A STUDY OF THE ANATOMICAL AND PHYSIOLOGICAL EFFECTS OF THE TOXICITY OF GALACTOSE ON SEEDLINGS

By

OWEN HARRISON LOVEJOY

Submitted to the Department of Botany and the Faculty of the Graduate School of the University of Kansas in partial fulfillment of the requirements for the degree of Master of Arts.

Approved By: W. C. Stevens 1917

Department of Botany

INTRODUCTORY AND HISTORICAL

Comparatively little study has been made of the effects of soil toxics on the structure and physiological behavior of the roots of seedlings. There is consequently a paucity of data available on the reactions of roots to soil media, since this field of Botany is a comparatively new one, only recently assuming substantial proportions on account of its economic importance.

At present this field of research is of great import in the subject of soil chemistry. Facts learned in this direction promise to have an ultimate bearing on the food supply of the world's increasing population.

The particular problem considered here is only indirectly related to the study of soils, since the seedlings used are nurtured in nutrient solutions under the sterile conditions and not in natural soils. However, the facts gained here may help us to better understand how plant tissues react toward certain environmental stimuli.

Stated briefly, the purpose of this research is to see how the root tissues of seedlings behave when subjected to the toxic influence of the hexose sugar galactose, and how certain inorganic salts affect the reactions when added to the media. It is understood, of course, that the seedlings and media are to be kept free from contamination by bacteria and fungi thruout the various experiments, by

methods explained later.

If we can succeed here in presenting a few new facts learned, we shall in so far help to add something to the knowledge of soil media and their effects on the development of plant tissues.

The problem was suggested by the researches of Lewis Knudson* in which various sugars were applied to nutrient solutions to determine their effects on the growth of young plants grown in the solutions. As will be explained later in greater detail, Knudson found that the sugar galactose behaved in a way radically different from the other sugars tested, in that it was actually toxic to the young plants, whereas, the other sugars were in most cases harmless, and in some cases at least actually stimulative to greater growth than that made by plants developed in normal solutions.

It is important here to tell something about the sugar galactose and its chemistry.** This sugar is formed by the hydrolysis of lactose,(milk sugar), and is found in nature associated with other sugars in certain gums and pectic

*Knudson, Lewis. The Toxicity of Galactose for Certain of the Higher Plants. Mo. Bot. Gar. Annuals 2:659-666 Nov. 1915.

**Haas, P. and Hill, T. G. , The Chemistry of Plant Products, P. 65.

substances. It is also found in several plants of the Pink family,(Caryophyllaceae), and is a component of the trisaccharide raffinose and the glucoside digitalin. Galactose is best prepared, however, by boiling lactose for six hours with four times its weight of 2% sulphuric acid. The solution is then evaporated, and a few crystals of galactose added to induce crystallization. After some time the crude galactose crystallizes out and may be purified by adding four fifths its weight of water, and mixing with two times its volume of 93% alcohol. The precipitated sugar is then filtered off and dried. Galactose crystallizes in minute hexagonal crystals, which melt at 168°C. It is strongly dextro-rotatory, A_d equals 83.8°, and exhibits muta-rotation. It ferments completely, but rather more slowly than glucose.

A more complete summary of Knudson's results now follows:* He found that seedlings of vetch,(Vicia villosa), showed a marked injury when grown in the presence of a nutrient medium containing 2% galactose, the toxicity being accompanied by killing of the roots and reduction of the top parts of the plant. He was led to carry his tests

*Knudson, Lewis. The Toxicity of Galactose for Certain of the Higher Plants, Mo. Bot. Gar. Annuals, 2:659-666 Nov. 1915.

further by the rather surprising discovery that lactose, a closely related sugar, actually proved to benefit rather than injure the plants. He grew his plants under sterile conditions on an agar medium made up with Pfeffer's nutrient solution at one half its normal strength, which is a neutrally reacting solution. His seeds were disinfected against contamination by a treatment with chloride of lime, (to be described later), and transferred to the sterile culture vessels with the usual bacteriological precautions. The culture vessels were tall narrow glass jars, to accomodate the growth of the leafy stems. After a period of thirty days, the plants growing on the galactose media were seen to have been unable to penetrate the agar, the roots dying soon after contact with the same. Many lateral, adventitious roots were thus forced, these dying likewise.

He made like tests with glucose, saccharose, lactose, and maltose, and found that these sugars greatly benefited, and even nourished the plants through absorption and assimilation.

Experiments with galactose involving varying per cents of the same, (from 0.125 to 2%), showed that marked toxicity was produced by percents of 1% or more. Canada field peas, corn, and wheat were tried with like results. Finally it was discovered that the sugar glucose, in equal percents with galactose, served to counteract utterly the toxicity

of the latter. This fact is especially significant, considering that both are hexose sugars, with the general formula $C_6 H_{12}O_6$, and have a very similar molecular structure, being isomers.

ACKNOWLEDGMENTS

The writer is indebted especially to Dr. Charles A. Shull, now Professor of Botany and Head of Department of Botany at Kentucky University, for the suggestion of this as a possible problem, as well as for his invaluable guidance, advice, and encouragement in the various phases of the work.

Especial acknowledgment is accorded to Mr. L. M. Peace of the Botany Faculty of the University of Kansas, for advice and assistance, not only in technique, but in furnishing the two splendid photographs, **I** and **II**, showing the principal tangible results obtained in the work. These pictures also bring out clearly a number of facts concerning the detail of the methods used.

METHODS AND MATERIALS

I shall first describe Knudson's method of culture of the seedlings in testing for the toxicity of galactose, and then compare the same with my own, giving reasons for varying the same.

Knudson used agar media exclusively, prepared with Pfeffer's nutrient solution made up to one half its normal strength. This solution reacts neutrally. To this solution was added the amount of galactose sugar to be tested. The solution with a 1% solution of agar, was placed in large glass cylinders,(of about 4l capacity), with 250cc of the solution per jar. The cylinders were then fitted with cotton stoppers and sterilized one hour in the autoclav at 15 lb. pressure. (The writer found that thirty minutes sterilization was amply sufficient in the autoclav at only 10 lb. pressure, and there was less danger of the heavy jars cracking at the lower pressure and shorter time period. In fact this pressure was used throughout the problem, with perfect freedom from infection from this source.)

The writer used the same method as Knudson in sterilizing the seed to be used, a method devised by Dr. J. K. Wilson*. The method consists of shaking up ten grams chloride of lime with 150cc tap water and filtering the supernatant liquid into a test tube where the seeds are treated for a period of several hours. (The writer found that one to two hours was sufficient for the proper sterilization of the Canada

*Wilson, Dr. J. K., Amer. Jour. Bot. 2:420-427;1915.

field pea, though as much as four hours has been recommended). The seed are then carefully transferred to the germinating jars by means of a small spoon. For this purpose the writer used a glass spoon, devised by Mr. Peace of the Department of Botany of Kansas University. First a bulb was blown by heating the end of a twelve inch glass tube, then the side of the bulb was reheated and blown out forcibly, the resultant rim being then heated down, leaving a bowl just large enough to receive one or two peas nicely, without these fitting so tight as to obstruct the process.

At each transferral of seed from disinfecting vessel to culture jar, the glass spoon was dipped in alcohol and flamed, thus providing double sterilization. Other bacteriological precautions were observed to prevent infection.

The writer found that agar at 0.75 % was more satisfactory than the 1% recommended by Knudson, as it, while giving a firm support for the germinating seed, still provided a medium easily penetrated by the tender rootlets. The greater amount of agar sometimes caused the young roots to travel across the surface some distance before starting downward, thus causing a possible source of delay in the apparent toxic effects of the galactose.

In these experiments it was found best to dispense with the tall jars used by Knudson, as we were not directly concerned with the tops of the plant but only with the roots.

Low shaped jars provided ample room for the tops of the small plants for periods of all the experiments carried out. In this connection several arrangements were tried to get good results with the growing roots. In general glasses of about 500cc capacity were most adaptable, with ground glass lids, as shown in the illustrations, with peripheral flange to keep them from slipping off during the sterilization operations. Not only did these prove more convenient in size, but they were much more adapted to the small autoclav at hand, it being possible thus to sterilize two dozen or more at once.

Before progressing far it was gound necessary to resort to water cultures, wherein the plants were supported over liquids instead of reclining on an agar soil, for certain results that could scarcely be obtained with the agar. In one case jars were provided with stoppers, with vertically sliding copper wires holding the seedlings, so as to make it possible to raise or lower the roots in the media at will. This would obviously have been impossible in a solid medium such as agar.

In preparing the water cultures the jars were equipped at first with various supports for the seedlings, mostly calling for the sprouting of the seeds in separate receptacles, and later transferral to the media. The most satisfactory supports for the seedlings were made with

copper braces wedged between the sides of the jars, and holding, just above the liquid thin discs of cork, notched to receive the seedlings. Each of these braces was cut from a single strip of brass, 1cm wide, and a little longer than the inner diameter of the glass. These strips were slit at each end a distance of 2cm, and bent so as to present two opposing prongs against the sides of the jar at either end of the brace. Then the disc of cork was fastened to the middle of the copper strip, by means of a prong of the copper itself, so as to be suspended horizontally just above the surface of the liquid. The writer found it convenient to notch the cork discs to receive three seedlings each, though one or two seedlings could be used if desired. Several methods were tried to support the seedlings before hitting upon the above, such as floating corks, and attachments from the tops of the jars. But this method had the advantage of allowing the root only to touch the medium, and at the same time permitted the use of close-fitting glass lids. The supports in general were placed in the jars about 2cm above the liquid.

The advantages of the water culture method were several. In this it was possible to select uniform hardy seedlings for the particular test, from a large number in the germinating jars, thus eliminating the seeds that might fail to germinate. Also the results could be observed of healthy

roots immersed in toxic media, as well as those growing into the latter from above. The appearance of the roots could be studied better in a clear liquid medium than in the less transparent agar. It was moreover found impossible to use the agar with certain chemicals such as $Al_2(SO_4)_3$, which prevent the "setting" of the agar at the lower concentrations. The "water culture" proved better in every way than the agar.

The preparation of the media required the most time and care. For both agar and water media the previously mentioned Pfeffers nutrient solution was used, prepared as follows: To six 1 water add 2g Ca NO_3, 0.5g KNO_3, 0.25g KCl, 0.50g K_2HPO_4, 0.5g Mg SO_4, 4mg Fe_2Cl_3. This stock solution was prepared and kept in quantity.

In preparing the agar medium the chemicals to be tested were added to the nutrient solution, in their proper proportions and in sufficient quantity to provide each culture jar with 200cc of the desired solution. Last, the washed agar was added, and the whole heated to dissolve the agar. When several reagents were to be tested, in different jars of the same experiment, the agar solution was boiled and distributed to the various jars, and the individual reagents added respectively before placing in the autoclav.

In preparing the water-culture media the nutrient solution was merely poured in proper quantity into the jars

and the chemicals weighed up and added to the individual vessels, before placing in the autoclav. The seedling supports were put in place before adding the liquids, so that the whole apparatus could be set in the autoclav without further adjustment.

A peculiarity was met with in the solution of calcium sulphate, which is a salt of very low solubility. At first small quantities of this were seen still undissolved after the setting up of the experiment, which remained insoluble in the medium. It was found, however, that if let stand over night before setting up, the salt would be thoroly dissolved. In such experiments as these, where great differences of result come from almost negligible variations in percents of constituents, such errors as the above would be most serious.

Considerable care was found necessary to raise the seedlings preliminary to setting up the experiments. We have already described the process of sterilizing the seeds preparatory to germinating the same under sterile conditions. The seed were then transferred to the culture jars by means of the glass spoon previously described, with bacteriological precautions. The culture jars in which the seedlings were sprouted, were of about 500cc capacity, glass covered and sterilized in the autoclav with each jar containing 200cc nutrient solution and 0.75% of agar. Enough dis-

infected seeds were placed in each jar to provide for one series of tests, allowing generously for now viable seeds. These jars were kept several days in the dark until the young roots had penetrated about 3cm into the agar on an average. This required about six to eight days. The seedlings were then transferred to the testing jars as follows; the bunsen flame was passed around the edge of each glass jar before opening. Then the seedlings were transferred by flamed tweezers, grasping the plumule lightly, and placed carefully with the cotyledons resting on the cork discs, the hypocotyls resting in the peripheral notches of the discs, and the roots projecting into the liquid. The jars were then flamed again, after replacing the lids, and set away in the dark to await further developments.

This method proved especially adapted to the study of young roots, not only in eliminating disturbing factors, but also in providing that plants of equal vitality might be selected and compared, and minuter distinctions made. Since all the roots could be placed in the media simultaneously, the toxic effects of the reagents could be timed with uniform results. This is important considering the marked response of the plant tissues observed to slight differences of the media.

THE EXPERIMENTS

(a) The Toxic Effects of Galactose Alone.

In carrying out the experiments two kinds of effects were studied,(a), the toxicity of galactose alone when applied in the nutrient solutions, and,(b), the effects of certain inorganic salts in their turn on the toxic effects of the galactose.

In the first case we shall take up the results obtained in studies made with the agar-nutrient media, and then those with the water-culture media.

As far as these experiments were carried out, the Canada field pea was the only plant employed, it having been used with good results by Lewis Knudson in his galactose studies.* In case of the agar media, record was kept of the number of days required for the seeds to germinate, and also the days till certain degrees of toxicity were apparent.

In the first tests, tall jars were used as sprouting vessels for the seedlings, fitted with cotton plugs, but soon it was found more convenient and efficient to substitute the glass vessels shown in the photographs, each being of 6cm interior dimension, and 10cm interior height, and fitted with the close fitting, ground glass lids. These were used successfully throughout the remaining work.

*Knudson, Lewis, The Toxicity of Galactose for Certain of the Higher Plants, Mo. Bot. Gar. Annuals 2:659-666 Nov. 1915

The first experiments were unsuccessful owing to impurity of the galactose used. When a fresh supply was obtained from the Chemistry Department of Kansas University, recommended there as being of a high grade, results were obtained quite in keeping with the observations of Knudson.

Jars were provided with 100cc each of the agar-nutrient solution. One half the jars were prepared with 1% galactose, and the rest left as checks. In two days the seed had sprouted, and three days later the characteristic yellowing of the roots had commenced to appear in the galactose jars, the checks remaining healthy and white with longer rootage than the others. More definite description of the toxic symptoms will be made later.

The preliminary tests were made with both Canada field pea,(Pisum satrium), and common popcorn,(yellow), It was seen that while both gave satisfactory results, the former with its heavier roots, lent itself better to purposes of observation, and facility in freehand sectioning in fresh condition. Also it proved that the smooth round seeds of Pisum satinum were more easily and quickly disinfected in the calcium chloride water, and gave better sprouting results. Therefore it was decided to limit the work to the use of the latter entirely.

Next was determined the lengths of time, at different concentrations of galactose, at which toxicity became

apparent in its different stages. A series of jars were prepared running from 0.05% to 0.20% galactose, along with sugar-free checks. In this, as in all the other agar tests, between two and three days were required for the seeds to sprout far enough for the roots to reach and penetrate the medium.

In eleven days from setting up the specimens in the weakest galactose solution had begun to turn slightly yellow, and in the other jars there was a splendid series of gradation of color, as the concentration increased, till a deep brown was to be observed in those of the 0.20% solution. The exact symptoms will be described in their proper place.

Further experiments of the same sort showed that no definite symptoms could be observed the sixth day after setting up at the 0.20% concentration of galactose, but a slight yellowing was apparent the seventh day, definite browning the eighth day, and a deep brown with shriveling of the root tips the nineth day. In a number of experiments, run in duplicate with three seeds to the jar, definite shriveling was uniformly seen by the tenth day at 0.20% concentrations. Further tests run at 0.30% showed a slight advancing of the stages, a very definite browning of the roots resulting in eight days instead of nine as above.

The symptoms of toxicity as observed in the specimens grown in agar media, showed the remarkable sensibility of the

plant's power of resistance to slight variations in the concentration of the galactose. This is demonstrated exceptionally well in the fourth series of tests, where the following concentrations of galactose were employed: 0.2%, 0.15%, 0.1%, 0.08%, and 0.05%.

On the eleventh day after setting up, these results were seen. The 0.05% specimens were apparently normal. The 0.08% specimens were very slightly yellow just at the root tips, and had slightly enlarged rootcaps. The 0.10% specimens were similar, except that the root caps were larger, and the tips somewhat yellower. The 0.12% specimens showed a slight browning of the submerged lateral branches of the roots, whose tips were covered with masses of sloughed off cells. Those in the 0.15% solution had a slight browning of the main roots, and their branch roots were now only 2cm long as compared to 6cm or more in case of the normal specimens. Those in 0.2% had deep brown roots, and the numerous brown rootlets were not nearly 1cm long.

That the cells of the browned tissues were actually dead was evident from the fact that very soon after removal from the jars they were seen to be teeming with bacteria and rapidly decaying.

It was also seen that, as reported by Knudson, the toxic effect was confined strictly to the parts of the root in immediate contact with the toxic medium. Also the roots in

concentrations of 0.1% or less, invariably produced profuse lateral roots, but in greater concentrations these were limited to mere stubs, due to the apparent paralyzing of the activity of the primordial mesistem.

We may note here that the tops of the plants were not appreciably affected by the toxicaction, but remained plump and firm. Grown in sunlight, the chlorophyll formation was not noticeably impaired.

The rootlets were seen to keep dying back at the tips, thus preventing increase in length, even before total toxicity occurred. The primordial mesistem in this case seemed stimulated to produce an abnormally large cap of cells, developing rapidly till cut off from food supply by general disintegration of the tissues.

In this same series of tests, after the plants had remained two weeks in media of galactose, 0.2% or stronger, and were a deep brown thruout the length of the submerged hypocotyl and root system, they were removed from the toxic solution, and placed in a vessel of the normal nutrient solution. The tap roots were apparently dead and stunted. The laterals were brown and of a maximum length of 1cm, as compared to as much as 10cm in those checks of the same age. By the next day the specimens had sent out branch roots from practically every root just back of the utmost shriveled tip.

It was seen that when new rootlets grew out of the dead

surface of older roots as above, they caused the dead tissues to split open longitudinally. These adventitious roots kept on growing in a perfectly healthy condition as long as they were kept in the nutrient normal solution.

In a later test with plants grown in 0.2% galactose, time was recorded as to the occurrence of the browning. The sixth day still showed the roots firm and white as the checks. The seventh day there was a distinct yellowing, beginning exactly at the surface of the medium and extending to near the tip of the roots. The 8th day the roots were still white and firm above the surface, but distinctly brown beneath. By this time the main root tips had dies back, but many rootlets were seen to branch out below the surface of the medium. The nineth day the main roots were shorter than the rootlets. Thus here, as in a number of the experiments, rootlets were able to develop to a considerable extent after the main root had been definitely checked in longitudinal growth. However, this phenomenon was seen only in rather low concentrations of the galactose.

In a number of cases where the surface layers of cells were dead, inward growth had caused longitudinal fissures in the surface, like those in the bark of a tree, caused by interval expansion. We shall now consider the anatomical effects of the galactose on the young roots, as observed in the agar media, reserving the water-culture effects for later consideration.

We have mentioned the rather common phenomena of the toxic action of galactose, the formation of large caps on the roots penetrating the medium. The microscope showed these to be masses of sloughed off cells, mostly elongated oblong in shape, and usually plasmolysed. New primordial tissues continued to develop for some time after the appearance of these caps. Upon exposure to air these root tips soon became teeming with bacteria and decayed rapidly.

It was apparent that the toxic action was immediately confined to the cells in contact with the medium, or separated from the same by dead cells only. The root tips and epidermal cells were thus first affected, the inner cells appearing perfectly normal and unplasmolysed.

In the toxic action toward the cell, the protoplast was seen at first apparently plasmolysed, then assumed a characteristic granular appearance, while the cell wall was still intact The inner functions of the root were not visibly interfered with, even when the epidermis and root hairs were shriveled and darkened.

So regular was the progress of the toxicity through the tissues that by periodic observation its course was traced through the epidermis, the outer layers of the cortex, and cell by cell through the inner tissues. The cambial layers seemed to be the limit of fatality. Each successive layer became brown and granular, and finally shriveled, till at length

little could be identified but the vascular tissues. The outer layers of tissue were missing in advanced stages of toxicity, apparently having sloughed off on the withdrawal of the specimens from the agar for study.

The microscopic study of the cells killed by galactose, showed a distinctly brownish color in the cell walls. There was a granular coating on the walls. Some of the intercellular spaces were entirely filled with a dark substance. Yellowish brown masses were seen clinging to the inside of the cells, perhaps the shriveled protoplasts.

The rate of progress of toxicity was found to be such that when 0.2% galactose was used, the dying of the cells had advanced through approximately six layers of cells by the ninth day from the setting up of the experiment. There had been no apparent browning until the seventh day, or four days after the roots penetrated the medium. By the time the six layers had been penetrated, the outermost layer of cells had in a few places utterly disintegrated. Where a number of layers of cells were killed, and inner expansion had produced the aforesaid longitudinal fractures, the microscopic appearance of the cross section was very similar to that of the cork cells formed on woody stems.

Any further description of microscopic observations will come under the head of microchemical effects, since from now on these two phases of the work will be rather closely related.

The above mentioned similarity of the dead root cells to cork cells suggested the possibility that the appearance of the came might be due to a futile attempt on the part of the plant to protect the living cells from further toxic effect. Therefore Molische's K O H test* for suberized tissues was applied to fresh cross-sections from affected roots. Concentrated solution of K O H was applied to sections under observation on the microscopic slide. The parts showing the toxic effects of galactose were turned a bright yellow. On heating the slide slightly over the microburner, the yellow was intensified in the cell walls, accompanied by considerable swelling. When heated further, yellow oily granular drops were exuded profusely from the cell walls. These drops were water soluble. The action was confined strictly to the affected regions.

Next are described the experiments made with seedlings in water-cultures in place of the agar. The first of these was made with three day old plants, placed as described on the brass supports, their roots hanging in immediate contact with the liquid. First the seedlings were suspended as seen in the photographs, directly on the brass supports. Later it was decided to utilize the notched cork discs in connection with the braces to prevent contact of the plants with the metal. Either method proved very satisfactory.

In all these tests the galactose-free checks developed long white roots with many laterals evenly distributed along

*Haas, P. and Hill, T. G., Chemistry of Plant Products.

the main root. In five days time the galactose-treated roots had begun to show yellowing and shriveling at a concentration of only 0.055% galactose, while the part of the root above the surface of the liquid was still white. A peculiar swelling, to be described more minutely later, was evident about 2cm above the liquid. Repeated tests gave similar results. Tests made with 0.1% galactose showed the roots becoming yellowish in three days, and putting forth numerous lateral roots above the surface of the medium, which phenomenon was a sign of lessened activity in the submerged root. In five days, the browning had deepened and advanced to a stage of shriveling. The lateral roots put out above the surface of the liquid shriveled at the tips on coming in contact with the medium. Further growth of the affected parts was definitely checked.

After seven days in the galactose medium, the diseased plants were transferred to a normal nutrient solution. The result differed somewhat from that obtained in case of the agar media, in that new lateral roots were put forth only above the browned areas, from the unaffected areas, showing that the toxic effects had permeated the submerged roots.

Tests made with 0.15% galactose caused browning and dying of the tips at five days or less. A series of solutions were prepared with galactose of the concentrations, (a), 0.05%, (b), 0.09%, (c) 0.12%, (d) 0.15%, and, (e), 0.2%. After two days the 0.05% specimens were not perceptibly injured; those at 0.09% showed a faint yellow coloration; at 0.12% a slight shrink-

age of the tips was seen, which was quite noticeable at 0.15%. At 0.2% the colorational effects were more pronounced, and the tips were still more shriveled. After another day, the specimens varied from a faint yellow to a pale brown, according to the concentrations of galactose. After a total of four days the 0.2% specimens were a deep brown and apparently dead. The 0.15% specimens were nearly as bad off, tho they had put out a few feeble laterals just below the surface of the medium, as the 0.2% specimens had failed to do, which reached an average length of 2mm. The 0.09% specimens had produced laterals longer, with root hairs, and not very brown, while the laterals of the 0.05% specimens were scarcely yellow.

Further tests proved that the toxic effects of galactose reached a maximum at between 0.2% and 0.3%, practically inhibiting further growth while the roots remained in the media.

A special experiment was devised to provide the young plants with adjustable wire supports, which could be raised or lowered from outside the jars to regulate the position of the roots relative to the surface of the liquid. The seedlings were placed with roots entirely above the liquids in the jars. Galactose was used at 0.2%. The roots on entering the toxic medium soon became dark and shriveled below the surface. Next day, when they were raised by the wire supports above the surface, they resumed terminal growth, putting forth white tips anew. On being once more submerged the tips were killed

again in a few hours. Later they disintegrated in the liquid, partly sloughing off and falling to the bottom of the jars. One root, left in the medium continuously shriveled and died.

The symptoms of toxicity where galactose alone was used, were similar with specimens grown in agar and water-cultures. One effect noticed in the higher galactose concentrations, was the tendency to inhibit lateral rootage below the surface of the liquid and increase same above, especially in advanced stages of the toxicity. Further discussion of symptoms will be included in the description of experiments dealing with inorganic salts and their effects on the toxicity of galactose.

As to microscopic aspects of the water-culture tests, we shall consider first the condition of hypertrophy of the hypocotyls mentioned earlier. We have said that the hypocotyls of certain poisoned plants exhibited a swelling some distance above the media as pictured in Plate I. These enlargements were due to the cortex cells which were so distended as to lose the characteristic hexagonal form, and become spherical in cross-section, thus obviously leaving intercellular spaces of considerable extent. Many of these cells measured as much as three times their normal diameter. The vascular and intravascular tissues were practically unaltered as to form and size of the cells. The entire area of cortical tissues was found to be enlarged to from one and one-fourth to one and one-half times its normal radial thickness. Still it

contained no more than its normal number of cells;i.e. no hyperplasia was apparent. This hypertrophied condition was most pronounced at a concentration of the galactose of 0.055%, and was not found at all at the higher percents.

The root tips of the water-culture seedlings in galactose showed strongly the aforementioned shriveling and granulation of the protoplasts of the exposed cells.

In cross-section the outer tissues of the roots were seen to disintegrate very rapidly, with the advance of the toxic effect, into a dark mass of shriveled, broken cells. This darkened mass was found to respond to the K O H saponification test very definitely, with great numbers of yellow globules, demonstrating the presence of large quantities of fatty materials. Sections of normal roots gave no such response to this test, nor did the normal cells of the galactose-affected specimens.

Another frequently observed effect of the toxic was the tendency of the cells near the root tips to split apart, though maintaining their original shape. Often whole strands of loosened cells would be seen free from the rest of a root tip, when examined as a whole.

When sections were studied of roots remaining in the media nine days, the toxic effect was seen to have not only permeated the root tissues, but to have actually been carried upward through the vascular system into the stem of the plant. Sections were examined from different parts of the stem to ascertain the

extent of the action. The characteristic brown coloration and shriveling of the cells was found to reach to the cotyledons, and as much as 3cm above the liquid. The tissues of the immediate vicinity of the water tubes were first affected, then the brown stain spread to the surrounding tissues till it reached in some cases to the cortex. These affected tissues responded positively to the K O H saponification test. The darkened areas about the vascular bundles were so great as to be discernible to the naked eye, and were seen to extend beyond the cotyledons into the epicotyl, (without affecting the cotyledons).

In the water media, as in the agar, the toxic effect was seen to progress always along a regular margin of cells inward through the cortex, reaching the cambial region first near the root tips, and killing the root back and causing it to shrivel.

(b) Effects of Inorganic Salts on the Toxicity of Galactose.

These experiments were limited to two classes, as far as it was possible to carry the problem; first to determine the effects of nitrates on the toxicity of galactose; and second, the effects of sulphates on the same.

A peculiar power of young roots, that of oxidizing substances brought in contact with them,* is definitely affected

*Schreiner, O., and Reed, H. S. Studies on the Oxidizing Power of Roots. Bot. Gaz. 47:355-388.

by soil conditions. The toxicity of galactose suggested that the sugar might in some way exert some influence on this oxidizing action of the roots and thus impair their functions. The oxidizing action of roots is no doubt of considerable utility to the plant, and its stimulus or inhibition would no doubt influence the plants welfare one way or the other. Now since the nitrate ion($-NO_3$) is known to stimulate root oxidization,** the question arose as to what effect the nitrates might have on the toxic symptoms of galactose. Knowledge of this might offer some explanation to the toxicity itself.

Sodium nitrate was first used in varying concentrations, added to the galactose media. In the first experiment the galactose was used at 0.3% and the sodium nitrate at a percent of molecular value equal to that ot the galactose. Checks were run as usual of normal nutrient solution. In this experiment to toxic effect was not affected by the addition of the nitrate, so it was varied so as to test different concentrations of the salt in different jars, varying from twice to four times the strength of the sugar. After eight days development the seedlings showed an increase in the toxic effect of the media with the increase of the sodium nitrate. In the higher strengths there was a swelling and abnormal yellowing of the roots, while the plumules, usually

**Bur. Soils Bul. 56:16-21 and 45-57

Oxidizing Effects of Roots.

inaffected by galactose alone, were darkened and swollen.

The above was repeated, with seedlings in water culture, this time employing the galactose at only 0.15%, and the sodium nitrate at the three strengths,(a),equal to that of galactose,(b),one-half the strength of galactose, and,(c), four times the strength of galactose. Here, too, the presence of the nitrate increased the coloration of the roots, and the specimens thus treated were less thrifty and vigorous than those with galactose alone. The nitrate of strength equal to that of the sugar apparently did not affect the toxicity, but the stronger solution seemed to kill the root tip and check growth.

The next tests were to determine what effects certain sulphates might have on the toxicity of galactose, since sulphates are said to retard root oxidation;* and thus might shed further light on the problem from that standpoint.

The first experiment was comparative, using calcium sulphate, calcium nitrate, and magnesium sulphate.

The three day old seedlings were placed on supports in the jars of media under the usual sterile conditions in four sets,(a), check with the normal nutrient solution,(b), galactose at 0.055% in nutrient solution,(c), same with calcium sulphate at 0.25%,(d), same as (b) with calcium nitrate at 0.25%,(e), same as (b) with magnesium sulphate at 0.413%,

*Hart, E. B., and Tottingham, W. E. Relation of Sulphur Compounds to Plant Nutrition. Dep. Agr. Agr. Res. Jour. 5:233-250:1915.

(or approximately equimolecular with the calcium sulphate at 0.25%).

In five days the specimens with galactose alone had become shriveled and yellow of roots, with the previously mentioned hypertrophy of the hypocotyls above the liquid. The check specimens showed the usual pure white, thrifty roots, which continued to grow rapidly and put out lateral roots. The specimens with calcium sulphate were generally thrifty, having even better roots than the check itself, and no trace of the toxic action. This direct overcoming of the toxicity of galactose led to a series of experiments along the same line. The calcium nitrate specimens developed long main roots, with less yellowing than with galactose alone, but the laterals were not normally numerous nor the condition in general so thrifty.

The specimens with $Mg.SO_4$ seemed to counteract the toxicity to some extent, producing a better root system than in the case of the galactose alone.

Plate I represents photographically the final results of the experiment. From left to right we have the arrangement of jars thus; (1), check, (2), with 0.055% galactose, (3), like (2) but with 0.25% calcium sulphate, (4), like (2) but with addition of 0.25% calcium nitrate, (5), like (2) but with 0.413% magnesium sulphate added.

The next experiment was also a sulphate test, employing $CaSO_4$ and $(NH_4)_2SO_4$. This was a comparative test also to

determine the relative effects of the Ca and $(NH_4)_2$ ions on the galactose toxicity.

As before jars were set up, (a), check, (b), galactose 0.11% in nutrient solution, (c), like (b) with added 0.25% $(aSO_4$, (d), $(NH_4)_2SO_4$ at 0.25% with the galactose. Observation in eight days showed the following results. The checks had white healthy roots copiously branched. The galactose specimens showed all submerged parts totally browned and shrunken. The branched roots above the medium did not penetrate below the surface. The $CaSO_4$ proved a good counteragent of the toxicity, the specimens looking as thrifty as those of the check except for a slight reddish coloration which did not penetrate the epidermis. The root tips were apparently unhurt, and continued unchecked their longitudinal growth. The lateral roots were equally healthy. The $(NH_4)_2SO_4$ specimens showed counteractive effects, but not to the same extent as the $CaSO_4$. The browning was quite pronounced here, and the lateral rootage somewhat inhibited, but the main roots were thrifty and continued growth. Microscopic study of the specimens grown as above showed the previously noted shriveling of the protoplasts of the galactose killed cells. In the specimens where the $CaSO_4$ had counteracted the toxicity the root tips did not display the usual disintegration and large root caps, as in case of the galactose specimens, but were firm with normal appearing cells.

Cross-sections made from the $CaSO_4$ -treated roots showed

that the outer cells were normal, with only occasional patches of a pale, brownish color appearing on the outer surface. Even normal, tho short, root hairs were seen in these brownish areas, and the epidermal cells were still plump and healthy looking where browned. When treated with the KOH test, the browned cell walls turned the characteristic bright yellow, but this color was confined definitely to the outer cell wall. Further heating produced the yellow globules of the suberin test.

The $(NH_4)_2SO_4$ specimens fared not so well, having their outer cell walls slightly disintegrated, and in some regions several layers of cortex cells dead with the usual appearance of galactose toxicity. However, this injury was not to be compared with that where no sulphate was used.

Next followed experiments to determine the $CaSO_4$ concentrations necessary to overcome the toxicity of certain percents of galactose. Six tests were run;(a), check as before, (b), galactose 0.1%, (c), same with 0.02% $CaSO_4$, (d), same but with 0.05% $CaSO_4$, (e), same but with 0.10% $CaSO_4$, (f), same but with 0.15% $CaSO_4$.

In three days the galactose specimens had begun to assume the yellowish tinge, and put out an abnormal number of lateral roots above the affected areas. There was a definite overcoming of the toxicity with the larger $CaSO_4$ percents. The specimens with o.15%$CaSO_4$ were in every way identical with the checks. Those with 0.10% were almost normal, though not

making quite the growth of those with 0.15%. Those with 0.05% showed a very slight yellowing, and had less thrifty rootage. The pure galactose specimens showed definite toxicity, with deadening and browning of the roots. The photograph, Plate II, shows the heavy, healthy rootage of the $CaSO_4$ specimens, as compared to those in pure galactose. On the left is seen the check, next the o.1% galactose, then respectively the 0.05%, 0.10%, and 0.15% $CaSO_4$ specimens toward the right. The slight yellowing of the 0.05% specimenscan be seen. The root development of the $CaSO_4$ specimens is much better than in the check itself.

In order to test the value of the sulphate ion further, another set of tests were made, using galactose at a stronger concentration than before, and sulphates of different sorts at equal strengths. Salts were selected of much greater solubility than Ca SO_4 as follows: Na_2SO_4, $CuSO_4$, $Na_2S_2O_3$, $(NH_4)_2SO_4$, $Al_2(SO_4)_3$, Mg SO_4. In five days the specimens with galactose alone showed marked toxic effects with darkening and clubbing of the roots. The Na_2SO_4seemed to inhibit the browning somewhat, but the roots were much stunted and laterals prevented. The $CuSO_4$ had penetrated and killed the entire seedlings, as was of course expected, due to the copper. The $Na_2S_2O_3$ showed a slight resistance to the toxic action of the galactose. The $(NH_4)_2SO_4$ prevented the browning at first, but growth was checked and later the toxicity had become complete.

The aluminum salt killed the roots much as did the copper, cut did not seem to penetrate the plumules, which remained alive and growing. The Mg SO_4 showed a checking of the root growth, with an apparent "second growth," with slight increase in length, giving the appearance of very small root tips growing from the browned stumps of older ones. This secondary increase was short-lived, the new tips never gaining more than one or two mm in length.

In general the Na_2SO_4 was the only sulphate which showed any really definite antitoxic effects, and even these results were not as definite as obtained with the $CaSO_4$.

An experiment was started to repeat the foregoing with the various concentrations of the ingredients correspondingly weaker, to eliminate possible toxic effects of the salts, but an accident prevented carrying out the work till too late to complete.

The next experiment was confined to the calcium sulphate and galactose in order to ascertain the concentrations at which counteraction would occur. A nearly saturated $CaSO_4$ solution was tested along with varying percents of the sugar. Equal percents were considered as equal value in the two compounds, since their molecular weights are nearly the same, that of calcium sulphate being 172plus, and of galactose 180. Galactose was prepared at the percents, 0.1%, 0.2%, 0.6%, and 1%. Six media were set up as previously; (a) check, (b), $CaSO_4$ alone, (c), same with 0.1% galactose, (d), same but with 0.2% galactose, (e), same but with 0.6% galactose, (f), same

but with 1% galactose. In these tests was seen a perfect gradation of results from normal roots with 0.1% galactose to complete toxicity in the case of 1%. After six days the roots with 0.1% galactose were long, white, and growing, those with 0.2% nearly as normal, while in the two other cases the poisoning was rather definite, so that no rootlets appeared below the surface of the liquids. Microscopic observation showed that there was no destruction whatever of the outer cells where 0.1% galactose was used, and but slightly in the case of the 0.2%.

Then followed another comparative test, with $CaSO_4$, $CaNO_3$, Na_2SO_4, and $Al_2(SO_4)_3$ in low concentrations. The media were set up as follows: (a), check, as usual, (b), galactose 0.1%, (c) like (b) with 0.1% $CaSO_4$, (d), like (b) with $CaNO_3$at 0.1%, (e), like (b) but with Na_2SO_4 at 0.177%, (f), like (b) but with 0.189% $Al_2(SO_4)_3$.

The results were that in three days the specimens with $CaNO_3$ and Na_2SO_4 showed a very slight stunting at the tips while there was a general cessation of growth in those with $Al_2(SO_4)_3$. In five days the pure galactose specimens showed the usual toxic symptoms of browning, and dying at the tips, while the $CaSO_4$ was seen to overcome this entirely. The $CaNO_3$ had partially overcome the toxicity, though growth was sufficiently checked to promote profuse adventitious rootage above the surface of the medium. With the Na_2SO_4, terminal growth was checked, with a slight and futile attempt at second

growth, as had been caused in case of the $MgSO_4$ previously tested. The $Al_2(SO_4)3$ prevented the browning completely, but also completely checked growth of the roots. Finally, the $CaSO_4$ completely checked the toxic action of the galactose. $CaNO_3$ did not prevent killing of the roots, though profuse laterals were forced, and the tops of the plants were extremely thrifty. The Na_2SO_4 did not prevent a finally complete toxicity.

Microscopic study of the cells of roots in the $Al_2(SO_4)_3$ medium showed no apparent abnormality. Those of the $Ca(NO_3)_2$ specimens were seen to be dead and darkened to the depth of the cortex, the same as those in the pure galactose. The cells of the Na_2SO_4 specimens were also dead, the outer ones very much shriveled.

The deadened cells invariably responded to the KOH saponification test, with yellow, water-soluble globules on heating.

Supplementary tests, made with agar media instead of the water-media, confirmed the above results, in that $CaSO_4$ counteracted completely, at 0.1% concentrations, galactose at 0.1%, and partly overcame the toxicity at 0.2% of galactose. Moreover, $CaSO_4$ at 0.05% invariably counteracted 0.1% galactose, and greatly retarded the same at 0.2%. In agar, concentrations of galactose of 0.4% or more, prevented the roots penetrating the medium in spite of the $CaSO_4$.

Next equal percents of $CaSO_4$ and galactose were used as follows; (a) check, (b), each at 0.1%, (c), each at 0.12%, (d), each at 0.14%, (e), each at 0.16%, (f), each at 0.2%. The experiment could not be carried out in full, as the $CaSO_4$ at above 0.14% did not wholly dissolve. The results of the weaker media were that the toxicity was definitely retarded as faras 0.16%, as seen by the microscope, and totally overcome as far as 0.12%.

$CaSO_4$ was then tested at 0.1% with the following percents of galactose; 0.1%, 0.12%, 0.14%, 0.16%. In three days those with 0.16% galactose had yellowed slightly, and a day later the 0.14% specimens had begun to yellow slightly. After a total of seven days it was seen that 0.12% was the maximum at which the toxic symptoms of galactose could be counteracted by 0.1% $CaSO_4$, and here finally a faint yellowing occurred.

Finally, when $CaSO_4$ was used at 0.12% with galactose at 0.1%, 0.11%, and 0.12%, respectively, the finer distinction was made that the salt would counteract the 0.11% galactose completely, and the 0.12% almost. In the latter case only the faintest yellowing was apparent.

Further experiments with $Al_2(SO_4)_3$ proved that this salt, while inhibiting entirely the symptoms of galactose toxicity, also completely checked the development of the roots. Where concentrations of over 0.03% $Al_2(SO_4)_3$ were used the tops of the plants were somewhat stunted. When $Al\ Cl_3$ was sub-

stituted for $Al_2(SO_4)_3$ at an equal percent, it proved much more injurious to the plants than the latter, and did not inhibit completely the toxicity of the galactose.

The microscope showed that the $Al_2(SO_4)_3$, while overcoming the browning action of the galactose, finally killed the roots, since the cells were seen to become granular and the walls thickened, though still white. The application of concentrated KOH and heating caused great swelling of the cell walls, with subsequent formation of the yellow, water soluble globules.

Another experiment was tried with $Al_2(SO_4)_3$, used at very low concentrations in order to check its own toxic effects. At 0.005 % it retarded definitely but did not overcome the galactose symptoms at 0.1%. $AlCl_3$ at 0.005% was still too toxic of its nature to permit the roots to develop. Both of these aluminium salts at 0.02% were entirely too strong to be of use in the experiments with galactose. $Al_2(SO_4)_3$ at 0.01% proved positively injurious to the seedlings, stunting the roots when tested with galactose at 0.1%. Finally, at concentrations of 0.004%, 0.006%, and 0.008%, the $Al_2(SO_4)_3$ was found unable to entirely counteract the browning effect of the sugar.

DISCUSSION

The big fact noticed in these experiments is definite manner in which the root tissues reacted to the minutest

variations in the media tested. The reader has observed that percent differences of little more than one thousandth of one % caused definite degrees of response in case of the roots subjected to the toxicity of galactose. For example, the roots were so sensitive to slight variations of concentration in the media, that had one interchanged the position of the jars of seedlings, in galactose at the percents 0.05%, 0.08%, 0.10%, 0.15% and 0.2%, from their consecutive places on the shelf, they could have been readily replaced according to their degrees of coloration. How delicately is plant nature adjusted to the soil that bears it!

Another point of interest was the regularity of the killing back of the cortex cells of the affected parts, leaving the inner layers of cells not only intact, but readily functioning to put forth new laterals at the first opportunity. Evidently the toxic element met opposition at each new cell attacked, since an average of twenty-four hours was required to penetrate each new layer of cells. Also much longer time was required to pass the epidermal layer than to penetrate the inner cells. This would suggest that the toxic action was principally toward the cell walls, especially since normal appearing protoplasts were seen in cells adjoining those shriveled and discolored. The thickening and granulating noticed in the affected walls, in sharp contrast to the smooth transparency of adjoining normal walls would support this statement.

In a way, the deadened cortical tissues of the root were very similar to cork, since, as shown by the KOH tests, the cell walls were thoroughly impregnated with a distinctly oily substance similar to suberin. Whether this substance was deposited as a protective means by the irritated protoplasts or not, remains to be determined in future experiments. The tendency of these dead tissues to split longitudinally due to inner expansion gave them an appearance much like that of cork, both in gross appearance and as seen microscopically.

An important observation physiologically was that the living parts of the plant above continued to draw the toxic fluid upward through roots already deadened, even though death and disintegration followed in its pathway.

Next we shall consider the effects produced by the inorganic salts on the toxicity of galactose. The experiments with $NaNO_3$ in connection with the galactose was suggested by the fact that nitrates are known to foster root oxidation,* which is one of the important physiological processes of young roots. This oxidizing power is due to certain enzymes which actually produce extra-cellular oxidization.

Roots have the power to oxidize certain organic substances, such as benzidine and naphthylamine, and quite possibly inorganic substances. Since unproductivity of soils is often known to be due to the presence of toxic organic

* Schreiner, O. and Reed, H. S. Studies on the Oxidizing Power of Roots. Bot. Gaz. 47:355-355.

compounds in the soil, the question was suggested as to whether there might be a close relation between our present problem and this oxidizing power of roots. In consideration of this it was decided to find out by tests with $NaNO_3$ any facts that might shed light on the matter.

The chief result of the experiments was that little effect on the toxicity was obtained by adding to the galactose media equimolecular amounts of the nitrate. However, the toxic symptoms were much increased by increasing the percents of nitrate. This would lead to the conclusion that if the root oxidization figures at all in the toxicity, increased oxidization would increase the toxic action of the galactose. From these things one can see that the following out of these nitrate studies would make a good problem of itself.

The next step was to test the galactose toxicity with other salts which tend to retard rather than increase root oxidization, so the sulphates were selected for another series of experiments.* In these, $CaSO_4$ was found to completely overcome the toxicity of low concentrations of the sugar. The principal obstacle encountered here was the fact that $CaSO_4$ has such a slight solubility that the experiments could not be run into very high concentrations of the salt. The results were, nevertheless, interesting,

*Russell, Edw. J. Effect of Inorganic Compounds on Plant Growth. Soil Conditions and Plant Growth:1912.

in demonstrating that an inorganic salt, or ion of the same, was really capable of reacting in such a way as to render the large sugar molecule incapable of its usual toxicity.

The further experiments with other sulphates showed that $CaSO_4$ was by far the most efficient counteragent to galactose. This suggests the possibility that the calcium ion might play an important part in the combative process. In case of the $Al_2(SO_4)_3$, there is no doubt that its own toxic effect was due solely to the aluminium ion, though it might possibly counteract the galactose toxicity at the same time. $MgSO_4$ was not so efficient in counteracting the toxicity as the $CaSO_4$, suggesting further the probable important role of the Calcium ion in the counteraction of galactose by $CaSO_4$. $(NH_4)_2SO_4$ gave even less favorable results than $MgSO_4$ in counteracting the toxicity.

Aside from experimental results, we may add that an important technical phase of the work was the devising of the culture method used, where the seedling roots could be observed under sterile conditions, especially in connection with their responses to various constituents of the culture media. Most of the results obtained would have been impossible if the agar medium alone had bean used. The reader will note by comparing the results and time periods obtained by either method, that the agar actually retarded the toxic effects, thus rendering possible the entering of factors that would confuse accurate results. For instance, at 0.1% concentration

in agar, the galactose required eleven days to cause the yellowing of the roots, while in water media the same result was obtained in one fifth that time. With the galactose at 0.2% the characteristic deep brown color was not reached till eleven days in agar, while in water culture that stage was reached in four days, or a little over one third the time. At o.15%, eleven days were required to reach a light browning in agar, while at the same percent in water culture a deep brown was reached in a little over four days.

SUMMARY

1. Water Media, properly provided for sterile cultures, was found to give far better results in root studies than agar.

2. Galactose, a hexose sugar was found to have certain definitely progressive effects on plant roots with definite rates of progress in toxicity.

3. The cells of the cortex were killed, layer by layer, till the cambial layer was reached, before death of the root occurred.

4. The untouched cells remained normal, and tended to put forth adventitious rootlets by splitting open the deadened tissues without.

5. The protoplasts affected became granular and shrunken, while the outer layers of dead cells tended to disintegrate.

6. Interior expansion of the living tissues caused longitudinal fissures in the outer dead tissues.

7. Seedlings grown in water culture caused the toxic symptoms in one third to one fifth the time required when grown in agar, the former method giving especially quick results in the lower concentrations of galactose.

8. A definite fatty constituent was found abundantly in the cell walls of the affected tissues, as shown by the KOH test for suberin.

9. $NaNO_3$, a substance which furthers root oxidization, tended

to increase the toxicity of galactose toward roots of seedlings, especially at strengths greater than the sugar.

10. $CaSO_4$ was found to definitely counteract the toxicity of galactose at low concentrations, though due to its own slight solubility it could not be used at higher percents.

11. The plant tissues were found to be sensitive to variations of the concentrations of the galactose and of its counteragent, $CaSO_4$, of less than one one-hundredth of one percent.

12. The counter effects of $CaSO_4$ were found to be such as to prevent the toxic from penetrating the tissues of the roots.

13. Other nitrates and sulphates were found to vary from the above in various ways, but interruption of the problem prevented further research.

BIBLIOGRAPHY OF LITERATURE CITED

1. Schreiner, O. and Reed, H. S. Studies on the Oxidizing Power of Roots. Bot. Gaz. 47:355-388.

2. Russell, Edward J. Effects of Inorganic Compounds on Plant Growth. Soil Conditions and Plant Growth:1912.

3. Hart, E. B. and Tottingham, W. E. The Relation of Sulphur Compounds to Plant Nutrition. Dep. Agr. Ag. Res. Jour. 5:233-250-1915.

4. Knudson, Lewis. Toxicity of Galactose for Certain of the Higher Plants, Mo. Bot. Gar. Annuals 2:659-666, Nov. 1915.

5. Haas, P. and Hill, T. G. The Chemistry of Plant Products, Page 65.

6. Shreiner, O. and Reed, H. S. Oxidizing Effects of Roots. Bu. Soils Bul. 56:16-21 and 45-57.

7. Wilson, Dr. J. K. Amer. Jour. Bot. 2:420-427:1915.

TABLE OF CONTENTS

A STUDY OF THE ANATOMICAL AND PHYSIOLOGICAL EFFECTS OF THE TOXICITY OF GALACTOSE ON SEEDLINGS.

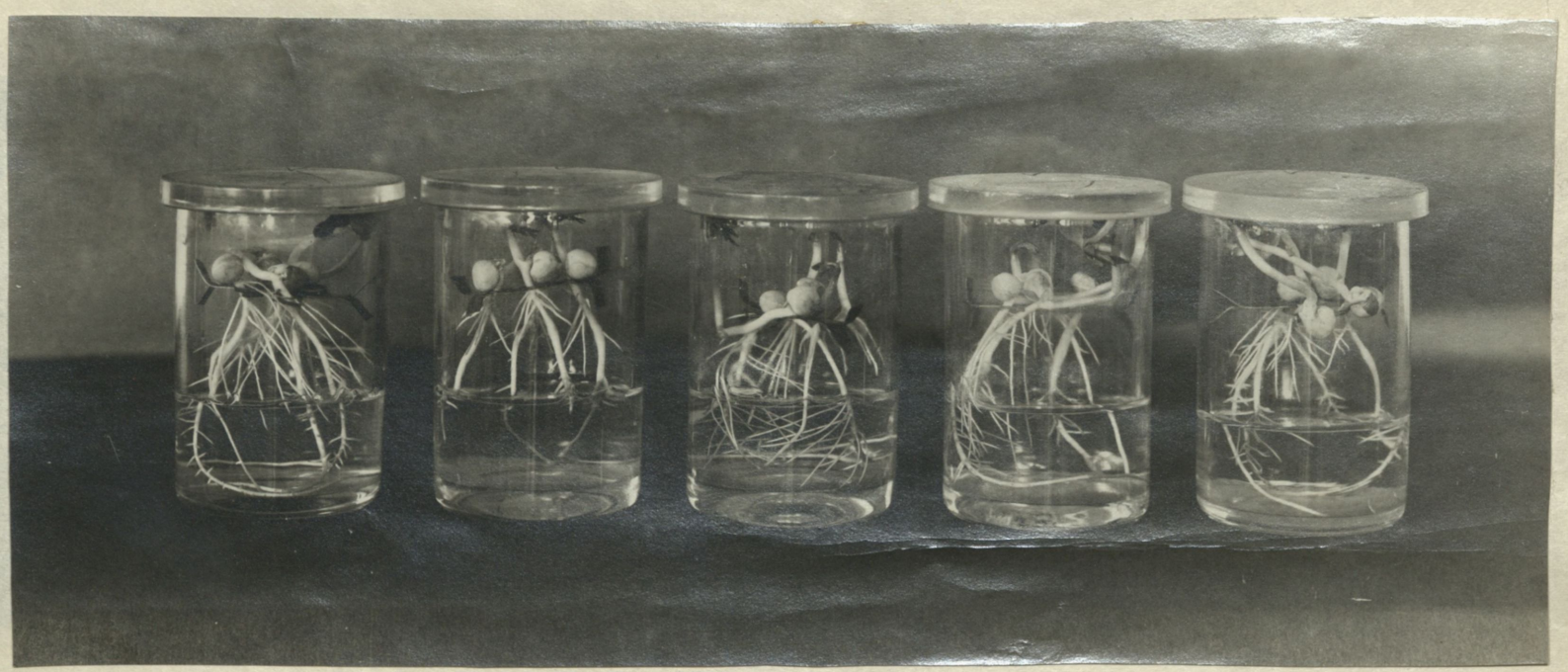

Plate I. Showing Effects of Inorganic Salts on Toxicity of Galactose. Described on Pages 28 and 29.

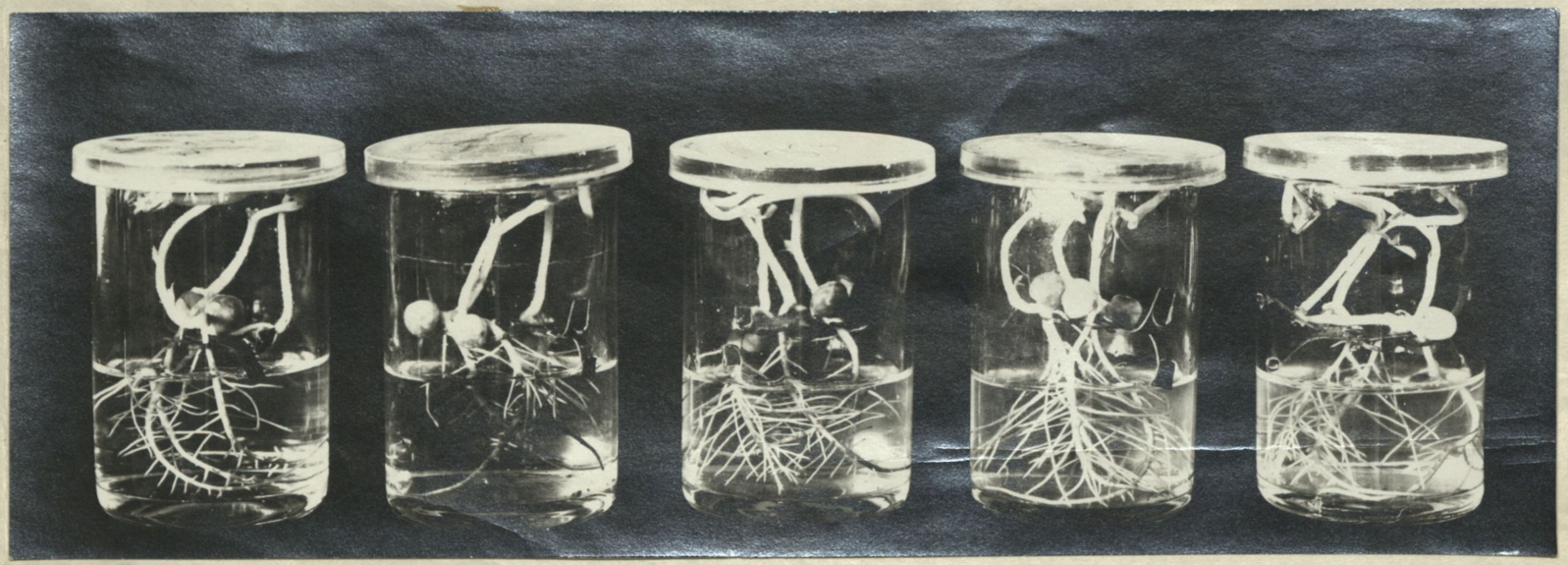

Plate II. Showing Effects of $CaSO_4$ on the Toxicity of Galactose. Described on Pages 31 and 32.