LIII. THE ABSORPTION AND TRANSLOCATION OF LEAD BY PLANTS.

A CONTRIBUTION TO THE APPLICATION OF THE METHOD OF RADIOACTIVE INDICATORS IN THE INVESTIGATION OF THE CHANGE OF SUBSTANCE IN PLANTS.

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The investigation of the absorption of lead by plants can be carried out quite simply by dipping them into a solution which contains a radioactive isotope of lead, and determining the radioactivity of the ash from various parts of the plant. In addition to its simplicity and the extraordinary rapidity with which the work can be carried out this method possesses the following advantages:—(*a*) By mixing suitable amounts of ordinary lead with the radioactive lead isotope, one can vary the lead concentration of the solution, as it were, between very wide limits. The assimilation of lead from a $N/1$ solution can be just as readily investigated as that from a solution many million times more dilute. (*b*) One can follow the change in localisation of the lead taken up by the plant, and thence draw conclusions as to the nature of its combination.

The experiments described in this paper were so carried out, that the plants, which had been cultivated in a culture solution, were washed with distilled water, and then the roots were immersed from 1 to 48 hours in a solution containing a mixture of lead nitrate and thorium B nitrate. In most cases *Vicia Faba* (horse-bean) was used. After this period of immersion the individual parts of the plant were first well rinsed with distilled water, and then ignited, and the intensity of the radioactivity of the ash was determined by means of an electroscope. This latter magnitude gives directly the lead content of the ash and thus also that of the corresponding part of the plant, when we know the radioactivity and the lead content of the solution in which the plant has been immersed.

Thorium B is a transformation product of thorium emanation, and is obtained in a very simple manner. A piece of platinum foil is charged negatively to a potential of 110 volts, say, and suspended in a vessel containing the preparation (radio-thorium, thorium *X*, etc.) from which the thorium emanation is generated. Under these circumstances the thorium *B* collects on the platinum surface, and can be removed with the aid of a few drops of dilute nitric acid. The normality as regards lead of a solution (thorium *B* is an isotope of lead, *i.e.* a substance showing completely the chemical properties of lead) prepared in this way is about 10^{-12}, and if we wish to increase it we only need add to the solution a known amount of lead nitrate. For example, if we assume that we have prepared in this manner a 10^{-6} *N* solution of lead nitrate, and that after evaporating it to dryness it shows a radioactivity of 10,000 relative units, then each relative radioactive unit would correspond to an amount of $2 \cdot 10^{-5}$ mg. of lead. We must of course take account of the fact that the material of the ash of the parts of the plants absorb part of the rays from the contained thorium *B*, but we can easily eliminate this disturbance by mixing the preparation used for comparison with the same quantity of ash as is contained in the sample the radioactivity of which we desire to know.

The following example shows the procedure during an experiment: *Vicia Faba* that had undergone cultivation in a nutrient solution for a fortnight was introduced, after careful washing, into 500 cc. of a 10^{-5} *N* radioactive solution of lead nitrate, which also contained 1/200 mol. of sodium nitrate. The temperature was 17°. The usual precautions such as screening the roots from light, etc., were also attended to. After 22 hours the plant was removed from the solution, and after careful washing with distilled water, the various parts—root, fruit, stem and leaves—were dried separately, ignited after the addition of a drop of concentrated sulphuric acid, and measured electroscopically[1]. The result of the experiment is shown in the following table.

Experiment (*a*):

Part of plant	Weight of ash in mg.	% of the total lead in the solution contained in the ash	Mg. of lead in the ash	Lead content of the ash in %
Roots	45	13·1	0·11	0·25
Fruit	5	0·10	0·0008	0·016
Stem	46	0·05	0·0004	0·001
Leaves	36	0·013	0·0001	0·0003

The purpose of the following experiments was to investigate the manner in which the assimilation of lead in the case of *Vicia Faba* varies with the lead concentration of the solution. In all of these experiments the volume of the lead solution was 200 cc. and the duration of the experiment was 24 hours.

The following collection of results shows that the individual experiments can be repeated, the agreement being quite satisfactory. Per cent. of the

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[1] Before the measurement one must wait about six hours in order to be certain that radioactive equilibrium has been established between thorium *B* and thorium *C*. The reasons for this are outside the scope of this paper.

lead content taken up by the root from a 10^{-6} N solution: 61·2, 62·3, 57·4, 59·6, 55·4, 57·8, 47·3, 62·2, 61·7, 62·6, 60·0, 51·2, 68·7, 57·6.

Experiment (*b*). With 10^{-6} N lead solution:

Part of plant[1]	Weight of ash in mg.	% of the total lead in the solution contained in the ash	Mg. of lead in the ash	Lead content of the ash in %
Root	41	60·0	0·02	0·052
Stem	12·6	0·04	0·000013	0·0001
Leaves	5·5	0·004	0·000001	0·00002
Experiment (*c*). With 10^{-5} N lead solution:				
Root	43	31·7	0·11	0·26
Stem	18	0·015	0·0004	0·002
Leaves	9·8	0·0012	0·00003	0·0003
Experiment (*d*). With 10^{-3} N lead solution:				
Root	39	11·9	3·9	10
Stem	18	0·02	0·007	0·04
Leaves	18	0·002	0·0007	0·004
Experiment (*e*). With 10^{-1} N lead solution:				
Roots	26	0·30	9·9	38
Fruit	18	0·11	3·6	20
Stem	11	0·065	2·2	20
Leaves	10	0·035	1·2	12

[1] The fruit was removed when the plant was introduced into the culture solution, since it constitutes a particularly good nutritive medium for troublesome moulds.

From the above experimental data it is seen that, whereas in the case of a 10^{-6} N solution more than half of the lead is taken up by the root, the percentage loss when a 10^{-1} N solution is used only amounts to 0·3, although the quantities of lead taken up by the root in the latter case are very much greater than in the former case. It is of interest to note that the *percentage* of lead which passes over into the stem and leaves from the concentrated solution of lead is not smaller than that from dilute solutions. This can be interpreted as meaning that with very dilute solutions the root itself is able to bind almost the whole quantity of lead, and thus renders extremely difficult the ascent of lead into the stem and leaves. On the other hand, when a concentrated lead solution is used, an ample sufficiency of unbound lead is available, and this can be carried upwards by the transpiration current. Except in the case of concentrated solutions, the root thus protects, as it were, the remaining parts of the plant, and this marked ability for "binding" lead is probably connected with an explanation of the relatively small toxicity of lead for plants, discussed on p. 444[1] [cf. Strasburger, 1891].

ON THE MODE OF COMBINATION OF LEAD IN THE ROOT.

The question as to whether the assimilated lead enters into an organic molecule, or whether it is retained by the plant in the form of a saline com-

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[1] Trees placed in solutions of copper sulphate or picric acid, etc., do not die until the poisonous substance has reached the highest points of the crown.

pound can easily be decided. In the first case, lead atoms which had once been taken up by the root would not be able to interchange places with other lead atoms, whereas in the second case an active kinetic interchange between the lead atoms bound in the plant and those present in the solution would necessarily take place.

In order to make the argument clearer, we shall designate the atoms of lead in molecules such as those of lead tetraphenyl as "red" ones, and those which occur in such a form as lead nitrate as "blue" ones. If we dissolve both compounds in the same solvent and then separate them by crystallisation, we should find only *red* atoms in the lead tetraphenyl and only *blue* ones in the lead nitrate, since the lead atoms in the lead tetraphenyl are available in an undissociable form. If, on the other hand, we dissolve equi-molecular amounts of lead chloride (with *red* lead) and lead nitrate (with *blue* lead), *i.e.* two salts in the same solvent, then after separation the two compounds would be composed half of *red* and half of *blue* lead atoms [Hevesy and Zechmeister, 1920]. The distinction between *red* and *blue* corresponds here to radioactive and to inactive lead.

If the root has taken up active lead and we place it in a solution of inactive lead, then, if the active lead lies stably embedded in organic molecules, no active lead will be able to pass over into the solution, or in other words we shall not be able to displace the active lead with the aid of inactive lead. Now experiment shows that, with the help of a solution which is relatively rich in lead (10^{-2} N), we can remove almost quantitatively the lead taken up by the root, whence we must conclude that *the lead in the plant root exists in the form of a dissociable saline compound*, perhaps attached to the cell walls.

For example, if we introduce a *Vicia Faba* (after careful rinsing) which has stood 24 hours in 200 cc. of an active 10^{-6} N lead nitrate solution into a much more concentrated 10^{-2} N inactive lead nitrate solution of the same volume, we find that 95 % of the active lead taken up by the root passes over into the 10^{-2} N solution; *i.e.* the active Pb-atoms are almost completely displaced from their places in the root by inactive atoms, which, of course, preponderate strongly (about 20,000 times), from the statistical viewpoint.

Now a 10^{-2} N lead nitrate solution is partially split up hydrolytically, and one might be inclined to ascribe the inverse dissolving action of lead nitrate to its acid content. However, with the aid of a 10^{-3} N HNO_3 solution it was possible to remove only 29 % of the lead content of the root, and by the use of distilled water as solvent only 18 % could be removed. The investigation of the assimilation of lead from solutions of different lead content showed that from 10^{-4} N HNO_3 64 %, and from 10^{-3} N HNO_3 practically the same amount, viz. 62 % is taken up by the root, when the normality of the lead ions in the solution is 10^{-6} N. From a 10^{-2} N HNO_3 solution, a concentration sufficient, in general, to kill the plant, only 26 % is assimilated by the root.

THE DISPLACEMENT OF THE LEAD TAKEN UP BY THE ROOT BY OTHER IONS.

Since it has been established that we can displace the lead taken up by the root by other lead atoms, it seemed to be of interest to investigate the ability of other ions to displace the assimilated lead. 10^{-2} N solutions were used throughout these experiments. The plant containing lead was placed for 24 hours in the solution under consideration and then both the amount of lead remaining in the plant and the amount displaced into the solution were determined. The results of these experiments are shown in the following table:

Solution used	% of the lead initially present in the root which remained after treatment
Lead nitrate (inactive)	5
Cupric nitrate	3
Cadmium nitrate	34
Zinc nitrate	38
Chromium nitrate	43
Barium nitrate	74
Sodium nitrate	76

When the reverse solution took place with the help of a 10^{-3} N $Pb(NO_3)_2$ solution, 14 % of the originally assimilated lead were still present in the root after 24 hours' treatment.

Only copper is able to displace lead in a similar degree to lead itself; all the other cations investigated show an appreciably smaller displacing power.

The extent of the re-solution of the lead taken up by the stem and leaves was not determined. Experiments which are being undertaken on the assimilation of lead by *algae* will, amongst other things, also serve to shed light on this point.

As is well known, different ions are assimilated to quite different degrees by plants, according to what other ions are present in the culture solution. The toxicity of individual types of ions is also arrested by others. One of the best known cases of this "antagonism" is probably that between $CaCl_2$ and NaCl. In this case the phenomenon of the suspension of the toxicity of NaCl by $CaCl_2$ is attributed to the ability of the $CaCl_2$ to alter the plasma-membrane in such a way that it is less permeable to NaCl [Osterhout, 1912]. Since it has been possible to show that in the case of lead a kinetic displacement of the assimilated ions by other ions occurs, we shall certainly have to reckon with the possibility that the antagonism is in individual cases occasioned by such kinetic effects.

LEAD ASSIMILATION AND TRANSPIRATION CURRENT.

From the fact that more than 50 % of the lead is taken up in 24 hours by the root in very dilute solutions of lead, *i.e.* a quantity of lead which was present in more than 100 cc. of liquid, we can conclude that it is *not the transpiration current* which transmits the assimilated lead, since the daily loss of water of *Vicia Faba* under present conditions is less than 1 cc. This independence is also shown in the following experiment. In one case the percentage assimilation of lead by the root was determined in the usual way, and in another after the root had been first separated from the stem under water. The volume of water was 500 cc., the lead concentration was $10^{-6}\ N$, and $p_{\mathrm{H}} = 4$. Duration of experiment = 1 hour.

Root as usual	6·8 and 7·5 %.
Root cut off	6·5 and 7·1 %.

It is seen that the amount of lead taken up was in both cases the same. Moreover, the lack of dependence of the assimilation of salt on the absorption of water by the plant has repeatedly been established [cf. Arrhenius, 1922].

THE TOXICITY OF LEAD.

In connection with the experiments described in the previous section it is of interest to note that, as has been shown by Bonnet [1922], the introduction of plants into $10^{-1}\ N$ $\mathrm{Pb(NO_3)_2}$ solution unfavourably influences the transpiration current. In contrast to more dilute solutions, such an appreciable concentration of lead shows distinct toxic effects on the plant[1]. *Vicia Faba* which had stood 24 hours in a $10^{-1}\ N$ $\mathrm{Pb(NO_3)_2}$ solution already showed a slight deviation from the geotropic direction, and the leaves situated closest to the root showed signs of withering.

The toxic action of lead on different plants, such as wheat, radishes, lentils, cabbage, etc., has been investigated quite recently by Bonnet [1922]. Just as in the present case, he introduced the plants into 200 cc. of water after their roots had attained a length of several centimetres. The water contained in solution a definite amount of lead acetate or lead nitrate, and he obtained the following results:

(1) After the plants had stood in $10^{-1}\ N$ solutions of lead salts, lead could readily be detected qualitatively in the root.

(2) Only traces of lead were found in the stem and in the leaves.

(3) $10^{-1}\ N$ solutions of lead killed, *e.g.* the wheat plant after 20 days, balsam after two days.

(4) Mg, Ca and K showed no antagonistic action to lead.

(5) The greater the dilution, the less lead was taken up by the plant.

Our present results confirm those of Bonnet. As regards the first result, we were able, thanks to the sensitiveness of the radioactive method, to detect with ease and quantitatively to determine the presence of lead even in the stem and in the leaves. It is interesting to note that Mg, Ca and K, which do not have an antitoxic action, have only a slight capacity of displacing lead, according to the experiments of the present author. In reference to

[1] Cf. also Lavison [1911] and older experiments of Phillips [1883], Knop [1885], Nolle, Bässler and Will [1884].

point (5), the radioactive methods enable us to carry out a quantitative investigation of the dependence of the assimilation of lead on the concentration of the solution within wide limits, in which all other methods fail. In this manner, it is found that only 1/500 part of the amount of lead is taken up from a 10^{-6} N solution as compared with a 10^{-1} N solution. Those experiments of Bonnet should be mentioned, from which we can see the influence of the assimilation of lead on the growth of plants. He finds the following values:

Plant: The bean.

	Length of root in mm.		
	Initially	After 1 week	After 1 month
In water	25	100	1000
In 10^{-3} N $Pb(NO_3)_2$	31	31	32

SUMMARY.

(1) The assimilation of lead from lead nitrate solutions by *Vicia Faba* has been investigated. A radioactive isotope of lead was mixed with the lead nitrate, and the amount of lead taken up was determined after ignition from the radioactive intensity of the ash of the various parts. This method makes possible the determination of exceedingly small amounts of assimilated lead.

(2) Whereas 0·3 % of the lead is taken up by the root from 200 cc. of a 10^{-1} N lead nitrate solution in the course of 24 hours, 60 % of the lead content of a 10^{-6} N solution is taken up in the same time. The leaves show a lead content of only a few hundredths or thousandths of 1 % of the amount of lead present in the solution.

(3) The assimilated (radioactive) lead can be displaced by introduction of the plant containing lead into another lead solution, whereby inactive lead atoms now take the place of the radioactive ones. From this it follows that the lead is not combined with carbon within the plant, but that it exists in the form of a dissociable salt which is soluble with difficulty.

(4) Even after 24 hours, a 10^{-1} N solution of a lead salt produces toxic effects on the plant, whilst more dilute solutions do not. Lead belongs to the least poisonous of the heavy metals.

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