ON THE RELATION OF CONCENTRATION OF SOLUTION AND TIME OF EXPOSURE TO THE TOXIC ACTION OF SODIUM CHLORIDE AND COPPER SULPHATE ON LUPINUS ALBUS AND SPIROGYRA.

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INTRODUCTION AND OBJECT.

The object of this research was mainly to find some relation between the strength of the solution and the length of the resistance period. Although a great deal of work has been done along this line of electrolytic dissociation with many chemicals and with both plants and animals, such as Lupinus albus L., Pisum sativum, Tea mais and Curcurbita pepo and Spirogyra among plants and Crustaceae, small fish, frogs and bacteria, no systematic results with one or a few chemicals have been obtained.

The theory of electrolytic dissociation was first formulated by Arrhenius (1) in working with molecular weights by freezing point methods. The relation of this theory to the toxic action exerted on plants was first demonstrated experimentally by a long series of experiments on plants by Kahlenberg and True.(2) They used Lupinus albus L. for their experiments and pointed out the fact that the action of antiseptics was due in many cases to the dissociation of the molecules and that the mere presence of a metal or other element in a solution does not decide its physiological action. Heald(3) repeated many of the experiments of Kahlenberg and True and confirmed the conclusions obtained by these authors. Heald used other plants, Pisum sativum, Zea mais, and Curcurbita pepo. Several investigations have been made showing the action of antiseptics first by Paul and Kroenig(4) on bacteria and also showed that Kahlenberg and True were justifiable in their conclusions. R. H. True and C. G. Hunkel(5) in a series of experiments on the poisonous effect exerted on living plants by phenols, show that the toxic effect is influenced by the dissociation of the molecules. The number of hydroxyl(OH) groups seem to have little to do with the toxic action of the phenols. The introduction of the methyl(CH_3) or the Nitro(NO_2) group seem to increase the toxicity. The action of antiseptics on algae has been worked upon by R. H. True(6) using Spirogyra which is very sensitive to acids but not so much to the phenols as the Lupines. In dilute solutions of acids for Spirogyra as well as the Lupines the number of hydrogen ions present is a measure of the toxic action of these solutions. Robert Gay made a series of experiments with hydroquinone and found that in solutions containing from 1/500 to 1/1500 grm. mol. per liter. it had a marked toxic effect on Staphylococcus pyogenes aurens. Coupin(7) discusses the toxic equivalent of NaCl or sea water for maratime and interior plants; maratime plants being able to stand much more salt than interior plants.

METHODS.

The problem is to find some relation between the toxic action and the dilution of the solutions and the length of the time that the Lupines are exposed in solutions of Sodium Chloride, a very weak poison, and solutions of Copper Sulphate, a strong poison representing the heavy metals.

The solutions were all made up upon the basis of gram molecules, of the pure salts, per liter of distilled water, Stock solutions of NaCl four normal in strength and of $CuSO_4$, one-hundredth of a gram molecule per liter, were made up. The solutions used were diluted from the stock solutions as needed.

The Lupines were grown according to the usual method. A number of Lupines were soaked over night in water and then planted. They were placed with the hilum downward on a sheet of cork, which had holes bored in it, and placed over a beaker of water, covered over with absorbant cotton to keep them moist and placed in a dark place which had an even temperature. After the tips were of the required length the seedlings were taken out, dried superficially,with filter paper and marked with India ink 15 mm. from the tip. They were then placed in a glass cylinder on glass pins which were placed in a cork having a glass rod running through the center. The other end of the glass rod was passed through a card board which was fitted rather snugly thus regulating the height of the Lupines. As the card board top only lay upon the glass container the oxygen supply was not cut off. while protection against too great evaporation was secured.

After the Lupines were left in the solution the required time the seedlings were placed in distilled water. After twenty-four hours the seedlings were examined. The length of growth beyond the 15 mm. mark, the general appearance as to color and turgidity were noted. If there was a definite growth the seedlings were thrown out but if their condition was in the least questionable they were returned to the cylinder and were allowed to stand at least five days.

The problem here to be solved was to ascertain the longest period of time in which Lupines or Spirogyra could survive when exposed to a series of concentrations of NaCl and $CuSO_4$

The plants were exposed to NaCl solutions varying in concentration from 4 normal to 1/16 normal strength as extremes, for periods varying between 15 seconds and a week. Lupines were exposed to solutions of $CuSO_4$ varying between 1/100 normal and 1/1000 normal.

In working with the Spirogyra the solutions were placed in glass beakers. The first step was to rinse the water out from the mass of filaments in a duplicate solution so as not to dilute the test solution. The time ofexposure was taken from the second it was placed in the solution until taken out of the second to be rinsed in distilled water. After a lapse of several hours the threads were examined under the microscope and treated with a 20 % sugar solution to test if the protoplasm had been affected by the salt. By withdrawing water from the cell sap the protoplasm is caused to shrink away from the wall, if living, producing plasmolysis. If the protoplast is dead, no plasmolysis occurs. If, after exposure for the required period, a majority of the cells were found to show undoubted plasmolysis, the specimen was regarded as living in this concentration for the given time.

EXPERIMENTAL RESULTS.

SODIUM CHLORIDE.--In working with Sodium Chloride Davenport(8) says that the action is probably solely osmotic but True(9) claims that the growth is diminished by the sudden change from water to the salt solution perhaps until the nedessary minimum turgor pressure is established. An irritable response seems also probable. The medium may exert a specific chemical change on seedlings placed in it. Bessey(10) claims "that sudden changes in the concentration of the surrounding fluid, stop the movements and cause the plasmodium of Myxomycetes to contract into one or more speroidal masses. When these influences cease, if they have not been so violent as to destroy the organization of the protoplasm, it returns after a greater or less length of time to its original form and the movements are resumed." This is the case with both the Lupines and the Spirogyra. In some cases the Lupines that showed no growth at the end of twenty-four hours if they were left in the distilled water began to grow after three or four days. In the case of the Spirogyra if examined a few minutes after it has been transferred from the solution to the distilled water the contents of the cells will appear to be disarranged but if left in the distilled water for several hours it often appears normal.

The NaCl solutions were the only ones used with the Spirogyra and the threads were exposed to a series of concentrations varying from 4 N. to 1/32 N. for periods varying from fifteen minutes to four days. The Spirogyra was killed in the strongest solution and was not even plasmolyzed. When left in the strong salt solutions the chlorophyl was destroyed and the threads became light brown. The Spirogyra was normal in a normal solution after be ing exposed for fifteen seconds. The boundries for the N/2 and N/3 solutions were not obtained but in the N/4 solution a majority were alive after three minutes exposure, in N/6 after ten minutes and in N/8 were still normal after four days. The results with the Spirogyra were not entirely satisfactory as duplicates varied on different days due probably to the change of environment. The best results were gotten from fresh material.

In working with the Lupines the shortest exposure to NaCl proved fatal in all concentrations above a normal solution. In the latter concentration, the radicles survived after an exposure for 30 seconds. As greater dilutions were used, the plants survived for increasingly long periods until the 1/16 normal solution was reached. In 1/16 normal concentration the Lupines survived for a week making a good growth. The relation between the concentration and the time linit is to be seen in the tables and curves following. The tables have the different times expressed in seconds, minutes and hours and the length of growth expressed in millimeters.

Of course there were a great.many experiments that are not indicated in the tables.

COPPER SULPHATE.--A great deal of work has been done on the effects of copper salts on plant life. Kahlenberg and True(11) worked with $CuSO_4$, $CuCl_2$ and $Cu(C_2H_3O_2)_2$ and found the toxic equivalent on Lupines. Heald(12) found the toxic equivalent for the same solutions using Pisum sativum, Zea mais, Curcurbita pepo. C. von Nägeli(13) found the water distilled from a copper vessel was fatal to Spirogyra placed in it. True found that beans held in place in solutions of KNO_3 with brass pins were poisoned when the metal projected in the solution. Cord(14) showed that plants of Lupine and Tea mais were more quickly acted upon in water cultures than in soil and the Lupines affected more quickly than the Tea mais. Coupin(15) gives the toxic equivalents of a number of the copper salts on wheat.

In all cases where copper salts have been used in experimental work they have been found to be toxic except in very dilute solutions. for a limited time. In no cases were the roots flabby as they were often in the NaCl solution but were flexible and were very smooth below the water line.

On account of limited time entirely satisfactory results were not obtained but I have tried to plat a curve showing the relation between the dilution and the resistance period and as some of the points were questionable they were marked so. This curve is more descending than the one for NaCl. The tables are made on the same plan as those for NaCl.

CONCLUSIONS.

1. There is a relation between the dilution of solutions and the resistance period as is shown in the curves and tables.

2. Solutions of NaCl are plainly toxic for Lupinus and Spirogyra and as the osmotic action is so slight it may not be taken into consideration.

3. Spirogyra readily adapts itself to the weak solutions for the length of time here investigated.

4. Copper sulphate is very poisonous to plant life-- even very dilute solutions acting for short periods of time.

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TABLE I.

NaCl 1 g. m. per 1.

30 sec	1 min	2 min	3 min	4 min	
27 mm	15 mm dead#	15 mm	15 mm	15 mm	
18 "	15 " "	15 "	15 "	15 "	
20 "	15 " "	15 "	15 "	15 "	
15 "	15 " "	15 "	15 "	15 "	
5 mm	0	0	0	0	average growth

#The tap root flabby, brownish in color and shrunken as if pinched where the roots were in solution.

TABLE II.

NaCl 1/2 gm. m. per 1.

45 sec	1 min	2 min	2 1/2 min	4 min	5 min	
25 mm	23 mm	19 mm	18 mm	20 mm	15 mm#	
55 "	22 "	17 "	19 "	20 "	15 "	
75 "	20 "	17 "	19 "	30 "	15 "	
25 "	-- "	19 "	20 "	24 "	15 "	
30 mm	6.6 mm	3 mm	8.4 mm	8.5 mm	0	Av. Gr.

#Dead--flabby and rusty in color.

TABLE III.

NaCl 1/3 g. m. per l.

1 min	2 min	5 min	8 min	10 min		15 min	
25 mm	25 mm	18 mm	22 mm	19 mm	#35 mm	15 mm dead	
40 "	25 "	19 "	19 "÷	16 "	15 " dead	15 "	
24 "	25 "	19 "	24 "	18 "	--	15 "	
--	27 "	18 "	30 "	19 "	25 "	15 "	
15 mm	10 mm	4 mm	9 mm	3 mm	10 mm	0	Av. Gr.

#The second reading in this case was taken five days later than the first.

÷The tap root bent L.

TABLE IV.

NaCl 1/4 g. m. per l.

30 sec	1 min	5 min	10 min	15 min	20 min	25 min	30 min
67 mm	20 mm	28 mm	39 mm	20 mm	20 mm	15 mm dead	15 mm dead
75 "	15 "	45 "	32 "	18 "	15 "	15 "	15 "
50 "	45 "	38 "	29 "	16 "	17 "	15 "	15 "
65 "	17 "	40 "	33 "	22 "	20 "	15 "	15 "
49 mm	9 mm	20 mm	18 mm	4 mm	3 mm	0 mm	0 mm Av. Gr.

TABLE V.

NaCl 1/5 g. m. per l.

30 min	45 min	1 hr	2 hrs	
20 mm	20 mm	20 mm	15 mm	
20 "	19 "	17 "	15 "	
20 "	17 "	20 "	15 "	
20 "	17 "	22 "	15 "	
5 mm	3 mm	7 mm	0 mm	Av. Gr.

TABLE VI.

NaCl 1/6 g. m. per l.

1 hr	1 1/2 hr	2 hrs	3 hrs	
20 mm	21 mm	21 mm	15 mm dead	
30 "	23 "	17 "	15 "	
30 "	23 "	17 "	15 "	
33 "	30 "	24 "#	15 "	
13 mm	9 mm	4 mm	0	Av. Gr.

#The tap root was bent ⌊ .

TABLE VII.

NaCl 1/8 g. m. per l.

30 min.	1 1/2 hrs	2 hrs	3 hrs	4 hrs	
33 mm	25 mm	24 mm	17 mm	17 mm	
38 "	20 "	20 "	21 "	15 " dead	
42 "	22 "	23 "	18 "	15 "	
35 "	broken	20 "	17 " #	16 "	
22 mm	7 mm	7 mm	3 mm	3 mm	Av. Gr.

#The tap root was bent (.

TABLE VIII.

NaCl 1/10 g. m. per l.

3 hrs	4 hrs	5 hrs	
23 mm	22 mm	18 mm	
20 "	20 "	17 "	
21 "	20 "	20 "	
21 "	19 "	20 "	
7 mm	5 mm	3 mm	Av. Gr.

TABLE IX.

NaCl 1/12 g. m. per l.

5 hrs	8 hrs	24 hrs	30 hrs	48 hrs	
19 mm	40 mm	35 mm	15 mm	15 mm	
17 "	48 "	30 "	15 "	15 "	
17 "	50 "	60 "	15 "	15 "	
20 "	48 "	45 "	15 "	15 "	
3 mm	31 mm	25 mm	0 mm	0 mm	Av. Gr.

Those left in 48 hours were badly discolored being rusty looking and translucent and were shrunken badly. They were similar to those Lupines that were put in the strong solutions such as the 4 U. Those left in thirty hours looked as if they had been pinched flat and were badly discolored.

TABLE X.

$CuSO_4$ 1/100 g. m. per l. $CuSO_4$.

15 sec	1 min	2 min	5 min	10 min	15 min	20 min	20 min
18 mm	19 mm	20 mm	22 mm	20 mm	17 mm	18 mm	15 mm
20 "	17 "	20 "	19 "	18 "	18 "	18 "	17 "
18 "	18 "	20 "	23 "	17 "	20 "	17 "	17 "
19 "	17 "	20 "	18 "	17 "	16 "	17 "	17 "
3.5 mm	2+ mm	5 mm	5.5 mm	3 mm	3 mm	2.5 mm	1.5 mm

The tip in all cases was turned a greenish brown and where was in solution was very smooth.

TABLE X1.

$CuSO_4$ 1/200 g. m. per l.

2 min	4 min	8 min	10 min	15 min	20 min	30 min
17 mm	20 mm	18 mm	15 mm	17 mm	17 mm	15 mm
20 "	20 "	21 "	16 "	17 "	18 "	15 "
35 "	19 "	23 "	17 "	17 "	20 "	15 "
38 "	20 "	20 "	17 "	16 "	--	15 "
12 mm	4 mm	5 mm	1 mm	2 mm	3 mm	0 mm

TABLE XII.

$CuSO_4$ 1/300 g. m. per l.

5 min	10 min	15 min	20 min	25 min	30 min
19 mm	17 mm	19 mm	17 mm	17 mm	15 mm
19 "	25 "	24 "	18 "	16 "	15 "
20 "	25 "	17 "	18 "	20 "	15 "
25 "	23 "	23 "	17 "	20 "	15 "
8 mm	10 mm	5.7 mm	2.5 mm	3 mm	0 mm

The tips in nearly all cases in this dilution were bent and all were discolored. The bend being caused by a more rapid growth on one side of the root than the other to accomodate this growth the epidermis was cracked thus giving the root an appearance of being honey-combed.

TABLE XIII.

$CuSO_4$ 1/400 g. m. per l.

1 min	5 min	20 min	25 min	30 min	45 min
32 mm	40 mm	17 mm	17 mm	17 mm	15 mm
24 "	40 "	17 "	16 "	17 "	15 "
25 "	38 "	17 "	18 "	17 "	15 "
35 "	40 "	17 "	16 "	17 "	15 "
14 mm	34 mm	2 mm	2 mm	2 mm	0 mm

TABLE XIV.

$CuSO_4$ 1/500 g. m. per l.

25 min	30 min	45 min
17 mm	17 mm	15 mm
16 "	16 "	15 "
18 "	17 "	15 "
16 "	17 "	15 "
2 mm	1.7 mm	0

TABLE XV.

$CuSO_4$ N/600

45 min	60 min
15 mm	15 mm
15 "	15 "
15 "	15 "
15 "	15 "
0 mm	0 mm

TABLE XVI.

$CuSO_4$ N/1000

2 hrs	24 hrs
15 mm	15 mm
15 "	15 "
15 "	15 "
15 "	15 "
0 mm	0 mm

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