EFFECT OF SEEDS UPON HYDROGEN-ION CONCENTRATION EQUILIBRIUM IN SOLUTION [1]

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INTRODUCTION

In an earlier paper [2] it was shown that the hydrogen-ion concentration of alkaline salt solutions in which seeds were immersed had changed markedly after about 15 hours of imbibition. The P_H changes recorded were all in one direction, namely, towards the acid side, and appeared to be fairly definite for each species of seed. The conclusion was drawn that these reaction changes were directly related to ion-absorption by the seeds.

The object of this paper is to show the rate of reaction changes and the existence of a definite equilibrium in solutions which have been in contact with seeds.

EXPERIMENTAL DATA

In view of the fact that a marked difference exists in the absorbing power of seeds of different species (seeds of the leguminous type show higher rates of absorption than seeds of the gramineous species [3]), four kinds of large seeds were selected, as follows: Corn (*Zea mays*), lupine (*Lupinus albus*), beans (*Phaseolus vulgaris*), and soy beans (*Soja maxima*).

The representative salt solutions, mineral and organic acids, covering the range of acids and salt radicles, are given in the tables. In the case of the mineral and organic acids an effort was made to bring the different acids to a dilution giving approximately a reaction of P_H 3.0. Some of the acids could be secured only at a nominal per cent and it was thought best to state the dilution in P_H values instead of in terms of normality. A large number of different salts were tried, but, for the comparison of the radicles, the representative potassium salts are selected and only two chlorides with other bases are entered in the tables.

Fifty seeds of each species were placed in small bottles each containing 100 c. c. of solution; 1.8 c. c. of this solution was pipetted off after definite time intervals, and the hydrogen-ion concentration of the solution was determined by the colorimetric method. The results of these determinations are given in the tables as the averages of at least two trials. In Tables I, II, and III the initial P_H values are compared with the readings at different intervals.

The rate of reaction change is not the same for all salt solutions, although a certain equilibrium is reached in the solutions after the seeds have been immersed sufficiently long (Table I). Corn changed the reaction of KCl to a point of equilibrium in about 15 minutes, while 15 hours were necessary in the case of K_2SO_4. For the mineral and organic acids similar differences were observed.

For mineral and organic acid solutions, considerably more time was necessary to reach the point of equilibrium than for salt solutions (Tables II and III.) On account of the rapid increase in the strength of the acids with every increment of the P_H values, a longer time for the reaction changes could be expected. Nevertheless, all acid solutions which had been in contact with the seeds after a certain period of time reached the same point of equilibrium as the salt solutions.

The P_H values observed in oxalic acid and potassium chloride solutions (corn and beans) plotted against time in minutes are presented in Figure 1. The curves show a fairly rapid rise during the first few time intervals with a flattening out toward the point of equilibrium. Ordinarily the solutions remain at this point indefinitely, but slight changes can be brought about by an increase of temperature. This explains why some of the salt solutions show slightly different P_H

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[2] RUDOLFS, W. EFFECT OF SALT SOLUTIONS HAVING DEFINITE OSMOTIC CONCENTRATION VALUES UPON ABSORPTION BY SEEDS. Soil Sci. 11: 277-293, illus. 1921.

[3] RUDOLFS, W. EFFECT OF SEEDS UPON HYDROGEN-ION CONCENTRATION OF SOLUTIONS. Bot. Gaz. 74: 215-220. 1922.

TABLE I.—*Reaction changes in representative salt solutions*

Time	K_2SO_4		K_2HPO_4		KNO_3		$KClO_3$		$CaCl_2$	
	Beans	Corn	Beans	Corn	Beans	Corn	Beans	Corn	Beans	Corn
	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H
Initial	5.9	5.9	4.1	4.1	5.4	5.4	6.5	6.4	6.0	6.0
After 30 sec	5.9	5.9	4.1	4.1	5.3	5.3	6.5	6.2	5.9	5.9
After 1 min	5.9	5.7	4.2	4.2	5.5	5.1	6.5	6.1	5.8	5.7
After 2 mins	5.9	5.5	4.2	4.2	5.5	5.2	6.5	5.7	5.8	5.6
After 3 mins	5.9	5.3	4.2	4.1	5.6	5.2	6.5	5.7	5.7	5.6
After 5 mins	6.0	5.4	4.0	4.1	5.7	5.4	6.3	5.4	5.9	5.6
After 10 mins	6.0	5.3	4.1	4.1	5.6	5.1	5.9	5.3	5.9	5.4
After 15 mins	6.0	5.2	4.0	4.0	5.6	4.9	5.9	5.1	5.9	5.4
After 20 mins	6.0	5.0	4.2	4.3	5.7	4.8	5.7	5.1	5.8	5.3
After 30 mins	6.0	5.1	4.4	4.3	5.7	4.8	5.7	5.0	5.8	5.3
After 1 hr	6.0	5.1	4.4	4.3	5.8	4.7	5.7	4.9	5.7	5.3
After 2 hrs	5.9	4.9	4.8	4.5	5.8	4.7	------	4.9	5.6	5.0
After 15 hrs	5.4	4.2	5.4	4.2	5.9	4.3	5.7	4.2	5.5	4.1
After 48 hrs	5.4	4.2	5.4	4.1	5.8	4.2	5.7	4.0	5.5	4.0

TABLE II.—*Reaction changes in mineral acid solutions*

Time	H_2SO_4		HCl		HNO_3		50 per cent H_3PO_4		H_3BO_3		KCl		$BaCl_2$	
	Beans	Corn	Beans	Corn	Beans	Corn	Beans	Corn	Beans	Corn	Beans	Corn	Beans	Corn
	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H
Initial	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	5.5	5.5	6.7	6.7	5.9	5.9
After 30 sec	3.1	3.0	3.1	3.0	3.0	3.0	3.1	3.0	5.7	5.4	6.6	6.4	5.9	5.8
After 1 min	3.1	3.0	3.3	3.1	3.1	3.0	3.5	3.0	5.9	5.3	6.5	6.1	5.9	5.6
After 2 min	3.2	3.1	3.5	3.2	3.2	3.1	4.4	3.1	6.0	5.3	6.5	5.2	5.9	5.5
After 3 min	3.2	3.1	3.6	3.2	3.3	3.1	4.5	3.1	6.2	5.3	6.5	4.9	5.9	5.4
After 5 min	3.3	3.1	4.0	3.2	3.5	3.1	4.7	3.1	6.3	5.2	6.3	4.6	5.9	5.3
After 10 min	4.1	3.1	4.6	3.3	3.6	3.2	5.2	3.2	6.4	5.1	6.2	4.1	5.9	5.2
After 15 min	4.6	3.1	4.9	3.4	3.8	3.3	5.7	3.2	6.6	5.1	6.2	4.0	5.9	5.0
After 20 min	4.7	3.1	5.2	3.4	3.9	3.3	5.7	3.5	6.5	4.9	6.1	4.0	5.9	4.6
After 30 min	5.1	3.2	5.5	3.4	4.0	3.3	5.8	3.7	6.5	4.7	5.9	3.9	5.9	4.3
After 1 hr	5.4	3.3	5.7	3.4	4.6	3.3	5.9	4.5	6.4	4.6	5.6	4.0	5.9	4.1
After 2 hrs	5.6	3.4	5.8	3.9	5.4	3.4	5.9	4.4	6.2	4.3	5.7	3.9	5.7	4.1
After 15 hrs	5.8	3.7	5.9	3.9	5.8	3.8	5.9	4.2	6.1	4.2	5.5	3.9	5.5	4.1
After 40 hrs	5.9	3.9	5.8	4.1	5.8	3.9	5.9	4.1	5.9	4.1	5.4	3.9	5.4	4.1

TABLE III.—*Reaction changes in representative organic acids*

Time	Bibasic acid		Hydroxy acids				Fatty acids				Amino acid		Aromatic acid	
	Oxalic		Citric		Lactic		Formic		Acetic		Asparagin		Benzoic	
	Corn	Beans	Corn	Beans	Corn	Beans	Corn	Beans	Corn	Beans	Corn	Beans	Corn	Beans
	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H
Initial	2.9	2.9	2.9	2.9	3.0	3.0	2.9	2.9	2.9	2.9	3.7	3.7	3.2	3.2
After 30 sec	2.9	3.0	2.9	2.9	3.0	3.0	2.9	3.0	2.9	2.9	3.7	3.7	3.3	3.5
After 1 min	3.0	3.1	3.0	3.0	3.0	3.1	3.0	3.1	2.9	2.9	3.7	3.7	3.5	3.7
After 2 min	3.1	3.2	3.0	3.1	3.1	3.1	3.1	3.2	2.8	2.8	3.7	3.8	3.6	3.7
After 3 min	3.1	3.4	3.0	3.1	3.1	3.1	3.1	3.4	2.9	2.8	3.8	3.8	3.6	3.7
After 5 min	3.3	3.9	3.0	3.2	3.1	3.2	3.2	3.6	3.0	3.0	3.8	3.8	3.6	3.9
After 10 min	3.6	4.2	3.0	3.3	3.1	3.6	3.3	4.4	3.0	3.0	3.9	3.8	3.7	4.3
After 15 min	3.7	4.5	3.1	3.7	3.1	3.9	3.5	4.7	3.0	3.0	3.9	3.8	3.7	4.5
After 20 min	3.8	4.8	3.1	3.9	3.1	4.0	3.5	4.9	3.0	3.1	3.9	3.9	3.6	4.6
After 30 min	3.9	5.1	3.1	4.2	3.1	4.2	3.6	5.4	3.1	3.3	4.1	4.0	3.7	4.8
After 1 hr	4.0	5.4	3.2	4.6	3.3	4.5	3.9	5.5	3.1	3.5	4.1	4.4	3.9	5.2
After 2 hrs	4.0	5.6	3.5	4.9	4.0	5.5	4.1	5.8	3.1	3.7	4.2	5.3	3.9	5.5
After 15 hrs	4.1	5.8	3.9	5.7	4.1	5.8	4.1	5.9	3.4	4.2	4.1	5.8	4.0	5.8

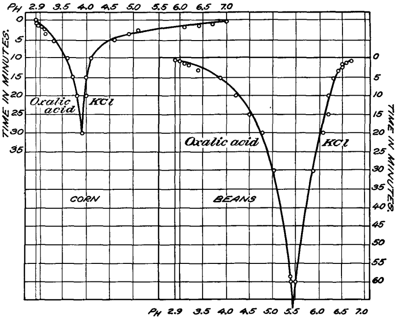

FIG. 1.—Chart showing the P_H values observed in oxalic acid and potassium chloride solutions (corn and beans) plotted against time

values upon standing when the temperature varies. Because variations in temperature cause apparent variations in ion absorption the question might be raised whether or not this is due to the comparative dryness of the seeds at the time of immersion. If the seeds are dry, water intake would presumably be greater and occur at a faster rate than when wet seeds are used. To test this point corn seeds were soaked for a certain period in distilled water before submersion into potassium sulphate. After the immersion a fresh potassium sulphate solution was used again to see when the limit of ion intake was reached. Table IV gives some of the data secured. It will be noticed that the changes produced by previously soaked seeds were very similar to the changes in the case of the air-dry seeds. The same seeds were then quickly taken out of the solution and again placed in a KCl solution of 7 atmospheres osmotic pressure. The observed changes of reaction were not as rapid and extensive as was the case during the first immersion. After 15 minutes the initial P_H value of 5.9 was changed to 5.0 and had risen again after 60 minutes to P_H 5.3. Immersion of these seeds in the same and in fresh solutions for 15 hours caused no further changes. There seems to be no doubt that the equilibrium was reached. Previous soaking in water seemed to have no influence as far as ion intake is concerned.

TABLE IV.—*Corn seeds soaked in distilled water for 30 minutes before submersion into potassium sulphate*

Time	K_2SO_4 (7 atm.)	Fresh solution	Time	K_2SO_4 (7 atm.)	Fresh solution
Minutes	P_H	P_H	*Minutes*	P_H	P_H
Initial	5.9	5.9	After 20	4.1	5.2
After 5	5.1	5.3	After 30	4.3	5.2
After 10	4.9	5.1	After 45	4.2	5.2
After 15	4.3	5.0	After 60	4.2	5.3

After the reaction changes were observed, the first impulse was to hold responsible the rapid changes of life phenomena in the dormant seed when brought in contact with the solutions, or possibly the action of enzymes which are characteristic during the processes of germination. The possibility of changing the salt solutions and acids at such a rate by these agents seemed remote; but, to make sure, seeds were killed by subjection to a temperature of 100° to 102° C. for periods of 48 and 96 hours. To prevent all possibility of enzyme action, beans were subjected to similar temperatures and then placed in formaldehyde. The data secured for seeds dried for 48 hours are given in Table V.

TABLE V.—*Reaction changes in solutions in which "fresh" and dried seed were soaked. The dried seeds were subjected to a temperature of 100° to 102° C. for a period of 48 hours*

Time	NaCl				KCl				Formic aldehyde	
	Lupine		Corn		Lupine		Corn		Beans	
	Fresh	Dried	Fresh	Dried	Fresh	Dried	Fresh	Dried	Fresh	Dried
	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H
Initial	6.6	6.6	6.6	6.6	6.7	6.7	6.7	6.7	3.6	3.6
After 30 sec	6.6	6.6	6.6	6.6	6.5	6.5	6.4	6.5	3.7	3.6
After 1 min	6.5	6.5	6.3	6.5	6.5	6.3	6.1	6.3	3.8	3.6
After 2 min	6.5	6.5	6.1	6.2	6.5	6.3	5.1	6.3	3.8	3.6
After 3 min	6.5	6.3	5.6	5.8	6.5	6.3	4.9	5.9	3.9	3.6
After 5 min	6.3	6.3	5.4	5.7	6.3	6.3	4.5	5.5	4.0	3.7
After 10 min	6.3	6.3	4.9	4.9	6.3	6.3	4.1	4.9	4.3	3.9
After 15 min	6.3	6.3	4.6	4.4	6.2	6.3	4.0	4.7	4.5	4.1
After 20 min	6.3	6.3	4.4	4.3	6.3	6.1	4.0	4.2	4.7	4.4
After 30 min	5.9	6.2	4.1	4.1	5.9	6.1	3.9	4.1	4.9	4.8
After 1 hr	5.5	6.3	4.1	4.1	5.5	6.1	3.9	3.9	5.0	4.9
After 2 hrs	4.9	4.9	-------	-------	4.9	5.2	-------	-------	5.4	5.3
After 18 hrs	4.7	4.6	-------	-------	4.7	4.6	-------	-------	-------	-------

A study of this table shows that the reaction changes were possibly somewhat slower in the dried seeds but that in general the reaction velocity did not undergo great changes. It might be that because of the drying of the seeds, slight chemical changes occured in the seed, so that the mechanical intake of the ions was retarded; or it might be that, on account of the drying, some of the retained moisture in the seeds was driven out and the moisture content had to be replaced to its original amount before the seeds were able to absorb the ions from the salt solutions and acids. It is interesting to note, however, that in all cases the final equilibrium was established regardless of the previous drying. Even in the case of dried seeds soaked in a formaldehyde solution the hydrogen-ion concentration was changed from P_H 3.6 to P_H 5.3 after 2 hours. None of the dried seeds germinated, while from 61 to 84 per cent of the "fresh" seeds germinated after being subjected to immersion in salt solutions for 2 hours.

It is known that when dry seeds are placed in moist soil or salt solutions they absorb moisture with great power. This absorption is not a simple phenomenon but implies forces like imbibition, capillarity, surface tension, osmotic pressure from internal salts, and possibly other forces. The amount of absorption depends on the salt concentration in the soil or solution. In previous papers[4] it has been shown that there is a difference in the absorbing powers of different species of seeds, that different salt solutions are differently affected, and also that there is variation in the amounts of salt solutions (in the form of ions) taken up by the different seeds.

Loeb[5] has suggested that in amphotheric membranes like those in the protoplasm of root hairs, and of vacuolate cells generally, the opposite sides of the membrane may be oppositely charged. Many different kinds of membranes are semipermeable and the property of all in common in that they are colloidal gels. Water can penetrate both phases of the colloidal gel, but salt molecules attempting to penetrate the membrane would be prevented by physical phenomena. From the data here presented, it seems clear that the ions of the solutions are rapidly absorbed by the seeds, but the material which makes up the seeds, and especially the seed coats, can not directly be compared with the colloidal gel or the semipermeable membrane of the cells of root hairs.

A study was therefore conducted to determine what part of the seed plays the most important rôle in ion ab-

[4] RUDOLFS, W. EFFECT OF SALT SOLUTIONS HAVING DEFINITE OSMOTIC CONCENTRATION VALUES UPON ABSORPTION BY SEEDS. Soil Sci. 11: 277–293, illus. 1921.
——— EFFECT OF SEEDS UPON HYDROGEN-ION CONCENTRATION OF SOLUTIONS. Bot. Gaz. 74: 215–220. 1922.

[5] LOEB, J. THE REVERSAL OF THE SIGN OF THE CHARGE OF MEMBRANES BY HYDROGEN IONS. Jour. Gen. Physiol. 2: 577–594, illus. 1920.

sorption. The seed coats of soy beans were carefully removed from the cotyledons and both cotyledons and coats placed in different acids and salt solutions; similar material was placed in distilled water. The water was not redistilled and was, as usual, slightly acid. The figures secured for beans soaked in hydrochloric acid are presented in Table VI.

TABLE VI.—*Reaction changes in 0.001 normal HCl and distilled water caused by dicotyledons of soy beans*

Time	HCl		Water	
	Coats	Dicotyledons	Coats	Dicotyledons
	P_H	P_H	P_H	P_H
Initial	3.6	3.6	6.6	6.6
After 30 sec	4.7	5.0	6.6	6.6
After 1 min	4.8	5.4	6.6	6.6
After 2 min	5.2	5.7	6.6	6.6
After 3 min	5.3	5.9	6.6	6.7
After 5 min	5.5	6.0		
After 10 min	5.6	6.0	6.7	6.6
After 15 min	5.9	6.1		
After 20 min	6.0	6.1	6.6	6.6
After 30 min	6.1	6.1	6.6	6.6
After 60 min	6 1	6.1	6.6	6.6

It can be seen at once that the cotyledons of the soy beans were more powerful in absorbing ions from this acid than were the coats. No reaction changes seemed to occur in distilled water. The dicotyledons of soy beans contain saturated acids, great amounts of oil and masses of protein. Since proteins are amphotheric, it seems justifiable to assume that the proteins are mainly active in ion absorption. It is well known that carbohydrates do not behave in a way similar to proteins. However, the influence of corn seed coats, the carbohydrates of the inner cells of the seeds, and pure starch upon the reaction changes of magnesium sulphate solutions as compared with redistilled water was determined. Table VII gives the condensed data secured in one series of trials.

The reaction changes brought about by the seed coats in the $MgSO_4$ solutions were similar to the reaction changes caused by the whole seeds, while the reaction changes caused by the endosperm of the corn seeds (carbohydrates mainly) were negligible. As could be expected, no changes occurred in the salt solutions or water with pure starch. The protein content of the corn seed coat seemed, therefore, responsible for the changes of hydrogen-ion concentrations.

It may perhaps be said that the characteristic external acidity which each species of seeds tends to preserve seems to be determined by the chemical properties of its chief constituent protein. The fact that a given species of seeds causes (and maintains in weak solutions) a certain equilibrium of hydrogen-ion concentration might possibly throw some light upon the question why certain plants are better able to withstand an acid or alkaline soil than others. If for instance a certain species of seeds maintains a characteristic P_H point 4.0, the seedling possibly would be able to survive in a more acid soil than a species of seeds with a characteristic P_H point of 5.8. However, a certain equilibrium caused by the seeds in the surrounding nutrient or soil solution does not necessarily mean that the growing plant causes a similar reaction, because the watery protein materials in the growing plant cell might have different characteristic P_H points. Moreover, the growing plant seems to be able to adjust or regulate internal changes readily. This is shown by Bauer and Haas,[6] who

TABLE VII.—*Influence of corn seed coats and starch upon the reaction of changes of magnesium sulphate as compared with distilled water*

Time	$MgSO_4$		Water		Pure starch	
	Coats	Endosperm	Coats	Endosperm	$MgSO_4$	Water
	P_H	P_H	P_H	P_H	P_H	P_H
Initial	6.7	6.7	7.0	7.0	6.7	7.0
After 15 min	4.6	6.7	6.9	6.9	6.7	7.0
After 30 min	4.2	6.6	6.9	6.9	6.7	7.0
After 60 min	4.2	6.5	6.8	6.9	6.7	7.0

[6] BAUER, F. C., and HAAS, A. R. C. THE EFFECT OF LIME, LEACHING, FORM OF PHOSPHATE AND NITROGEN SALT ON PLANT AND SOIL ACIDITY, AND THE RELATION OF THESE TO THE FEEDING POWER OF THE PLANT. Soil Sci. 13: 461-477, illus. 1922.

state: "In the case of the soy bean roots, while the hydrogen-ion concentration usually showed a direct relation to the acidity of the soil, the total acidity (of the plant) usually varied in the opposite direction. The data of these experiments strikingly show the power possessed by plants to regulate internal acidity. Marked differences in the acidity of the soil caused only small differences in the acidity of the plant juices." It might be that the acidity or alkalinity of soils as such is not always the limiting factor in soil productivity. It is recognized that the measure of soil acidity alone may be useful in determining the amount of lime necessary to adjust a soil for a crop, but the establishment of the characteristic P_H point for the seeds as revealed in these tests together with the determination of the hydrogen-ion concentration of the soil solution might serve as a better indicator in the use of particular crops in particular soils.

SUMMARY

When seeds were immersed in representative salt solutions, mineral and organic acids, and the changes in hydrogen-ion concentration and reaction changes were recorded, it was found that different seeds are able to change the hydrogen-ion concentration of the solutions to definite points, and that certain equilibrium is reached in all solutions after the seeds have been immersed sufficiently long. The changes of the solutions in which previously soaked seeds were immersed are very similar to the changes in solutions caused by air-dry seeds.

The reaction caused by dried seeds (dried at 100° to 102° C. for 48 and 96 hours) are similar to the reaction changes caused by fresh seeds, although the rate of reaction is slightly less. The cotyledons of soy beans were more powerful to absorb ions from the solutions than were the seed coats; and the reaction changes caused by seed coats of corn were similar to the changes brought about by the whole seeds, while changes caused by the endosperm of the seeds (carbohydrates mainly) were negligible. The chemical properties of the chief protein constituent of the seeds seem responsible for the changes in hydrogen-ion concentrations of the solutions.