated.

The chloracetic acids.—These acids are formed by the replacement of one, two, and three atoms of H, respectively, in the acetic acid radical by the element Cl; thus—



It is a rule that the halogen substitution-products of carbon compounds have a toxic value which bears a close relation to the number of H atoms in the organic radical or hydrocarbon which have been replaced by the halogen. To quote from Davenport ('97): "Beginning with methane, CH_4, we find this substance —marsh gas—innocuous when mingled with air. As the H atoms become replaced by one or more Cl atoms, the poisonous properties increase,—

CH_3 Cl is slightly anesthetic,

$CHCl_3$ = chloroform,

CCl_4 is very dangerous, stupefying involuntary muscles." Many such examples might be quoted, establishing this rule. It was a matter, then, of great surprise to find what, at first sight, seemed to be a direct exception to this rule in the action of the chloracetic acids on the mold fungi. Their critical points were determined as follows:

(Acetic acid - - - - -	25.6	*83*	314)
Monochloracetic acid - -	8.8	*58*	64
Dichloracetic acid - - -	10.4	*64*	64
Trichloracetic acid - -	37	*90*	115

As soon as the experiment was complete the stock solutions used were placed in the hands of a chemist who formed the potassium salt of each by just neutralizing with KOH. These salts are all quite highly ionized, the cathion being ionic $\overset{+}{K}$ and the anion the acid radicals of the respective acids. The toxic

values of these anions, as nearly as could be determined with Aspergillus and Œdocephalum, were as follows:

Monochloracetic - - - -	$-CH_2ClCOO$,	$\frac{1}{32}$ $\overset{+}{H}$
Dichloracetic - - - -	$-CHCl_2COO$,	$\frac{1}{21}$ $\overset{+}{H}$
Trichloracetic - - - -	$-CCl_3COO$,	$\frac{1}{11}$ $\overset{+}{H}$

Here the toxic values, although not high, are in the expected order.

Let us next glance at the toxic properties of these acids in a practically completely ionized condition. Kahlenberg and True ('96) found that with Lupinus $\frac{n}{6400}$ monochloracetic permitted growth. The same concentration of dichloracetic was evidently on the line, one plant being killed by it while the check survived. $\frac{n}{6400}$ trichloracetic proved fatal. Ionization being practically complete at this great dilution, we have to deal with ionic $\overset{+}{H}$ and the anions only. The toxic values of such solutions should be equal to that of HCl + the value of the anion. This is exactly what we find. Monochloracetic with its non-toxic anion has the same toxic value as HCl for Lupinus. The others are somewhat more toxic; due doubtless to the influence of the anions, which are apparently relatively more toxic towards the higher plants than towards the molds.

At the concentrations at which they are effective towards the fungi, however, we have an entirely different condition as regards ionization, as may be seen from the following:

Monochloracetic inhibits at	$\frac{n}{35}$	and is	20	per cent.	ionized.
Dichloracetic	" $\frac{n}{32}$	"	70	"	"
Trichloracetic	" $\frac{n}{22.8}$	"	88.8	"	"

This variation in the ionization (Ostwald '89) is the key to the explanation of this apparent deviation from the rule referred to above.

The introduction of Cl into the acetic radical results in two distinct changes in the chemical and physical properties of the acid. (1) A great increase in the toxic properties of the un-ionized molecules. (2) A great increase in the ionization of

the acid in aqueous solution of given concentration. That the great ionization often masks the effect of the increase in the toxic properties of the un-ionized part is easily understood when we recall that the toxic value of the ionized portion of an acid is not greater than the toxic value of the ionic $\overset{+}{H}$ + the toxic value of the anion.

Putting all together, we get in brief the following: (1) The replacement of one H in the acetic radical by Cl doubles the toxic properties of the un-ionized portion, and increases the ionization in a $\frac{n}{35}$ solution from 2 per cent. to 20 per cent.; the resultant of these two factors is an increase in the toxic value of about 40 per cent. in a $\frac{n}{35}$ concentration. (2) The replacement of two atoms of H by Cl more than trebles the toxicity of the un-ionized molecule and causes ionization to advance from 2 per cent. to 70 per cent. in a $\frac{n}{32}$ solution. This gives a net increase of about 30 per cent. in toxic properties at this concentration. (3) When all three H atoms are thus replaced by Cl the un-ionized molecule has a toxic value of over five times its original value and ionization advances from 2 per cent. to 88.8 per cent. in a $\frac{n}{22.8}$ solution. At this concentration the effect of the greater ionization is *more* than can be made up by the increased toxic properties of the 11.2 per cent. remaining in molecular form, hence a fall in the toxic properties of this acid at $\frac{n}{22.8}$ concentration as compared with the original acetic.

The values of the un-ionized molecules as worked out in table I, p. 325, show that these acids are very far from being an exception to the rule that the toxic properties of compounds increase with the introduction of Cl into the organic radical. These values are as follows:

Acetic acid,	2.8	times the value of ionic	$\overset{+}{H}$.	
Monochloracetic acid,	4.7	"	"	"
Dichloracetic acid,	9.5	"	"	"
Trichloracetic acid,	14.1	"	"	"

The addition of any highly ionized acid to a solution of a weaker acid throws back the ionization of both, but more particularly that of the one having the low ionization. This method of decreasing the ionization, and thereby increasing the toxic properties, was used with di- and trichloracetic acids. To solutions of these acids HCl was added — molecule for molecule — and the resulting mixture was tested with the five molds. The *calculated* increase of toxic properties due to the *forcing back of the ionization* of dichloracetic acid was found to be 168 units. The experimental test gave an increase of toxicity for the mixture of 147 units more than the *additive* toxic properties of the two acids mixed. In the case of trichloric acid the experimental test gave a slight excess (13 units) over the calculated increase.[3]

Krönig and Paul's ('97) work on anthrax spores is of especial interest here. A preparation of anthrax spores immersed in a $\frac{n}{1}$ solution of trichloracetic acid for 2 hours and afterwards cultured in a favorable medium proved to be entirely sterilized, not a spore surviving. In another test when a similar preparation of spores was immersed in the same concentration for 20 minutes, comparatively few survived. Tests were also made with the same acid at $\frac{n}{16}$ concentration. Spores immersed in this concentration for 56 hours showed much less injury than those immersed for 20 minutes in $\frac{n}{1}$ solution, thus showing clearly that the *efficacy of the acid as a disinfectant was more greatly reduced than could be accounted for by dilution only*. Their work on the other acetic acids, although not extensive, is quite in harmony with the results here recorded for the molds.

Hydrocyanic acid, HCN; 0.36, *3*, 20. This poison, so deadly to the higher vertebrates, has long been known to be much less toxic to less highly organized structures. Extremely fatal to man even in minute quantities, the more lowly organized

[3] For details of these tests and other data regarding the relation of electrolytic dissociation to the physiological action of acids, see article by the writer in Journal of Physical Chemistry **3**:263. May 1899.

Ascaris resists a 3 per cent. solution for 75 minutes. A myriapod (Fontaria) excretes HCN when irritated! The accumulation of many such data has led to the general acceptance of the theory that HCN acts chiefly or wholly upon the aldehydes of the nerve centers (Loew '93). This is no doubt quite satisfactory from the point of view of the animal physiologist, but it leaves us without any explanation for its violent toxic properties toward plants, which have no nerve centers.

Little work seems to have been done with it on plants. Kahlenberg and True ('96) found that toward Lupinus it had double the toxic value of ionic $\overset{+}{H}$, $\frac{n}{6400}$ proving fatal. To the molds, however, it is relatively a much more powerful agent, having 76.6 times the value of $\overset{+}{H}$, thus ranking as one of their most fatal poisons. The data on the ionization of this acid are meager. At $\frac{n}{32}$ it gives about one sixteenth the electrical conductivity of acetic acid at the same concentration (Ostwald '85). From this we would judge that the ionization is practically zero at the concentrations with which we have to deal.

The value of the $\overline{C}N$ ion was determined by means of the potassium salt, which is quite highly ionized (Kohlrausch '79). It was found to be approximately $8\overset{+}{H}$ for the molds. Were HCN fully ionized we would expect its solutions to have a toxic value of about $9\overset{+}{H}$. The fact that the practically un-ionized solutions with which we deal have a value of over *eight times* that calculated for the entirely ionized acid tells for the extremely toxic influence of the un-ionized molecule, HCN.

Aspergillus showed a high specific resistance to this agent, $\frac{n}{32}$ being necessary to kill all the spores. Œdocephalum was particularly sensitive, being injured by $\frac{n}{32768}$, inhibited by $\frac{n}{2048}$, and killed by $\frac{n}{256}$.

In table I the toxic values of the un-ionized molecules of the

TABLE I.

RELATIVE TOXIC PROPERTIES OF UN-IONIZED MOLECULES.

I. As measured by their inhibiting powers.

HCN	$\frac{1}{2}H_2SO_4$	HNO_3	Trich.	Dich.	Monoch.	Acetic	HCl.	Agent	
$\frac{n}{683}$	$\frac{n}{10}$	$\frac{n}{14.5}$	$\frac{n}{22.8}$	$\frac{n}{32}$	$\frac{n}{35}$	$\frac{n}{24}$	$\frac{n}{8.8}$	Concentration inhibiting germination	
7666.	112	163	255	359	396	277	100	Relative toxic value. HCl = 100	
—	62	90.6	88.8	70	20	2		Per cent. ionized at inhibiting point	
—	62	90.6	88.8	70	20	2		Toxic value of cathion in terms of ionic $\overset{+}{H}$	Ionized
—	—	—	8	3.3	.6	—		Toxic value of anion, ditto	
—	62	90.6	96.8	73.3	20 6	2		Total, cathion + anion	
7666	50	72.4	158.2	285.7	375.4	275		Residual units	Un-ionized
100	38	9.4	11.2	30	80	98		Per cent. un-ionized	
76.6	*1.3*	*7.7*	*14.1*	*9.5*	*4.7*	*2.8*		*Value of ionized molecules in terms of ionic $\dot{H}$*	

TABLE I.—(*Continued.*)

RELATIVE TOXIC PROPERTIES OF UN-IONIZED MOLECULES.

II. As measured by their killing powers.

HCN	$\frac{1}{2}H_2SO_4$	HNO_3	Trich.	Dich.	Monoch.	Acetic	HCl.	Agent	
$\frac{n}{102}$	$\frac{n}{3.5}$	$\frac{n}{5.3}$	$\frac{n}{18}$	$\frac{n}{32}$	$\frac{n}{32}$	$\frac{n}{6.5}$	$\frac{n}{3.3}$	Concentration killing spores	
3070	104	160	534	960	960	196	100	Relative toxic value. HCl = 100	
—	54.	90	88	70	19.9	1		Per cent. ionized at death-point	
—	54	90	88.	70	19.9	1		Toxic value of cathion in H	Ionized
—	—	—	8	3.3	.5	—		Toxic value of anion in H	
—	54	90	96.	73.3	20.4	1		Total cathion + anion	
3070	50	70	438	887	940	195		Residual units	Un-ionized
100	46	10	12	30	80.1	99		Per cent. un-ionized	
30.7	*1.1*	7	*36.5*	*29.6*	*11.7*	*1.97*		*Value of un-ionized molecules in terms of ionic* $\overset{+}{H}$	

different acids, as shown by their inhibiting and killing powers toward the spores of the five molds, are approximated. Too much importance must not be attached to the *exact numerical* value here expressed for the different molecules, for several reasons. We do not know the reaction of the acids toward the nutrient medium in which we grow our plants; we do not know the exact effect of the salts and sugars present on the ionization of the acids; and were it possible to eliminate every factor causing doubt or error, we should undoubtedly find the relative toxic properties of the molecules varying with almost every plant tested. Let me repeat: the exact numerical values here given are *not* significant. The order and general proportions of these values *are* significant. The emphasis is laid on part I of this table for reasons already given.

Line 1 gives the strength of the various acids required on an average to inhibit germination. Line 2 is developed from line 1. It gives the relative toxic properties of the acids expressed numerically, HCl being taken as 100 for a basis of comparison. The ionized and un-ionized portions are considered separately, lines 4, 5, and 6 being devoted to the former, and 7, 8, and 9 to the latter. Line 4 gives the toxic value of the cathion in units of ionic $\overset{+}{H}$; line 5 that of the anion. Line 6, being the total of 4 and 5, gives that portion of the total toxic value of the acid which is to be attributed to ionized portion. Line 7 gives the "residual units." In other words, that part of the total toxic value to be accounted for by the un-ionized portion. Line 8 gives the percentage of such un-ionized molecules present at the inhibiting point. Line 9, the quotient of the residual units divided by the percentage of un-ionized molecules, gives in terms of ionic $\overset{+}{H}$ the toxic value of the different acid molecules.

Part II is worked out similarly, and has reference to the toxicity of the acids towards the molds, as shown by their power to *kill* the spores.

[*To be concluded.*]