

## CHEMICAL EQUIVALENT BASE EXCHANGE REACTIONS IN PLANTS

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The problem of determining the causes of the movement of mineral elements through membranous tissues within the plant has elicited considerable scientific inquiry. Moreover, the factors which govern the concentration of the several ions in plant sap and of those fixed by the plant tissues have received much attention. Any observations, therefore, which relate to these phases of plant physiology and nutrition are of interest.

During recent years much work has been done on the relationships existing between the soil solution and the exchange complex of the soil, the compounds which function in the stoichiometric exchange of bases. The soil compounds associated with base exchange reactions may be divided into organic and inorganic groups. The latter have been extensively investigated, but the former have received comparatively little attention. While the absorbing power of so-called humic bodies has been recognized for many years, comparatively little study has been devoted to quantitative values and to the nature of the absorbing compounds. At the time the exchange property of the clay fraction of the soil was demonstrated the same property was assumed for the organic fraction, but later some doubt arose as to the validity of this assumption. This problem has been rather intensively studied at the Arizona Agricultural Experiment Station (3) and it has been demonstrated beyond question that the organic fraction of the soil possesses a chemically equivalent exchange property, and that it is a linear function of the carbon content of the organic material.

Of the carbon compounds which compose the organic fraction of the soil the pentosans, lignins, proteins and cellulose are most abundant. Of these, lignin appeared most likely to possess base exchange properties and it was therefore chosen for study. While the chemical composition of lignin has not been definitely established, it is known to possess phenolic hydroxyl groups through which basic lignates may be formed. Lignin as isolated from a number of soils and from plant materials was studied and all samples were found to possess the property of exchanging one base for another in chemically equivalent proportions. On quantitatively determining the percentage of lignin and the replacement capacity of highly organic soils, a linear relationship between them was noted. The base-absorbing capacity of lignin varied with the source, and with the method used in preparing it from the mother substance. For example, lignin extracted from soils and plant material by alcoholic NaOH varied in exchange capacity from 38 to 178 M.E. (milliequivalents) per 100 grams, while that extracted by aqueous

NaOH varied from 327 to 420 M.E. per 100 grams. It was found that the maximum absorbing capacity of lignin depended upon complete hydrolysis of the complex. This is shown by the fact that the exchange capacities of lignins extracted with alcoholic NaOH were all increased to approximately 200 M.E. when hydrolyzed with HCl, while the capacities of the aqueous soluble lignins were increased to approximately 400 M.E. If we assume that lignin in raw plant material is in part combined with cellulose as ligno-cellulose this hydrolytic effect of HCl is easily explained.

Throughout the investigations (3, 4), which have been very briefly presented, the fact that lignin is a constituent of most plants was outstanding. This fact suggested that lignins, and possibly other organic compounds, notably the lipoids and pectins, may function in base exchange reactions within the plant. Absorption capacity would be limited by the combination with cellulose. Thus lignin may be said to exist in the plant in both active and inactive forms with the base exchange capacity varying accordingly.

### Experimental

To investigate the base exchange phenomenon in plant material, alfalfa, one of the principal crops of the Southwest, was chosen for study. Five-gram samples of air dried, finely ground alfalfa were treated as follows:

1. Without any preliminary washing, duplicate samples were leached with neutral normal barium acetate and neutral normal ammonium acetate, 250-cc. volumes being used. Hydrogen was determined by electrometric titration of the barium acetate leachate and calcium, magnesium, potassium and sodium determined in the ammonium acetate leachate by gravimetric methods.

2. Duplicate samples were leached with 500 cc. of neutral 95 per cent. ethyl alcohol, after which they were leached with barium and ammonium acetates as in 1. The alcohol and acetate leachings were analyzed separately.

3. Duplicate samples were leached with 500 cc. of distilled water and then leached with barium and ammonium acetates as in 1. The water and acetate leachates were analyzed separately.

In each case where ammonium acetate was used, the samples were subsequently leached with alcohol to remove the excess ammonium acetate, and then the absorbed ammonium determined quantitatively. The data are given in table I.

That alfalfa possesses the property of exchanging its bases and hydrogen with other bases in chemically equivalent amounts is shown by a comparison of the total amount of ammonium absorbed and the sum of the displaced bases and hydrogen in column 3. In other experimental work similar data

TABLE I

TOTAL BASES, THE RELATIVE SOLUBILITIES IN ALCOHOL AND WATER, AND THE FIXED EXCHANGEABLE BASES

RESULTS ARE RECORDED IN MILLIEQUIVALENTS PER 100 GRAMS

	TOTAL BASES AND HYDROGEN	INSOLUBLE IN ALCOHOL BUT EXCHANGEABLE	INSOLUBLE IN WATER BUT EXCHANGEABLE
Exchange* capacity ...	44.1	41.6	39.6
Hydrogen .....	16.0	Trace	7.0
Calcium .....	78.0	63.0	27.8
Sodium .....	49.9	5.7	3.2
Magnesium .....	11.1	3.7	3.1
Potassium .....	30.2	3.9	0.15
		Dissolved by alcohol	Dissolved by water
Calcium .....		11.8	52.5
Sodium .....		51.1	52.3
Magnesium .....		9.9	9.7
Potassium .....		28.4	28.6
Total .....	185.2	177.5	184.35

\* Calculated from the  $\text{NH}_4$  absorbed from ammonium acetate.

have been obtained by determining the saturation capacity of alfalfa for barium and calcium ions. Column 1 of table I shows the total amount of bases and hydrogen present both as soluble and as fixed forms. In this case the soluble cations are approximately three times the fixed ions on a milliequivalent basis.

Alcohol was used as a leaching solvent to determine whether or not the soluble unfixed cations could be removed preparatory to determining the fixed cations. It will be noted that the calcium is difficultly soluble in alcohol, while the sodium, potassium and magnesium are largely removed by this solvent. Alcohol, therefore, fails to qualify as a preliminary leaching liquid in the determination of exchangeable bases.

Column 3 closely represents the ratio of bases in fixed and soluble forms, although there is some evidence that a part of the fixed potassium is displaced by soluble calcium during the leaching operation.

In addition to demonstrating an equivalent exchange property of bases, the data show that calcium is the predominant fixed base. The essential need of this high calcium absorption is manifested in the property of the plant material when saturated with calcium and with a univalent base,

such as sodium or potassium. Leaching was very rapid with the calcium saturated alfalfa, but when saturated with sodium or potassium the plant tissues became swollen and dispersed, and leaching velocity was greatly reduced. Tissue permeability is therefore a function of calcium saturation and will be reduced if too great a displacement of calcium by sodium or potassium takes place.

#### Common ion effect

In base exchange reactions the energy of absorption of the exchange complex is of the order  $\text{Ca} > \text{Mg} > \text{K} > \text{Na}$  and is therefore, for the bases which have been discussed, greatest for calcium. On the other hand, absorption is also influenced by the concentration of the salt of a common base and other electrolytes in the control solution. The property of ionization of basic lignates and the effect of common ions on this property has been demonstrated (4). Similar experiments with alfalfa were also conducted.

Six 2-gram samples of alfalfa were leached with neutral 0.5 normal calcium acetate, after which the excess salt was removed by leaching with water. These calcium saturated samples were then placed in 500-cc. volumes of varying concentrations of KCl solution, shaken occasionally for 4 days and the displacing effect of the K ions ascertained by determining the Ca in solution. The results are given in table II.

TABLE II  
REPLACEMENT OF CA FROM CA-SATURATED ALFALFA IN CONTACT WITH VARYING CONCENTRATIONS OF KCL  
2 GRAMS ALFALFA 500 CC. OF SOLUTION

EXPERIMENT NO.	KCL PER 500 CC.	FIXED CA IN 2 GM. ALFALFA	CA DISPLACED BY KCL
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
1 .....	None	0.0160	0.0016
2 .....	0.5	"	0.0032
3 .....	1.0	"	0.0036
4 .....	2.5	"	0.0041
5 .....	5.0	"	0.0044
6 .....	10.0	"	0.0045

A similar experiment was conducted in which the potassium-saturated alfalfa was prepared by leaching with a solution of potassium acetate and finally with 95 per cent. alcohol to remove excess of salt. These samples were then placed in contact with 500-cc. volumes of varying concentrations of  $\text{CaCl}_2$  solution, shaken occasionally for 4 days and displaced potassium ascertained by determining the calcium left in solution. The results are given in table III.

These data clearly illustrate the greater energy of absorption for calcium. With a solution of 10 gm. KCl per 500 cc. in contact with 2 gm. calcium-

TABLE III

DISPLACEMENT OF K FROM K-SATURATED ALFALFA IN CONTACT WITH  $\text{CaCl}_2$  SOLUTIONS  
2 GRAMS ALFALFA PER 500 CC. SOLUTION

EXPERIMENT NO.	$\text{CaCl}_2$ PER 500 CC.	CA ABSORBED BY ALFALFA	ABSORBING CAPACITY OF 2 GM. ALFALFA IN GM. CA
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
1 .....	0.1	0.00780	0.0169
2 .....	0.5	0.00997	0.0169
3 .....	1.0	0.01490	0.0169

saturated alfalfa, only one-fourth the calcium was displaced. On the other hand, when the potassium-saturated alfalfa was placed in contact with solutions of  $\text{CaCl}_2$  the displacement of K was almost complete in the presence of 1 gm.  $\text{CaCl}_2$  per 500 cc. These data lend confirmation to those in table I and illustrate why the absorption capacity of the plant is largely saturated with calcium. Water permeability and turgidity of the tissues apparently demand a near saturation with calcium.

The reactions described, the data presented and the observations which have been made, may be used to explain a number of experimental observations to be found in the literature where mention is often made of the abnormal changes in permeability of plant cells subjected to single salt cultures. According to OSTERHOUT (5) the antagonistic effect of salts is due to the presence in the cell of a compound regulating permeability. HANSTEEN-CRANNER (2) suggests that lipid and pectic materials govern permeability of cell walls. Alkalis act as dispersants and calcium as a coagulant. PRIESTLEY (6) found a similar property for pectic acids, namely, the formation of gelatinous soluble salts with sodium and potassium, and of insoluble flocculent salts with calcium. These statements agree with our own observations on the property of the alkali- and alkali earth-saturated alfalfa. GENAUD (1) has recently demonstrated a rapid ionic interchange between the cellular membrane of yeast cells and the surrounding fluid. The cationic interchange usually involves equimolecular quantities. He also found that bivalent ions penetrate more easily than univalents. The cell membrane and cell vacuole behave like insoluble organic acids, exchanging their cations for those of the environing medium until an equilibrium, due to mass action, is obtained.

It is also of interest to note that STILES (8) greatly emphasizes the failure of osmotic views to afford a complete explanation of the cell reaction to its dissolved substances. He observed (7) that when storage tissue is immersed in a solution of a single salt, the ions of the salt are not absorbed in equivalent amounts. The balance of the ionic charges in the external solution is maintained by the diffusion of ions from the tissue. He

also mentions that salts may combine chemically with the cell constituents. STILES also quotes similar work by REDFREN (7). In the latter's experiments it was found that on immersing tissues in a solution of  $\text{CaCl}_2$  the calcium was absorbed in excess of the Cl and that Mg and K ions had diffused out of the tissue, being replaced in the tissue by calcium.

These few references serve to show that there is every reason to suppose that certain tissue constituents play a part in the interchange of substances between plant membranes. It is shown by our experiments that absorbed bases may be displaced by other bases or by hydrogen, and that this interchange may play a part in ionic movement.

### Summary

Air dried plant tissues of alfalfa show a definite property of absorbing bases or hydrogen and exchanging them for other bases in chemically equivalent proportions. It is suggested that living tissues may exhibit this same property and that it may be related to the phenomenon of the permeability of plant tissues and cationic concentration within the plant system. The diffusion of bases through the plant tissues may be aided by an actual combination of the base with a relatively insoluble anion, such as lignin for example, to form a definite chemical combination, or to form an absorption complex with other electronegative colloids which are tissue constituents.

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