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SOME EXPERIMENTS ON THE TOXICITY OF VARIOUS CHEMICALS TO
MONILIA SITOPHILA (MONT.) SACC.

by

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1.

SOME EXPERIMENTS ON THE TOXICITY OF VARIOUS CHEMICALS TO
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From the work that has been done by various biological investigators our knowledge concerning the relations which exist between living organisms and the chemical aspect of their environment has been greatly extended. Although many of the phenomena recorded have not been finally proven to be scientific facts and many of the relations that have been established have been shown to be true only in the case of certain organisms, much valuable data has been accumulated. Many of these investigations bear upon the problem of toxicity of chemical substances. Hence, before giving an account of the experiments, which it is the purpose of this paper to present, it may be well to speak briefly of the injurious influence which certain chemical compounds have been found to exert upon living organisms.

The influence of the mineral nutrients upon growth has been the subject of much investigation. It has been found that certain of these are absolutely necessary to all life.(13) Nevertheless, most, if not all, of the chemical compounds that have been used in the preparation of nutrient solutions are at certain concentrations very injurious. There are in addition, however, many substances that are poisonous to living organisms, even in very dilute solutions.

In 1899 Clark (2) gave an account of some experiments in which he shows the toxicity of some twenty-five different

chemicals to the growth of six different molds, including Penicillium glaucum. In testing the toxicity of these substances he grew his molds on a solid medium, made by adding twelve grams of agar to one liter of sugar-beet infusion. He did not determine the toxicity of any substance ordinarily used as a nutrient. In fact, he seemed to think a nutrient could not be toxic, for he states that potassium is not toxic since it is a necessary food element.

Loeb (11) gives in a paper that he published in 1900 an account of some experiments that show the toxicity of sodium chloride to the marine fish, Fundulus. He found that this animal was able to live in sea-water to which five per cent of sodium chloride was added, but that it soon died in a pure sodium chloride solution of the same concentration as that in which this salt exists in sea-water. In a five-eighths normal sodium chloride solution, these animals lived less than twelve hours; in a one-sixteenth normal sodium chloride solution, they lived about seventy-two hours; and in pure water they were still alive after ten days when the experiment was terminated. He further learned that the addition of a small amount of potassium chloride and calcium chloride caused the toxic effects of sodium chloride to disappear. These results were in full accord with the assumption, which he had formerly made, concerning the existence of ion-proteids.

Loeb had assumed that the molecules of salts do not exist exclusively as such in the cell, but at least some

of their ions are in loose combination with proteids. He had also supposed that one kind of ion may be easily substituted for another and that by such substitution the physical properties, or at least some of the physical properties, of the proteids change. Some of the evidence that led him to make these assumptions was gained through experiments on the effects, which different ions produce, on the absorption of water by animal muscles (10). In one of these experiments he found that in a .7 per cent. sodium chloride solution a muscle would absorb about 7 per cent of its own weight of water; that in an equimolecular solution of potassium chloride it absorbed from 40 to 50 per cent. of its own weight; that in an equimolecular solution of calcium chloride it lost about 20 per cent. of its own weight.

Because of the fact that the addition of a small amount of potassium chloride and calcium chloride to a sodium chloride solution caused the toxicity of that solution to disappear, Loeb concluded that the greater part of the toxic effect of sodium chloride was due to the sodium-ions. In a later paper (12) however, he came to the opposite conclusion, for he decided that the poisonous effects of sodium chloride are largely due to the chlorine-ions. He came to this conclusion because he found that the amount of calcium necessary to destroy the poisonous effects of sodium salts having ions of different valencies, increases more rapidly than the valency increases. For example, in the case of sodium acetate, sodium sulphate and sodium citrate the antitoxic doses of calcium stand in about the relation of 1:4:16.

In some other experiments (12) Loeb found calcium sulphate effective in lessening the toxicity of sodium chloride to the developing eggs of *Fundulus*. Calcium nitrate was almost as effective for this purpose as calcium sulphate, but sodium sulphate had no antitoxic effect whatever. This and other similar experiments led him to decide that the toxic effects of salts with a monovalent kation and a monovalent anion can be destroyed only by bivalent or trivalent kations, but not by monovalent, bivalent or trivalent anions. Trivalent kations, however, were found to be more efficient than bivalent kations in producing these antitoxic effects.

Osterhout (15) has shown that pure sodium chloride solutions are poisonous to a large number of different plants, including flowering plants and algae. He found that each of the salts of sea-water is poisonous where it alone is present in solution, but that in a mixture of these salts in the proper proportions the toxic effects are mutually counteracted. Such a mixture he calls a physiologically balanced solution, and concludes that such solutions have the same fundamental importance for plants as for animals.

Osterhout (17) considered toxicity to be due to the metal-ion, and because he found in some experiments that he performed, potassium chloride and sodium chloride much alike as regards their toxicity to plants, he concludes that potassium and sodium, which are known to have very

different nutrient values, are quite similar so far as toxicity to plants is concerned. In a series of experiments (16) on wheat, he has reported antagonism between the following pairs of salts:

NH ₄ vs. Ca	NH ₄ vs. Na	Mg vs. K	Na vs. Sr
K vs. Ca	NH ₄ vs. K	Na vs. Ba	K vs. Sr
Na vs. Ca	Na vs. K	K vs. Ba	Mg vs. Sr
Mg vs. Ca			

It seems strange that Osterhout should decide that the toxicity of the salts with which he experimented is due to the metal-ion, without performing experiments similar to those that caused Loeb to reach the opposite conclusion.

Miss Magowan (14), Lipman (9), Kearney and Cameron (7) and other workers on toxicity have performed many experiments to show the injurious effects of inorganic salts to a large number of different plants. Some of these workers have also investigated the inhibiting effects of various inorganic salts on the toxicity of various other inorganic salts.

Although it has been found that the addition of a small amount of one salt to a pure solution of another salt generally lessens the toxicity of the latter, this result does not follow in all cases. Kellerman (8) reports that monocarbonate of lime seems to increase the toxicity of copper sulphate to numerous saprophytic bacteria. Lipman (9) has found in his experiments with Bacillus subtilis, that no antagonism exists between magnesium and calcium, but that the toxic effect of each is increased by the addition of the

other.

Brooks (1) has shown in some experiments, in which he used five different molds, including Monilia fructigena, and Penicillium glaucum, that in most cases the injurious action of a toxic agent increases very rapidly with a rise in temperature.

Fluri (4) reported in 1908 that the salts of aluminum render the protoplasm of Spirogyra and other water plants permeable, and are, therefore, injurious to these plants. He finds, however, that if glucose, glycerine or isodulcitol are mixed with the aluminum salt, it loses its power of rendering the protoplasm permeable. Thus the toxicity of aluminum salts to these plants depends upon the presence or absence of glucose, glycerine, or isodulcitol.

The problem of toxicity is by no means simple. A number of conditions influence the effects that are produced by various deleterious agents. The degree of purity of the toxic agent, the concentration of the toxic solution, the temperature at which the experiment is performed, and doubtless many other factors must be taken into account in interpreting the results of experiments of this nature.

The complicated nature of this problem has seldom been recognized. Those who have undertaken a study of the poisonous effects of various substances upon fungi, have generally, in the beginning of their work, determined on some suitable medium on which to grow the particular form or forms with which they have intended to experiment. To this

standard medium they have added different amounts of the several substances whose toxicity they have wished to determine. In many cases such standard media consist in part of vegetable infusions about the chemical constitution of which little is known. Clark (2), as has already been stated, used as his standard medium a sugar-beet agar. Brooks (1) used, in his work on the effects of temperature on toxicity, a sugar-beet infusion. Lipman (9) in his determination of the toxic effects of salts as related to ammonification by *Bacillus subtilis*, used a one per cent. solution of Witte's peptone. Our knowledge concerning the toxic effects of different substances upon fungi has been obtained largely through experiments of this nature.

On account of the facts reported by Fluri (4) regarding the influence of glucose and other organic substances on the toxicity of aluminum salts to *Spirogyra* as well as to other plants, it has seemed worth while to investigate the influence of certain organic substances on the toxicity of inorganic salts. It has, therefore, been the object of the work which I shall now report upon: 1) to determine the influence of various media on the growth of *Monilia sitophila* (Mont.) Sacc. and, in some cases, of a few other molds: 2) to determine the toxic effects of a number of different salts, with special reference to the influence of a few organic substances, such as are often used in the preparation of culture media.

All cultures have been grown in Petri dishes, which were previously immersed for at least ten hours in cleansing solution made according to Duggar. (3). All glassware used

in connection with any of these experiments was previously cleaned in this manner and in all cases rinsed thoroughly, first in tap-water and then several times in distilled water. In the preparation of all media redistilled water was used, except where otherwise stated. In the work on toxicity the following chemicals, all of which were obtained from Bausch and Lomb Optical Company, were used:

Urea nitrate	$\text{CO}(\text{NH}_2)_2, \text{HNO}_3$	
Aluminum nitrate	$\text{Al}(\text{NO}_3)_3, 9\text{H}_2\text{O}$	c.p.
Barium nitrate	$\text{Ba}(\text{NO}_3)_2$	c.p.
Ferric nitrate	$\text{Fe}_2(\text{NO}_3)_6, 9\text{H}_2\text{O}$	c.p.
Sodium nitrate	Na NO_3	c.p.
Potassium nitrate	KNO_3	highest purity
Ammonium nitrate	$\text{NH}_4 \text{NO}_3$	c.p.
Zinc nitrate	$\text{Zn}(\text{NO}_3)_2, 6\text{H}_2\text{O}$	c.p.
Silver nitrate	Ag NO_3	c.p.
Calcium nitrate	$\text{Ca}(\text{NO}_3)_2, 4\text{H}_2\text{O}$	c.p.
Ammonium tartrate	$\text{C}_4\text{H}_4\text{O}_6 (\text{NH}_4)_2$	c.p.
Corn starch		
Peptone		
Glucose		c.p.
Fructose		c.p.
Galactose		

A few general remarks may be made at this point concerning the mold, Monilia sitophila. One of the most striking characteristics of this fungus is its rapidity of growth and spore production. In some early experiments it seemed that its spores, when placed in nutrient agar (nutrient agar, as here used, means the kind of agar ordinarily used for growing bacteria), at room temperature, were able to germinate and produce a mycelium which, in turn, would produce more spores, all in less than 12 hours. It was later found, however, that these early spores were produced by a mycelium which came, not from a typical spore, but from large mycelial cells which are often transferred along with the spores when inoculations are made. These large cells are capable of germination and, when placed on nutrient agar, germinate several hours earlier than the normal spores.

Monilia sitophila is able to use as a partial source of its food supply a rather large number of organic compounds. Went (20) has found that it grows on media containing, together with certain mineral nutrients, any one of a large number of different substances, e.g., maltose, trehalose, raffinose, saccharose, cellulose, starch, fats and proteids. For this reason this mold is especially suitable for use in experiments where the organic part of the medium is to be varied.

The optimum temperature for this fungus, according to Went (19), is about 30° C. Nevertheless, it grows quite well at lower temperatures, and in the following experiments all cultures, whether ^{of} Monilia or other fungi, were kept at room temperature. (about 22° C.)

10.

Data.

It was found that Monilia sitophila, Mucor spinosus and an unidentified species of Aspergillus, would produce a small amount of growth on a medium made by adding 2 grams of agar threads to 100 cc of distilled water. (Growth has in all cases been measured by the diameter of the colony.) To this agar medium other substances were added in order to determine their influence on the growth of these forms. When the spores of these molds were placed on a medium, made by adding to each 100 cc of a 2 per cent. agar solution, 1 gram of potassium nitrate, 0.5 grams of acid potassium phosphate, 0.25 grams of magnesium sulphate, and a trace of iron chloride, no growth was obtained except in the case of Mucor spinosus. On this medium, Mucor spinosus made considerable growth and produced a good crop of spores. If the potassium nitrate was left out of the medium both Monilia sitophila and Aspergillus gave good growth. Mucor spinosus gave better growth than when the potassium nitrate was added. When the salts were thus added without the potassium nitrate, much better growth was secured in all cases than when 2 per cent agar alone was used. (A solution made by adding the salts mentioned above in the quantities there mentioned, to distilled water will hereafter be designated, standard solution.) This experiment was often repeated, and, although a very small amount of growth was sometimes obtained with Monilia on standard solution plus 2 per cent. of agar, the same general results were always ob-

11.

tained. It was found that when the salts of the standard solution were added directly to sufficient water to make that solution, better growth was obtained than when the different salts were first added to a small amount of water and the dilution made at a later time. It was convenient to make up the standard solution in double strength and then to add to 50 cc of such a solution 50 cc of four per cent. agar; but such a medium gave almost no growth. If two grams of agar were added directly to the standard solution, then a medium was obtained that gave somewhat better growth. In all cases where the potassium nitrate was left out, rather good growth was obtained.

This seemed to indicate that the potassium nitrate in the standard solution was detrimental to the growth of these molds. For this reason an experiment was performed, in which a number of different nitrogen containing compounds were substituted for potassium nitrate in this solution. Monilia was used for inoculating the media of this experiment. The results are shown in Table I.

Table I. Nitrogen Supply.

<u>Medium</u>				<u>Time</u>	<u>Growth</u>
Standard					
Solution	-KNO ₃	+ 2%	agar	48 hrs.	Very poor
"	"	+	" + 1% peptone	"	" " "
"	"	+	" + 1% asparagine	"	" Good
"	"	+	" + 1% ammonium tartrate	"	" Excellent
"	"	+	" + 1% " chloride	"	" Excellent

Since the addition of ammonium tartrate and ammonium chloride gives much better growth than when no nitrogen compound was used, it seems that Monilia sitophila is able to use nitrogen in the form of ammonia. None of these nitrogen containing compounds hindered the growth of Monilia. Asparagine, ammonium tartrate and ammonium chloride are positively beneficial to it, while peptone seems to have no effect one way or the other. In later experiments, however, it was found that if the amount of peptone is increased, growth will be aided.

In order to determine the influence of various organic substances on standard solution-agar, as a medium for Monilia, the cultures indicated in Table II were made:

Table II.

Medium	Growth after	Medium
Standard solu- tion. + 2 % agar	11 days.	
" + " " +5% grape sugar	-	Solid
" + " " + " lactose	+	"
" + " " + " maltose	+	"
" + " " + " starch	+	"
" + " " + " peptone	+	"
" + " " + " asparagin	+	"
" + " " + " am. tartrate	-	liquid
" + " " + " glycerine	+	solid
" + " " + " pot. bicarbonate	0	liquid
" + " " + " urea	0	"
" + " " + " pot. oxalate	-	"
" + " " + " cane sugar	+	solid
" + " " + " pot. tartrate	-	liquid
" + " " + " mannit	+	solid

Medium	Growth af-	Medium
Standard solu-	ter 11 days.	
tion, + 2% agar + 5% galactose.	+	Solid
" + " " + " gum arabic	+	"
(0 = No growth)		
(- = Little growth, i.e., colony less than 2 cm in diameter.).		
(+ = Good growth, i.e. " more " " "		
in diameter.)		

This experiment shows that the different substances added to the standard solution agar, have had considerable influence on the value of the medium. All of the media enumerated in Table II were sterilized at 100° C for fifteen minutes on three successive days. It will be noticed that in some cases the medium did not solidify and that in all such cases growth was either entirely lacking or very small in amount.

This experiment was often repeated, and it was found that the solidifying power of most of these media depended on the length of time that they were heated and the temperature to which they were brought in sterilization. The growth made by Monilia on the media that failed to solidify was in most cases quite different in amount from the growth made upon the same medium as a solid. Better growth was, in most cases, obtained when the medium was solid. When agar loses its power of solidifying, it has undoubtedly undergone some kind of chemical change. These experiments showed that in such cases the agar itself becomes a determining factor. For this reason agar was dispensed with in all subsequent experiments.

ment of the mycelium.

ed.

Culture No.	Medium	Growth after 11 days.
1.	1.33 molar solution potassium nitrate + 5% starch	-
2	1.06 " " " " + " "	-
3	.80 " " " " + " "	+
4	.53 " " " " + " "	+
5	.26 " " " " + " "	+
6	.13 " " " " + " "	+
7	5 per cent starch	+

Table IV.

It is interesting to note that after three days no growth had taken place on culture No. 1 and only a very small amount of growth had taken place on cultures No. 2, 3 and 4. At the end of 11 days, however, there was a small amount of growth on culture No. 1 and abundant growth on all media of less concentration.

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Table V.

Culture	Medium	Growth after
No.		11 days.
1	.667 molar solution of sodium nitrate	5% starch 0
2	.533 " " " " "	" " 0
3	.400 " " " " "	" " -
4	.267 " " " " "	" " +
5	.133 " " " " "	" " +
6	.067 " " " " "	" " +
7	.034 " " " " "	" " +
8	5 per cent. starch	+

Here again it was found that growth appears only after a considerable period of time on the media of higher concentrations. At the end of three days there was no growth in cultures Number 1, 2, 3 and 4 and very little growth in culture No. 5.

Table VI.

Culture No.	Medium	Growth after 7 days.
1	.187 molar solution barium nitrate + 5% starch	0
2	.156 " " " " + " "	-
3	.125 " " " " + " "	-
4	.094 " " " " + " "	-
5	.062 " " " " + " "	+
6	.031 " " " " + " "	+
7	.007 " " " " + " "	+
9	5 per cent. starch.	+

Table VII.

Culture No.	Medium	Growth after 4 days.
1	.5 molar solution of urea nitrate + 5% starch	0
2	.25 " " " " " + " "	0
3	.125 " " " " " + " "	0
4	.063 " " " " " + " "	0
5	.05 " " " " " + " "	-
6	.025 " " " " " + " "	+
7	.013 " " " " " + " "	+
8	.006 " " " " " + " "	+
9	5 per cent. starch.	+

No further changes of any consequence had taken place in the cultures enumerated in Table VII after 11 days.

Table VIII.

Culture No.	Medium	Growth after 9 days.
1	.05 molar solution of ammonium nitrate + 5% starch	0
2	.038 " " " " " + " "	-
3	.025 " " " " " + " "	+
4	.013 " " " " " + " "	+
5	.006 " " " " " + " "	+
6	5 per cent. starch.	+

Table IX.

Culture No.	Medium	Growth after 7 days.
1	.0075 molar solution of aluminum nitrate + 5% starch	0
2	.0063 " " " " " + " "	0
3	.0050 " " " " " + " "	0
4	.0038 " " " " " + " "	0
5	.0025 " " " " " + " "	0
6	.0013 " " " " " + " "	-
7	.0006 " " " " " + " "	+
8	5 per cent starch.	+

Table X.

Culture No.	Medium	Growth after 11 days
1	.00133 molar solution of ferric nitrate + 5% starch	0
2	.00067 " " " " " + " "	-
3	.00053 " " " " " + " "	0

Culture No.	Medium	Growth after 11 days
4	.00040 molar solution of ferric nitrate + 5% starch	-
5	.00027 " " " " " + " " "	+
6	.00013 " " " " " + " " "	+
7	.00007 " " " " " + " " "	+
8	5 per cent. starch	+

Culture No.2 in Table X showed a very small amount of growth, while culture No.3, which contained less ferric nitrate, showed no growth. Such variations are difficult to explain, but are frequently met with in experiments of this nature. A M/100 or a M/1000 solution of ferric nitrate when first prepared shows very little color, but after standing from one to two weeks, they both take on a reddish color. This is due to the production of colloidal ferric hydroxide, which is slowly formed in dilute solutions of ferric nitrate. For this reason fresh solutions of ferric nitrate were used in the preparation of media.

Table XI.

Culture No.	Medium	Growth after 11 days
1	.00133 molar solution of silver nitrate + 5% starch	0
2	.00067 " " " " " + " " "	0
3	.00053 " " " " " + " " "	0
4	.00040 " " " " " + " " "	0
5	.00027 " " " " " + " " "	0

19.

Culture No.	Medium	Growth after 11 days
6	.00013 molar solution of silver nitrate + 5% starch	-
7	.00007 " " " " " " + " "	+
8	5 per cent. starch.	+

No growth had taken place in cultures Number 6 and 7 after three days incubation.

Table XII.

Culture No.	Medium	Growth after 11 days.
1	.000338 molar solution of zinc nitrate + 5% starch	0
2	.000254 " " " " " " + " "	0
3	.000169 " " " " " " + " "	0
4	.000082 " " " " " " + " "	0
5	.000043 " " " " " " + " "	0
6	.000034 " " " " " " + " "	0
7	.000025 " " " " " " + " "	-
8	.000017 " " " " " " + " "	+
9	.000008 " " " " " " + " "	+
10	.000004 " " " " " " + " "	+
11	5 per cent starch.	+

Copper nitrate has been used in a number of experiments. It was not used in molar solutions and its toxicity cannot, therefore, be compared with the toxicity of other nitrates.

Table XIII.

Culture No.	Medium	Growth after 4 days.
1 .5	molar solution of ammonium tartrate	5% starch 0
2 .25	" " " " "	" " 0
3 .125	" " " " "	" " 0
4 .0625	" " " " "	" " 0
5 .0500	" " " " "	" " 0
6 .0250	" " " " "	" " -
7 .0125	" " " " "	" " +
8 .0063	" " " " "	" " +
9	5 per cent. starch	+

No growth of any consequence took place in any of the cultures of Table XIII after an incubation of eleven days.

Table XIV.

Valence of kation. Toxic concentration of salt*.

monovalent	1.33	molar solution of potassium nitrate
divalent	.467	" " " calcium "
monovalent	.40	" " " sodium "
divalent	.156	" " " barium "
monovalent	.050	" " " urea "
monovalent	.038	" " " ammonium "
trivalent	.0013	" " " aluminum "
trivalent	.00067	" " " ferric "
monovalent	.00013	" " " silver "
divalent	.000025	" " " zinc "
monovalent	.02500	" " " ammonium tartrate

*The toxic concentration of a salt, as here used, means the greatest concentration in which growth has been obtained.

Table VIII of the above series shows ammonium nitrate to be very toxic. Only a small amount of growth was obtained on a medium containing less than .3 of one per cent. of this salt. Ritter (18) has found that the ammonium salts of mineral acids as a source of nitrogen for molds is inverse to the strength of the acid forming the negative ion of the salt. He attributes this to the toxic effect of the acid liberated through the assimilation of the ammonium-ion. For example, he found monoammonium or diammonium phosphate to be far better as a source of nitrogen than the ammonium salts of sulphuric, hydrochloric, or nitric acid.

Table XIV of the above series shows in the case of each salt the greatest concentration at which growth was obtained when the salt was offered in a starch medium. The nitrates are given in the order of their toxicity. A comparison of equimolecular concentrations shows that of all the nitrates used, potassium nitrate is the least toxic and zinc nitrate the most toxic to Monilia on a 5 per cent starch medium. It is plain that so far as the nitrates are concerned, there is no relation between toxicity and the valence of the kation or its atomic weight. Silver, which has an atomic weight of

107.93 and is monovalent is, with the exception of zinc, the most toxic of all the elements used as nitrates in molar solutions. But aluminum nitrate, the kation of which has an atomic weight of only 27.1 and is trivalent, is almost as toxic.

Having thus determined the degree of concentration at which the different salts enumerated in Table XIV are toxic to Monilia sitophila in starch media, it was decided to vary the organic part of the medium for the purpose of determining whether or not such substances as starch, peptone and sugar have any influence upon the toxicity of some of these salts.

Table XV.

Culture No.	Medium	Growth after 11 days.
1	.167 molar solution of barium nitrate + 5% peptone	0
2	.133 " " " " " + " "	0
3	.067 " " " " " + " "	-
4	.033 " " " " " + " "	+
5	.017 " " " " " + " "	+
6	.007 " " " " " + " "	+
7	5 per cent peptone.	+

There was considerable precipitate in all of the media of Table XV.

Table XVI.

Culture No.	Medium	Growth after 11 days.
1	.167 molar solution of barium nitrate + 5% glucose	-
2	.133 " " " " " + " "	-
3	.1 " " " " " + " "	-

Culture No.	Medium	Growth after 11 days.
4	.067 molar solution of barium nitrate + 5% glucose	-
5	.033 " " " " " + " "	-
6	.017 " " " " " + " "	+
7	.007 " " " " " + " "	+
8	.005 " " " " " + " "	+
9	.004 " " " " " + " "	+
10.	5 per cent. glucose.	+

No concentration of barium nitrate shown in Table XVI was of sufficient strength to prohibit growth. There was, however, a very small amount of growth on culture NO. I of that table. This indicates that the toxic dose of barium nitrate in this medium is not of much greater concentration than .167 molar. There was no precipitate in any of the media shown in Table XVI.

Table XVII.

Culture No.	Medium	Growth after 11 days.
1	.167 molar solution of barium nitrate + 5% fructose	-
2	.133 " " " " " + " "	-
3	.1 " " " " " + " "	-
4	.067 " " " " " + " "	-
5	.033 " " " " " + " "	-
6	.017 " " " " " + " "	-
7	.007 " " " " " + " "	+
8	.005 " " " " " + " "	+
9	.004 " " " " " + " "	+

Culture No.	Medium	Growth after 11 days.
10.	.003 molar solution of barium nitrate + 5% fructose	+
11	5 per cent. fructose	+

The small amount of growth obtained on culture No.1 of the above Table indicates that a slightly greater concentration would have prohibited growth. Barium nitrate was not offered in concentrations greater than .167 molar in a fructose medium. There was a slight precipitate in all the media of Table XVII.

Table XVIII.

Culture No.	Medium	Growth after 11 days.
1	.167 molar solution of barium nitrate + 5% galactose	0
2	.133 " " " " " + " "	0
3	.1 " " " " " + " "	0
4	.067 " " " " " + " "	-
5	.033 " " " " " + " "	-
6	.017 " " " " " + " "	+
7	.007 " " " " " + " "	+
8	.005 " " " " " + " "	+
9	.004 " " " " " + " "	+
10	.003 " " " " " + " "	+
11	5 per cent. galactose	+

There was no precipitate in any of the medium described in Table XVIII.

Table XIX.

Culture No.	Medium	Growth after 11 days
1	.167 molar solution of aluminum nitrate + 5% peptone	0
2	.133 " " " " " + " "	0
3	.1 " " " " " + " "	0
4	.067 " " " " " + " "	0
5	.033 " " " " " + " "	0
6	.017 " " " " " + " "	-
7	.007 " " " " " + " "	+
8	.004 " " " " " + " "	+
9	.003 " " " " " + " "	+
10	5 per cent. peptone	+

There was a slight precipitate in cultures No. 6-9 inclusive.

Table XX.

Culture No.	Medium	Growth after 11 days
1	.167 molar solution of aluminum nitrate + 5% glucose	0
2	.133 " " " " " + " "	0
3	.1 " " " " " + " "	0
4	.067 " " " " " + " "	0
5	.033 " " " " " + " "	0
6	.017 " " " " " + " "	0
7	.006 " " " " " + " "	0
8	.003 " " " " " + " "	0
9	.0007 " " " " " + " "	-
10	5 per cent. glucose	+

There was no precipitate in any of the media of Table XX.

Table XXI.

Culture	Medium							Growth after
No.								11 days.
1	.167 molar solution of aluminum nitrate + 5% fructose							0
2	.133	"	"	"	"	+	"	0
3	.1	"	"	"	"	+	"	0
4	.067	"	"	"	"	+	"	0
5	.033	"	"	"	"	+	"	0
6	.017	"	"	"	"	+	"	0
7	.007	"	"	"	"	+	"	0
8	.002	"	"	"	"	+	"	0
9	.001	"	"	"	"	+	"	0
10	.0007	"	"	"	"	+	"	-
11	5 per cent. fructose							+

There was no precipitate in any of the media
of Table XXI.

Table XXII.

Culture	Medium							Growth after
No.								11 days.
1	.167 molar solution of aluminum nitrate + 5% galactose							0
2	.133	"	"	"	"	+	"	0
3	.1	"	"	"	"	+	"	0
4	.067	"	"	"	"	+	"	0
5	.033	"	"	"	"	+	"	0
6	.017	"	"	"	"	+	"	0
7	.007	"	"	"	"	+	"	0
8	.004	"	"	"	"	+	"	0
9	.003	"	"	"	"	+	"	0

Culture No.	Medium	Growth after 11 days.
10	.0013 molar solution of aluminum nitrate + 5% galactose	-
11	.0007 " " " " " + " "	+
12	5 per cent. galactose	+

There was no precipitate in any of the media of Table XXII.

Table XXIII.

Culture No.	Medium	Growth after 11 days.
1	.0067 molar solution of ferric nitrate + 5% peptone	+
2	.0053 " " " " " + " "	+
3	.004 " " " " " + " "	+
4	.0027 " " " " " + " "	+
5	.0013 " " " " " + " "	+
6	.0007 " " " " " + " "	+
7	.0004 " " " " " + " "	+
8	.0003 " " " " " + " "	+
9	.0001 " " " " " + " "	+
10	5 per cent. peptone	+

There was much precipitate in all media of Table XXIII, except in the case of the medium of culture No. 10, which showed a slight precipitate.

Table XXIV.

Culture No.	Medium	Growth after 11 days.
1	.00667 molar solution of ferric nitrate + 5% glucose	0
2	.00533 " " " " " + " "	0
3	.00400 " " " " " + " "	0

Culture No.	Medium	Growth after 11 days.
4 .00267	molar solution of ferric nitrate + 5% glucose	0
5 .00133	" " " " " + " "	0
6 .00067	" " " " " + " "	0
7 .00053	" " " " " + " "	-
8 .00040	" " " " " + " "	-
9 .00027	" " " " " + " "	-
10 .00013	" " " " " + " "	+
11 .00007	" " " " " + " "	+
12	5 per cent. glucose	+

There was slight precipitate in the first five cultures of Table XXIV. In the other cultures there was no precipitate.

Table XXV.

Culture No.	Medium	Growth after 11 days.
1 .0067	molar solution of ferric nitrate + 5% fructose	0
2 .0007	" " " " " + " "	-
3 .0001	" " " " " + " "	+
4 .	5 per cent. fructose	+

There was slight precipitate in all media of Table XXV, except the medium of culture No. 4.

Table XXVI.

Culture No.	Medium	Growth after 11 days.
1 .00667	molar solution of ferric nitrate + 5% galactose	0
2 .00533	" " " " " + " "	0
3 .00400	" " " " " + " "	0

Culture No.	Medium	Growth after 11 days.
4 .00267	molar solution of ferric nitrate + 5% galactose	0
5 .00133	" " " " " + " "	0
6 .00067	" " " " " + " "	0
7 .00053	" " " " " + " "	0
8 .00040	" " " " " + " "	-
9 .00027	" " " " " + " "	+
10 .00013	" " " " " + " "	+
11 .00007	" " " " " + " "	+
12	5 per cent. galactose	+

There was a slight precipitate in the first eight cultures of Table XXVI. In the other cultures there was no precipitate.

Table XXVII.

Culture No.	Medium	Growth after 11 days.
1 .133	molar solution of ammonium tartrate + 5% peptone	0
2 .067	" " " " " + " "	0
3 .060	" " " " " + " "	0
4 .053	" " " " " + " "	0
5 .047	" " " " " + " "	0
6 .040	" " " " " + " "	0
7 .033	" " " " " + " "	0
8 .027	" " " " " + " "	-
9 .020	" " " " " + " "	-
10 .013	" " " " " + " "	-

Culture No.	Medium	Growth after 11 days.
-------------	--------	-----------------------

11	.007 molar solution of ammonium tartrate 5% peptone	
----	---	--

12	5 per cent peptone	
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There was no precipitate in any of the cultures of Table XXVII, except in Nos. 7, 8, 9, and 10.

Table XXVII.

Culture No.	Medium	Growth after 11 days.
-------------	--------	-----------------------

1	.2 molar solution of urea nitrate + 5 % peptone	-
---	---	---

2	.133 " " " " " + " "	+
---	----------------------	---

3	.067 " " " " " + " "	+
---	----------------------	---

4	.033 " " " " " + " "	+
---	----------------------	---

5	.027 " " " " " + " "	+
---	----------------------	---

6	.020 " " " " " + " "	+
---	----------------------	---

7	.013 " " " " " + " "	+
---	----------------------	---

8	.007 " " " " " + " "	+
---	----------------------	---

9	5 per cent. peptone	+
---	---------------------	---

There was no precipitate in all the cultures of Table XXVIII.

Table XXIX.

Toxic concentration*	Salt.	Organic constituent of medium.
----------------------	-------	--------------------------------

.156 molar	barium nitrate	starch
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.067 "	" "	peptone
--------	-----	---------

.167 "	" "	glucose
--------	-----	---------

.167 "	" "	fructose
--------	-----	----------

.067 "	" "	galactose
--------	-----	-----------

.0013 "	aluminum nitrate	starch
---------	------------------	--------

.017	molar	aluminum nitrate	peptone
.0007	"	" "	glucose
.0007	"	" "	fructose
.0013	"	" "	galactose
.00067	"	ferric nitrate	starch
.0067**	"	" "	peptone
.00053	"	" "	glucose
.0007***	"	" "	fructose
.0004	"	" "	galactose
.05	"	urea nitrate	starch
.2	"	" "	peptone
.025	"	ammonium nitrate	starch
.027	"	" "	peptone

* The toxic concentration of a salt is here taken to mean the greatest concentration that permits growth.

** Higher concentrations of ferric nitrate were not used in peptone media.

*** Not determined within narrow limits.

Table XXIX shows the greatest concentration at which barium nitrate, aluminum nitrate and ferric nitrate permit growth in media containing 5 per cent. starch, 5 per cent peptone, 5 per cent. glucose, 5 per cent. fructose or 5 per cent. galactose.

Table XXIV shows that growth took place in a medium containing 5 per cent. peptone in a 0.0067 molar solution of ferric nitrate, while Table X shows that in 5 per cent. starch no growth was obtained on media containing much less ferric nitrate.

An experiment has been performed for the purpose of determin-

ing the influence of peptone on the toxicity of ferric nitrate in 5 per cent. starch media.

Table XXX.

Culture No.	Medium	Growth after 11 days.
1 1 % peptone + 5 % starch + .0067 molar ferric nitrate		0
2 2 % " + " " + .0067 " " "		-
3 3 % " + " " + .0067 " " "		-
4 4 % " + " " + .0067 " " "		+
5 5 % " + " " + .0067 " " "		+
6 6 % " + " " + .0067 " " "		+
7 7 % " + " " + .0067 " " "		+
8 8 % " + " " + .0067 " " "		+
9 8 % " + " " + .0067 " " "		+
10 5 % starch + .0067 " " "		0

Summary.

In the following paragraphs, some of the more important results of this paper are enumerated.

1. If the toxic concentration of zinc nitrate in starch media be taken as 1, then the toxic concentrations of the other nitrates in the same kind of media may, by comparing equimolecular concentrations, be expressed approximately by the following numbers: silver nitrate, 5; ferric nitrate, 26; aluminum nitrate, 52; ammonium nitrate, 1520; urea nitrate, 2,000; barium nitrate, 6240; sodium nitrate, 16,000; calcium nitrate, 18,680; and potassium nitrate, 53,200.

2. The toxicity of the nitrates used does not vary as the valence or as the atomic weight of the kation.

3. The spores of Monilia germinate and produce growth only after they have lain for several days on starch media containing calcium nitrate at a concentration of 0.467 molar. The same is true of starch media containing sodium nitrate at a concentration of 0.4 molar, or silver nitrate at a concentration of 0.00013 molar. This indicates that the spores are able to accommodate themselves to the toxicity of these salts.

4. The toxicity of barium nitrate, aluminum nitrate, ferric nitrate and urea nitrate to Monilia depends on the organic substance contained in the medium in which these salts are offered.

5. Barium nitrate is more toxic in peptone media than in starch media.

6. Barium nitrate has practically the same toxicity in starch media that it has in either glucose or fructose media. Its toxicity in peptone is the same as its toxicity in galactose.

7. Aluminum nitrate and ferric nitrate are more toxic in starch media than in peptone media.

8. The toxicity of aluminum nitrate in galactose is the same as its toxicity in starch.

9. The toxicity of aluminum nitrate in fructose is much greater than in peptone.

10. The toxicity of ferric nitrate is practically the same in starch as in glucose, fructose, or galactose. Its toxicity in peptone, however, is much less than in any of these substances.

11. Barium nitrate is more toxic in peptone media than in starch media, while aluminum nitrate and ferric nitrate are more toxic in starch media than in peptone media.

12. The toxicity of aluminum nitrate in glucose or fructose is greater than in peptone, while the toxicity of barium nitrate in glucose or fructose is much less than in peptone.

13. Urea nitrate is four times as toxic in starch as in peptone.

14. The toxicity of ammonium tartrate is practically the same in starch as in peptone.

Discussion.

Thus it is shown that organic substances are capable of varying the toxicity of inorganic salts in much the same way that inorganic salts have been found to vary the toxicity of other inorganic salts. For this reason simple synthetic media should be used in all experiments where the object is to determine the concentration at which a given substance is toxic to a fungus. In no case should media containing vegetable infusions be used, because such infusions contain varying amounts of many different organic substances as well as inorganic salts.

It should be remembered that certain reactions may, and often do take place, when an inorganic salt is added to a solution containing an organic substance, and that the compound or compounds resulting from such reactions may themselves be toxic, or may influence the toxicity of the inorganic salt. It is, therefore, incorrect to say that, in experiments like

those above described, the toxic effects observed are directly due to the inorganic salt, or to one or both of its ions.

But it is, nevertheless, true that the inorganic salt is, at least indirectly, the cause of the effects observed. Only a trace of silver nitrate, less than one one-thousandth of one per cent. will, when added to a five per cent. starch medium, prevent growth of Monilia. On the other hand, some of the nitrates, such as calcium and sodium nitrate, are not very toxic. The toxicity varies as the metal-ion is varied, and with this fact in mind it is difficult to refrain from the conclusion that it is chiefly the metal-ion that produces the deleterious effects. This is exactly what most workers on toxicity have concluded, but as has already been pointed out, Loeb (12) came to the opposite conclusion.

It is, however, possible to explain the variation in the toxic action of the several salts of a given acid when used in equimolecular solutions, without deciding that the toxicity is, in each case, largely due to the metal-ion. If it be assumed that the different nitrates, when in solution, enter the protoplasm of the cell with equal facility, then it must be concluded that the metal-ion, either alone or in combination with its anion, either directly or indirectly, causes the variation in the toxicity of the several salts. But for what reason should this assumption be made? The phenomenon of selective absorption has often been noted in plants, and it is not impossible to imagine that the variation in the harmful effects of various chemical agents upon plants is, at least in part, due to a variation in the capacity of a plant for absorbing these different salts. Monilia sitiphila is

able to grow on a medium containing a rather large amount of potassium nitrate, but this does not prove that potassium nitrate is less toxic than the other nitrates used. It may only indicate that the protoplasm absorbs a very small amount of this substance. On the other hand, the great toxicity of silver nitrate may be accounted for if it be supposed that the protoplasm readily absorbs this substance. There is thus a possibility that the variation in toxicity may be due to a variation in the extent to which each substance is absorbed by the cell. In this way we may explain the variation shown by the different salts of a given acid. This explanation does not attempt to show why a salt when absorbed by the cell produces harmful effects on its protoplasm.

What takes place within the cell when a toxic substance is absorbed is not known and thus the problem as to why certain substances, in small quantities, are detrimental to life, is still unsolved. The ion-proteid theory of Loeb attempts an explanation. This theory assumed that the ions of the absorbed salt enter into combination with certain proteids and that the physical properties of these ion-proteids, substances that are essential constituents of living protoplasm, vary as the salt, whose ions enter into such combinations, is varied.

But there are undoubtedly other ways of explaining toxicity. It has occurred to the writer as being entirely possible that the different nitrates, at their toxic concentrations, influence the power of the protoplasm to absorb water and other substances. As has already been stated, Loeb (10) found that different salts influence the power of muscles to absorb water.

It may be that when the cell has taken up a certain amount of a given nitrate it loses its power of absorbing water and food from the particular medium upon which it is placed. Some spores of Monilia were placed in a medium which was made by adding five grams of corn starch to ninety-five cubic centimeters of a 0.003 molar solution of aluminum nitrate. Upon this medium no germination had taken place after fifteen days, but spores placed in a medium made by using a 0.001 molar solution of aluminum nitrate germinated and produced a very small amount of growth. That the failure of the spores to germinate on the medium containing the greater amount of aluminum nitrate is not due to their death, is shown by the fact that when such spores are placed in a nutrient agar medium, they germinate in less than forty-eight hours. The same results were obtained when spores were taken from starch media containing other nitrates, in such concentration that no germination had taken place after fifteen days. This shows that the failure of the spores to germinate is the presence of small quantities of some of the different chemical substances used is not due to the death of their protoplasm. It is entirely possible that in certain concentrations the nitrates are detrimental to the growth of Monilia sitophila, not because they are directly harmful to the protoplasm of that mold at the concentrations used, but because they, in some manner, effect its absorptive powers. It may be that the protoplasm of a cell is so changed by the absorption of a certain amount of aluminum nitrate that it is no longer able to absorb water and other substances, which are

essential to its growth and development.

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