SCIENCE

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THE SIGNIFICANCE OF CALCIUM FOR HIGHER GREEN PLANTS[1]

In view of the time limit reasonably set for this paper, I shall not attempt to review the very extensive literature that in one way or another deals with the relation of calcium to the plant world, but shall content myself with pointing out certain of the land marks that occur at certain intervals along this oft-traveled road. And, at the beginning, I may as well give Jost's summing up of the situation as he saw it in 1906, [2]when he says, "We are bound to admit that its function has not yet been discovered."

To Salm-Horstmar[3] seems to belong the credit of proving in 1856 that calcium is necessary for phanerogams and is distinctly not replaceable by magnesium.

Almost simultaneously in 1869 Adolph Mayer [4] and Raulin [5] showed that this rule was not of general application since certain non-chlorophyllose types were found to thrive without it.

Mayer grew yeast normally in media from which calcium was lacking and Raulin did the same with Aspergillus. It remained for Molisch[6] in 1895 to demonstrate that not all green plants require calcium by cultivating

[1]Address of the Vice-President and Chairman of Section G, Botanical Sciences, American Association for the Advancement of Science, Toronto, December, 1921.

[2]Jost, Ludwig, "Lectures on Plant Physiology," Gibson's transl. Oxford, 1907: 85.

[3]Salm-Horstmar, "Versuche und Resultate über die Nahrung der Pflanzen, Braunschweig." 1856.

[4]Mayer, Adolph, "Untersuchungen über Alkoholgährung." 1869: 44.

[5]Raulin, *Ann. d. Sci. Nat.*, V, Ser. I, 11: 224, 1869.

[6] Molisch, *Stizb. d. Wien. Akad.*, Abt. I, 104: 733. 1895.

certain algæ in media from which this element was absent.

In the meantime the distilled water problem had arisen to vex all physiologists and in their attempt to deal with it the zoologists had thrown some light on the calcium problem as well. Perhaps fundamental to all was the work of the English physiologist, Sydney Ringer, who, as a by-product of a long series of experiments, developed the generally-used normal saline solution known by his name. While working on the characteristic effects produced by various salts in prolonging the life of organisms in water cultures, he noted the favorable action of calcium salts.[7] He observed that in distilled water calcium and other salts were extracted from fish placed in it, and records that epithelial and mucous cells seemed to become detached from the gills. In later experiments carried out on Tubifex, a freshwater worm, he noted that a far more striking change took place. After a time spent in water from which calcium salts were excluded, the worms disintegrated. When to distilled water a calcium salt was added the worms not only lived but behaved very much as they did in river water.[8]

His explanation of the fundamental causes here operating was couched in rather general language, but one gathers that he conceived them to be of a physico-chemical nature, and the seat of operation was thought to be in the cells of the animals. There is much in Ringer's work to repay the student of general physiology.

The fundamental features observed by him were confirmed by Herbst in 1900[9], when he showed that in certain sea-urchin larvæ grown in sea water from which Ca was lacking, the epithelial tissues dissolved into their component cells. When these dissociated, but still living, elements were returned to calcium-containing sea water, they adhered again to each other at their points of contact. Herbst assumed that a *Verbindungsmembran* exists between the cells when Ca is present, that this membrane is dissolved when Ca is lacking in the external medium, thus releasing the cells of the complex. When Ca is restored, this membrane is reconstituted and again cements the cells at their points of contact.

It is interesting to note in connection with these observations of Herbst those of Knüdson,[10] who found that in Pfeffer's solution the root cap cells of corn and Canada field peas are sometimes sloughed and remain in the medium isolated but living for as long a period as seventy days or more. While it does not appear that a Ca shortage existed in these root cap cells, the possibility of such a shortage would be well worth investigating.

In 1905 and 1906, while engaged in a study of the physiological properties of distilled water, the author, with the kindly aid of his colleague, Dr. Lyman G. Briggs, applied the method of electrical conductivity to the investigation of ion changes in solutions in which seedlings were growing. It was observed that the conducting capacity of distilled water in which seedlings were grown increased, due, it was believed, chiefly to the leaching of ions from the cells of the seedlings. It was noted furthermore that this leaching was checked when a small quantity of a Ca salt was added to the distilled water.[11]

The use of the conductivity method was extended by H. H. Bartlett and the author [12] to a study of ion changes taking place in distilled water and in solutions of calcium nitrate and magnesium nitrate planted with pea seedlings. Owing to the fact that the method as applied to this type of work had not then been carefully studied, more attention was given here to the method. The conclusion was reached that equilibrium concentrations of Ca and NO_3 ions in one case and of Mg and NO_3 ions in the other instance existed for peas below which the roots would leach ions into

[7] Ringer, Sydney, *Journ. of Physiol.* 4: IV. 1883.

[8] Ringer, Sydney, and Sainsbury, H., *Journ. of Physiol.*, 16:4. 1894.

[9] Herbst, C., *Arch. Entwicklungsmech* 9: 424. 1900.

[10] Knüdson, L., *Am. Journ. Bot.*, 6: 309. 1919.

[11] True, Rodney H., *Am. Journ. Bot.*, 1: 255-273. 1914.

[12] True, Rodney H., and Bartlett, Harley Harris, Bureau of Plant Industry, U. S. Dept. of Agri. Bull., 231: 1-36. 1912.

either solution, or into solutions containing both salts mixed in various proportions and above which the roots would absorb ions. It was shown that Ca differed essentially from Mg ions in being harmless in concentrations that proved fatal in the case of magnesium.

The conductivity method was next applied to the problem of absorption by phanerogamic seedlings from solutions of the ordinary nutrient salts; these being studied singly in various concentrations[13] and mixed in a variety of proportions and concentrations.[14] I will not try to deal here with the results gained beyond presenting a brief summary of such points as bear on the question now in hand. As a result of the study of several sorts of seedlings grown in solutions of single salts it may be said that in solutions of potassium and sodium salts no concentration was observed in which the seedlings were able to carry on sustained absorption, in the end yielding markedly more ions to the medium than they were able at any time to appropriate.

In solutions of Ca and Mg salts there was a well defined equilibrium concentration below which the roots were not able to absorb and in these sub-minimal solutions ions leached out into the medium. In solutions stronger than this equilibrium concentration, absorption took place in greater or less measure. It appeared that Ca was more favorable generally than Mg. At no concentration tried, the strongest being about 900×10^{-6} gram norm. per liter, was there any evidence of injury. Where the concentration of Mg was raised in order to ascertain the maximum quantity of absorption, characteristic injury appeared and death more or less promptly thereafter. A similar injury appeared tardily in weaker solutions on longer duration.

In mixtures again, absorption or leach depended on the presence of Ca or Mg ions. Again Ca in high proportion never brought injury. Mg injury appeared less often than in simple solutions. There was little evidence that any such thing as a very definite Ca-Mg ratio exists. In mixtures containing Ca and other nutrient ions, especially when all or a large proportion of the required ions were present, the total quantity of ions absorbed far exceeded the quantity of Ca ions present. This indicated that in such mixtures Ca ions in some way secured conditions that bring about the absorption of ions, that, offered in unmixed solutions, would be unabsorbed, or would cause an active leach of other ions from the plant cells.

Thus we may fairly say that the presence of Ca ions in some way makes those absorbable that would otherwise be unabsorbable and enables the plant to retain ions that it would otherwise be unable to retain. The Ca ions may be truly said to make the others *physiologically available* to the plant. Stating this in terms of the soil, we may say that when the required minimum of Ca ions is not present in the soil solution other nutrient ions present are largely out of reach and such a deficient soil solution may finally leach mobilized nutrients from seedlings. If the required minimum of Ca ions is not present, other nutrient ions may be present in abundance but be *physiologically unavailable* because of the inability of the plant to appropriate them.

Having thus far established the relation between Ca ions and the ability of the roots of the seedlings studied to retain ions gained by the mobilization of their reserves and to absorb others from the nutrient medium outside them, let us turn to a somewhat more detailed study of this phase of calcium action.

Analytical data have long since indicated a close chemical relation between the calcium-content of higher plants and the cell wall. The calcium content is relatively low in young meristematic tissue and increases greatly in those parts characterized by mature cell walls.

A more critical study of cell walls by Fremy, Mangin, Bertrand and others has shown that these are by no means homogeneous structures, either chemically or structurally speaking, but consist characteristically of an outermost layer lying on the boundary line between adjoining cells and other layers lying between it and the

[13] True, Rodney H., and Bartlett, Harley Harris, *Am. Journ. Bot.*, 2: 255-278. 1915.

[14] True, Rodney H., and Bartlett, Harley Harris, *Am. Journ. Bot.*, 2: 311-323. 1915.

[15] True, Rodney H., and Bartlett, Harley Harris, *Am. Journ. Bot.*, 3: 47-57. 1916.

plasma membranes. This outermost boundary layer consists of a calcium salt of a weak organic acid known since work of Mangin as pectic acid. Not only is this structure Ca pectate, but in cases other layers of similar chemical character occur in the thickening materials laid down in the more interior parts of the wall. This Ca pectate has been shown to be a stiff adhesive colloid that is formed when pectic acid meets Ca ions. According to authors from Fremy to Bertrand this acid appears when the neutral mother substance pectin is acted on by the enzyme pectase.

Now in view of the observations of Mangin, Bertrand, and others, and latterly those of Sampson [16] there seems to be considerable freedom in the shifting of cell wall materials into and out of the pectic acid condition, and when Ca ions are present, with the consequent appearance of calcium pectate layers. These chemical shifts are frequently explicable only by relaying them back to internal irritable causes. They appear then in cases to be self-regulated chemical responses to stimuli, perhaps due in the first instance to external conditions, but in their immediate application, to internal causes. Thus Sampson finds in the abscission tissue of coleus leaves following the shock due to inflicted injury a change of more or less of the cellulose of cell wall tissues to pectic acid with a disappearance of calcium ions from the cells of the abscission layers and from their walls. Sampson favors the view that the change of cellulose into pectic acid arising from the irritation that sets in motion the train of abscission phenomena is responsible for the disappearance of Ca. Pectic acid being present greatly in excess of the quantity of Ca ions present can not be converted by these ions into the firm colloid, calcium pectate, but creates a thin, mechanically weak colloidal medium which mutually interdiffuses with the Ca ions and in proportion as the pectic acid exceeds the Ca dilutes and removes it from its original seat.

In this connection it should be noted as a general observation that the conversion of cellulose into pectose is a usual feature in aging cell walls (Sampson: 48). The shift from pectose to pectic acid follows easily.

The change in firmness of fruits and vegetables seen to follow the action of parasitic or of saprophytic fungi seems to be a related phenomenon. Here some form of Wiesner's theory of the generation of organic acids which take possession of the Ca tied up in health in the Ca pectate layers seems likely to apply. With the removal of the Ca by acids formed directly or indirectly by fungi, the pectate layers become pectic acid or something closely akin. Since these substances lack mechanical strength, a slump of the tissues follows.

It was my good fortune to be able during the winter of 1919-20 to associate Dr. Sophia H. Eckerson of the University of Chicago with our work on this calcium problem, then being carried on in the U. S. Department of Agriculture and with her permission I beg to refer here to some of her findings. She grew seedlings of wheat, maize and white lupine in series of solutions closely paralleling others that were receiving attention, or had received attention in conductivity experiments. Dr. Eckerson applied the methods of microchemistry to the study of seedlings grown in potassium solutions in which Bartlett and the author had found a leaching of ions from the seedlings into the solution. She observed (1) that ions readily entered the cells of the roots, (2) that within twenty-four hours Ca ions began to diffuse out of the calcium pectate middle lamella, (3) *K* pectate was formed instead of the Ca salt and this substance being relatively soluble in water soon dissolved, (4) at this stage, *sugars, amino-acids,* and *salts,* chiefly Mg, diffused rapidly out of the roots. Thus we find Dr. Eckerson's microchemical evidence giving us the stages of an event already found to exist by means of our grosser conductivity work. It was established beyond doubt that not only was the cell wall modified and in part dissolved by the replacement of Ca ions by K ions in the solution, but it was shown that the damage goes far more deeply into the cell. Analyses of the leach into distilled water by lupine roots has already demonstrated to us that no less than two thirds of

[16] Sampson, Homer C., *Bot Gaz.*, 66: 32-53. 1918.

the materials yielded were organic and perhaps in large part non-electrolytes. Dr. Eckerson finds that the leach into K solutions are largely organic and non-electrolytic. These solutions must have come in considerable part from the cell contents. The permeability of the cell walls had been greatly modified, also the osmotic properties of the plasma membranes.

These modifications were seen in the passing of materials from within outward. Dr. Eckerson tested the permeability in the opposite direction. Corn seedlings after five days in a KNO_3 solution were placed in a 1 per cent solution of copper sulphate. In one hour the Cu ions had penetrated all of the root tissue. Similar seedlings after five days in a $Ca(NO_3)_2$ solution showed the penetration of Cu ions only after twenty-four hours in a similar copper solution. This seems to make it clear that permeability for ingoing ions is also greatly increased by the changes that we have described.

Experimental work on Mg solutions showed that Mg pectate replaced Ca pectate in solutions of Mg salts. It is known that while Mg pectate is not soluble like K pectate and is less permeable it is slightly more permeable than the firmer Ca pectate.

Dr. Eckerson found in addition to this that the fatal result repeatedly seen in our other work to come after a longer or shorter time to seedlings grown in Mg solutions of more than minimal concentration did not occur until the Ca of the middle lamella had been wholly replaced by Mg. When this had come to pass the cells died. We could perhaps imagine that sufficient uncaptured Mg ions were then free to penetrate the deeper structures of the cell to bring about the fatal upset.

The conclusion seems well founded that the integrity of the calcium pectate forming the middle lamella was maintained when a sufficient quantity of Ca ions was present in the culture solution and with it the normal retention of its contents by the cell. When according to the laws of mass action this quantity of Ca ions fell below the equilibrium concentration, other kations present replaced the Ca in the colloid compound forming the middle lamella. As a result of a long series of experiments in various culture solutions, it may be said that no kation other than Ca has been found that can replace it in this relation without an injurious or fatal change seen in permeability relations, or without the appearance sooner or later of other toxic response. Mg comes most nearly to replacing Ca, but fails, partly because of the greater permeability of its pectate, chiefly because of the ultimately toxic action of the Mg ions when they reach the deeper lying structures.

In view of what has been said, what are we justified in thinking concerning the phenomena that lie deeper than cell walls, what about the living content of the cell? I think we are justified in regarding the cell wall and the plasmal membranes that secrete it, and in closest contact with which it lies, as standing in the closest relation. Cell walls, except in specialized locations, are seldom decisive in determining what ions pass through them. They influence, as we have seen, up to a certain quantity the ions that pass into them, through the chemical changes which take place in the walls themselves and thus far may be regarded as having a certain quasi-determining influence. Beyond that, after chemical demands in the walls have been satisfied, more deeply lying equilibria are concerned. As an ion-containing structure, the cell wall maintains ion-equilibria subject to the laws of equilibria in colloids, with the living membranes with which it stands in most intimate chemical and biological contact. When ion equilibria in the wall are disturbed, this disturbance is transmitted to the equilibria of the protoplast that lays it down, modifies it and remains in closest relation to it. Hence it is not surprising that a drastic change in the very chemical composition of parts of the wall itself if continued should work through and perhaps profoundly affect the equilibria of the protoplasm.

This close relation of protoplasm and cell wall has already been seen in the cases of wall change initiated from within in response to irritation. When cells are melted apart by self-regulatory processes it seems hardly necessary to argue the intimate relation of wall change to protoplasm change. In response to the formative laws governing the organism a dozen or more layers of cells surrounding the embryo of the wheat or maize are completely absorbed and in the end the innermost remaining walls of the ovary are literally cemented to the outer unabsorbed layer of the inner integument.[17] Here is emphatic control of cell walls by the life inhabiting them, control exerted chiefly through the agency of the Ca-ion-equilibria of the tissues concerned. Finally this control in the wheat as in Herbst's sea urchin embryos is shown by the fusing together of *outer surfaces of cell walls.* Here we seem to have clean cut instances to show how in the formative processes the living material is able to command the structure it forms about itself. The outer walls of cells originally located far from each other are brought together by the solution of intervening structures. The substances necessary for the formation of the cementing layer seem to be extruded from the protoplasm through the wall to the outside surfaces where they unite to form the coagulum seen. Perhaps the Ca ions and the pectase thrust through from the interior of the cell meet at its frontier the pectin which under enzyme action yields pectic acid in the presence of the Ca ions. The product of such an occurrence would be seen in the cementing layer formed on the outside of each of the now neighboring cells.

In conclusion, I should like to refer briefly to some of the more practical results that seem to flow from the considerations that have been here set forth.

It appears that a certain quantity of Ca ions must be present in the medium for the maintenance of the chemical and functional integrity of the cell wall, as well as the chemical and functional integrity of the deeper lying living parts of the cells of absorbing roots of higher green plants. When this is so maintained, absorption takes place in the manner we are accustomed to call normal. When this necessary minimal supply of Ca ions in the medium is lacking, be it in soil solution, water culture, or sand culture, the function of absorption is upset and a more or less marked leaching of ions from the plant follows. In the absence of this necessary minimum of Ca ions, the soil solution or culture solution may be rich in all other required ions, but these are useless to the plant. They are unabsorbable. This brings us face to face with a condition of affairs in plant nutrition that has not been recognized and therefore has not been characterized. We may fairly say that Ca ions make *physiologically available* other equally indispensable nutrient ions. The practical consequences that follow from this way of looking at the fertilizer problem have not thus far been realized. We learn why from earliest times civilizations have grown up on soils rich in limestone dèbris. We learn why agriculture has readily succeeded in some regions, not in others. We understand why, by the use of lime, lands have been rendered capable of supporting largely increased populations. We are now able to correlate these broad facts with those of cell physiology and to suggest perhaps not *the calcium function* sought by Jost, but one way perhaps of many in which higher green plants find calcium necessary.

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[17] True, R. H., *Bot Gaz.*, 18: 212-226. 1983.

THE METHOD OF SCIENCE IN AGRICULTURE[1]

To be practical has been the great goal of agricultural investigation from the beginning. It was entered upon with a practical purpose, and in a large degree practical results early came to the expectation of the farming people. Here was a type of science which was not working in the clouds for its own sake, but down in the dirt where the problems of farming lay.

It is fortunate that this has been so—that this close sympathy and this urge to meet the needs of the art have been felt so keenly. It has given life as well as purpose to our branch of science, and the wide extent to which its

[1] Address of the Vice-president and Chairman of Section O—Agriculture, American Association for the Advancement of Science, Toronto, 1921.