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# The distribution of watersoluble radioactive substances in plant tissue — some experiments with an auto-radiographic method

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# *The distribution of watersoluble radioactive substances in plant tissue — some experiments with an autoradiographic method*

## Introduction and methods

In recent years autoradiography has frequently been used to study the distribution of radioactive nutrients absorbed by plants. The method generally employed has been very simple. The material containing the radioisotope is dried in a botanical press or between layers of glass cloth in an oven, and thereafter autoradiographed by apposition on film (BOYD 1955; WIEBE 1955). The disadvantages of this method are obvious. The relatively large and uneven thickness of the unsectioned material results in diffuse pictures and errors due to selfabsorption, the latter especially in the case of isotopes with weak radiation. During the drying procedure destruction of tissue occurs. Probably displacements of watersoluble compounds also take place. In addition such a survey method cannot elucidate questions regarding the distribution of the isotope within the plant tissue. With this latter aim in sight histological procedures must be used in combination with autoradiography. As a matter of fact a few attempts have been made to correlate the site of accumulation to the anatomical structure of the tissue by autoradiographing sections of plant tissue containing radioactive substances. (RABIDEAU 1950; STEFFEY 1953). But all the methods sofar used suffer from the disadvantage that in at least some phase of the procedure the tissue comes in contact with fluids, where some of the radioactive material from the section is extracted. (Boyd 1955; Kaminski 1955). Thus the pattern which the radioisotope has attained in vivo is disturbed, and the autoradiograph does not give a true picture of the distribution.

This problem is of especial interest when studying the distribution of watersoluble radioisotopes, e. g. in plant root material, the main object of this study. With a suitable method problems related to the intake and accumulation of ions as well as to the ways of transport of the ions within the root might be attacked.

These methodological difficulties are largely overcome by using a method devised by Ullberg, which permits a fixation of the watersoluble radioisotopes in situ. (Ullberg 1954). According to this method the material is sectioned

in the frozen state, the sections are taken up on tape and autoradiographed by the apposition technique. The present paper is a short account of an attempt to adapt the method for plant tissue.

Young pine plants *(Pinus silvestris)* from aseptic cultures in Erlenmeyer flasks (Melin's nutrient solution, Melin 1936) and roots of *Vicia Faba* at different stages of development were used as test objects. The seeds of the beans were soaked in water, germinated in ordinary garden soil and cultivated furthur in a photothermostat in aereated nutrient solution. The nutrient solution used for the beans was that of Hoagland, diluted to 1/5 of the original strength.

The radioisotopes, phosphorus — 32 or sulphur — 35, were added to undiluted nutrient solution in carrierfree form as phosphoric acid and sodium sulphate respectively. The concentration used was 3 mC/l. The pine plants were removed from the aseptic culture flasks and allowed to absorb the isotope from aereated nutrient solution for 24 hours (with 12 hours illumination) through the root. The bean roots were excised and put into Petri dishes with 30 ml undiluted nutrient solution containing the isotope. The absorption period for the bean roots was 3 hours at a room temperature of 20—22° C. The material to be sectioned was rinsed in distilled water and embedded in thin layers of cotton wool soaked in water at 0° C. It was gently straightened between two object glasses, and quickly frozen down with carbon dioxide snow in a freezing chamber at 6—8° C below zero. All furthur manipulations with the material took place at this temperature. The upper object glass was removed by gentle thawing. The other object glass with ice block on was mounted on a stage of fabric bakelite by means of freezing with a little water. The stage was fixed in a Leitz Grundschlitten microtome (type 1300), whereafter the material, which must not be allowed to thaw, was sectioned with a sharp knife. In order to get whole sections of the friable material a strip of Scotch tape was fastened on the flat section surface before cutting. The indicator scale of the microtome was generally set at 25—30 $\mu$, which appeared to be the best thickness. After cutting the sections thus fastened on the tape were allowed to dry in the freezing chamber for 24 hours.

For the exposure of the sections the technique devised by Ullberg was followed in detail. The dry sections were thus put in contact with the film (Gevaert Dentus Rapid) without intervening layers. Film and section were pressed against each other in an autoradiographic press. The exposure took place in the freezing chamber.

For the development and fixing Gevaert's salts were used (G 230 and G 305 A). The time of development was five minutes.

In some experiments parallels without radioisotope were run. No chemical fogging was detected after exposure for a week.

After the exposure some of the sections were removed from the tape, transferred on to an object glass, stained with crystal-violet and erythrosin and mounted in Canada balsam. (Palmgren 1954).

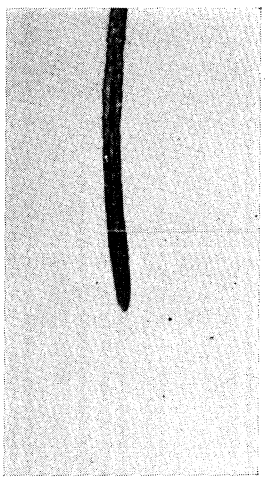

Figure 1. Autoradiograph of a young lateral root of *Vicia Faba,* showing the distribution of absorbed S —35 as sulphate. 2.5 ×.

**Results and discussion**

Fig. 1 is a photograph of an autoradiogram of a longitudinal section of a young lateral root of *Vicia Faba.* The isotope was sulphur — 35. The autoradiogram reveals the distribution of accumulated sulphate. Comparison with the section showed that the heaviest accumulation is not in the tip itself (with the calyptra and the apical meristem) but in a rather diffuse zone between 2 and 5 mm from the tip. (This zone corresponds in its lower part to the zone of elongation). In older parts farther from the apex the accumulation was lower than in younger parts. Replications gave similar results. Scrutiny in the autoradiogram of a short zone a couple of centimetres behind the tip magnified in detail revealed that the epidermis had accumulated more sulphate than the cells in the cortex inside.

If lateral roots, in which the elongation was arrested were chosen, a picture different from that of rapidly elongating roots was obtained. See

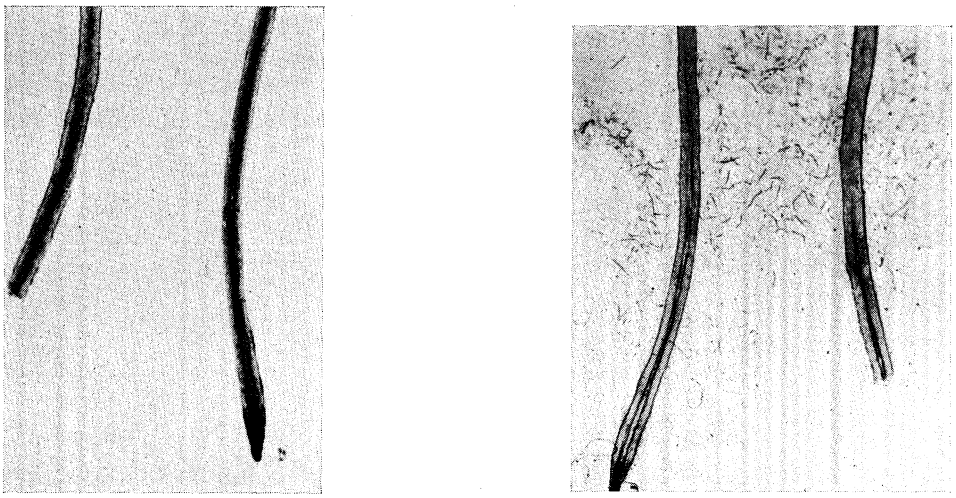

Figure 2 a. Autoradiograph of a lateral root of *Vicia Faba* with arrested growth, showing the distribution of absorbed labelled sulphate. 2.5 ×.
b. Photograph of the corresponding section. 2.5 ×.

fig. 2 a. The cause of the slowing down of growth was not studied. The corresponding section (fig. 2 b) showed that the differentiation of the vascular elements had proceeded closer to the apex in these roots than in a young growing root such as the one autoradiographed in fig. 1. Lateral root primordia had also been initiated as close as only a few mm from the apex. This observation is in accordance with previous experience from other root material. (Esau 1953). The autoradiogram (fig. 2 a) showed that the largest accumulation was located in a short zone in the apex. At levels farther from the apex the concentration was lower in cortex whereas most of the isotope seemed to be gathered in the stele. The observations contrast sharply with the state of things in young roots where a relatively long zone of the root tip showed an even blackening. It seems clear that the difference in accumulation pattern between the two roots is intimately connected with ageing phenomena in the cortex cells in the one with arrested growth. On the other hand it seems conceivable that the accumulating capacity of the tissue in the stele is unaltered or larger in the older root.

Fig. 3 shows an autoradiogram obtained by exposing a section of a root fragment (tap root with young lateral root), given radiosulphate. The lateral root was in good growing condition. The exposed section is chosen to show the distribution of the isotope in the point of connection of the vascular elements between the lateral and tap roots. Of the lateral root the section only comprises the outer cell layers of the apex, but it is seen here,

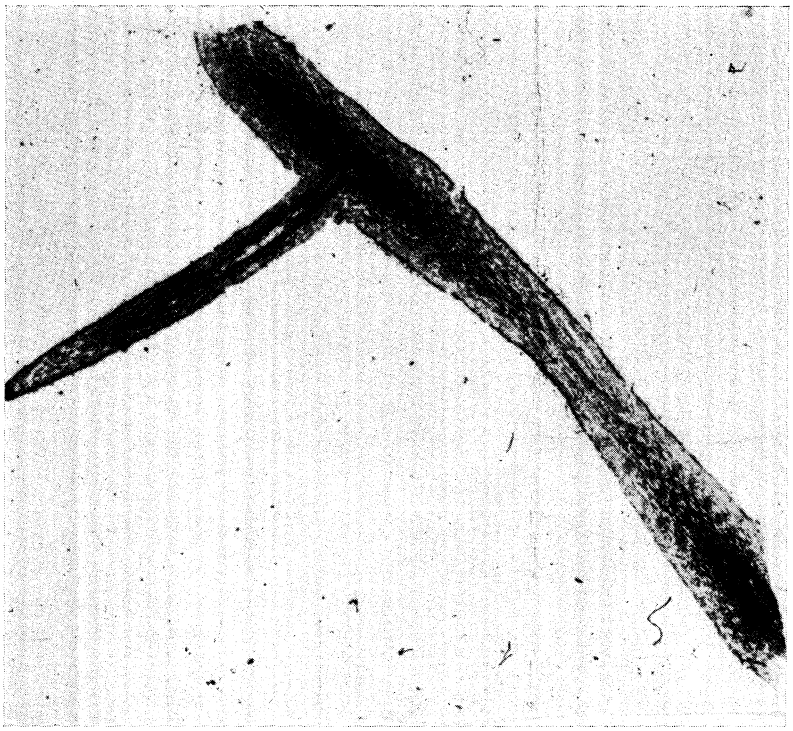

Figure 3. Autoradiogram of a section of a tap root fragment of *Vicia Faba* with an attached lateral root, showing the distribution of labelled sulphate. 10 ×.

too, that the largest accumulation in the young root is not in the apex but a bit farther up. (The white area within the stele of the lteral root is caused by rupture in the section). The accumulation in the epidermis is especially marked in the apical part of the lateral root.

As for the tap root fragment it is clearly seen that the sulphate concentration is higher in the stele than in the surrounding cortex. Owing to the fact that the section was cut obliquely through cortex and stele it is possible to compare isotope concentration within different parts of the cortex. (For the watery appearance see below). It is thus seen from the autoradiogram that a falling concentration gradient of radiosulphate exists from the inner to the outer cortical cells. The concentration is higher in the stele, and as far as could be seen there was no steep fall from the endodermis to the innermost cells of the cortex.

Higher magnification often reveal a reticulate pattern coinciding with that of the cellwalls in the section. Though it is generally supposed that ions can migrate in the cellulose walls, this autoradiographic pattern cannot be

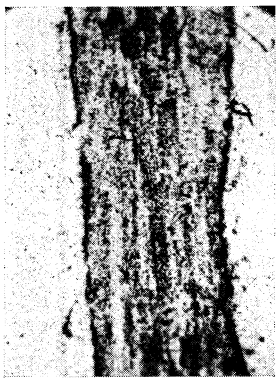

Figure 4. *a.* Autoradiogram of section of cortex tissue from root of *Vicia Faba,* and *b.* corresponding section, showing that absorbed radiosulphate is located in the protoplasm. 15 ×.

taken as evidence for this. The cause is that during the drying procedure of the section plasma probably adheres to the walls. Thus one cannot exclude the possibility that the reticulate pattern must at least in part be ascribed to isotopes in the plasma. Direct evidence for the fact that the plasma has absorbed radiosulphate is seen in fig. 4 a und b. The picture appears watered A closer comparison between autoradiograph and the stained section showed that the lighter bands corresponded to parts in the section where the cells were empty, the darker regions on the other hand to cells with the plasma in situ. The plasma has been removed at the sectioning by the knife owing to brittleness of the embedding material.

The strongest accumulation is often located within the stele, in a position corresponding to the margin of the stelar region. Closer examination reveals that the sieve tubes are the structures responsible for the strongest blackening. This is clearly seen in figures 5 a and b. The pictures show a point where the vascular elements of a lateral root are connected with those of a tap root. The concentration in the xylem ducts is far lower than in the sieve tubes. Endodermis was identified in the section on the basis of its staining properties. It is stained by crystal violet like the xylem. As far as could be seen it was not represented in the autoradiogram by any marked structures.

Figure 6 a is an autoradiogram obtained by exposing a section (30 $\mu$ thick of a shoot of *Pinus silvestris* (figure 6 b) which was allowed to absorb phosphorus —32 phosphate via its root. The most obvious structure in the basal part of the autoradiogram is a longitudinal dark band, bordered by still darker lines. The lines correspond to the phloem and cambium tissue in the section. Here as in the bean roots the strongest accumulation is thus

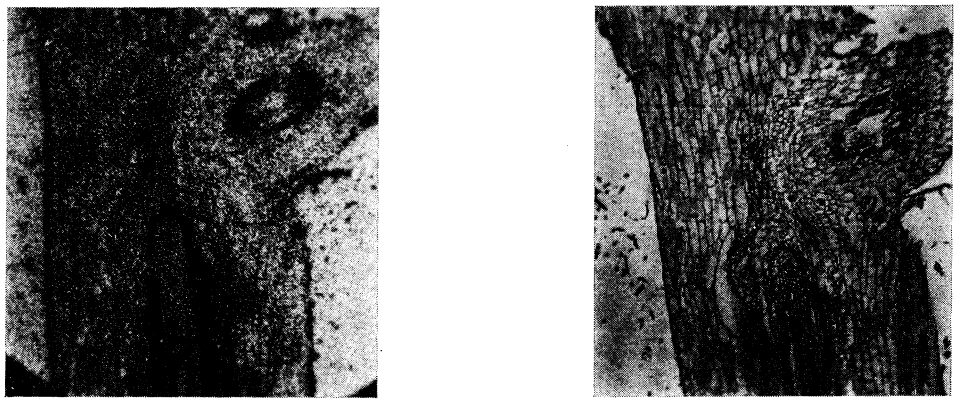

Figure 5 a. Autoradiogram of root section of *Vicia Faba*, and b. corresponding section, showing the distribution of absorbed radiosulphate. Note the high concentration in the phloem. 20 ×.

located in the phloem. The concentration in the xylem part could not be distinguished from that of the ground tissue. In the parenchyma outside the sieve cells the concentration is still lower than in the xylem. In the upper part of the autoradiogram the two black lines converge into a single one in the section corresponding to cells from phloem and procambium. (Figures 7 a and b). The largest accumulation is located in the buds and bud traces. The lower meristematic part of a young needle has also retained much phosphorus especially in its vascular part.

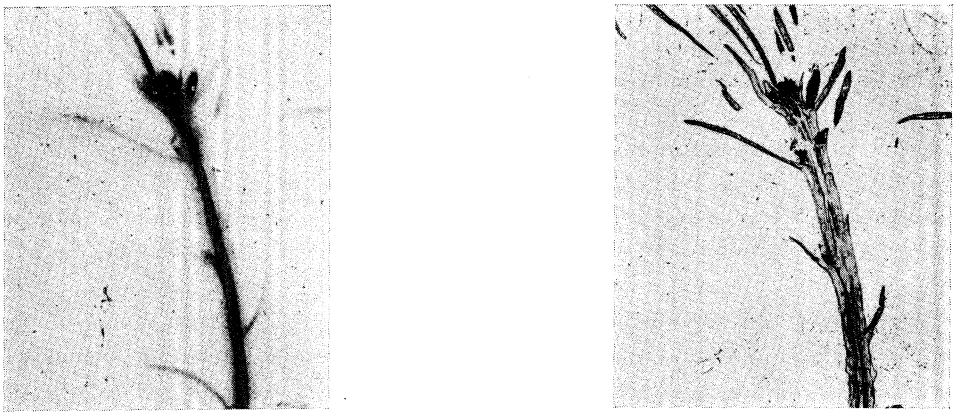

Figure 6 *a.* Autoradiogram of a section of a shoot of *Pinus silvestris*, and *b.* corresponding section showing the distribution of absorbed radiophosphate. 2 ×.

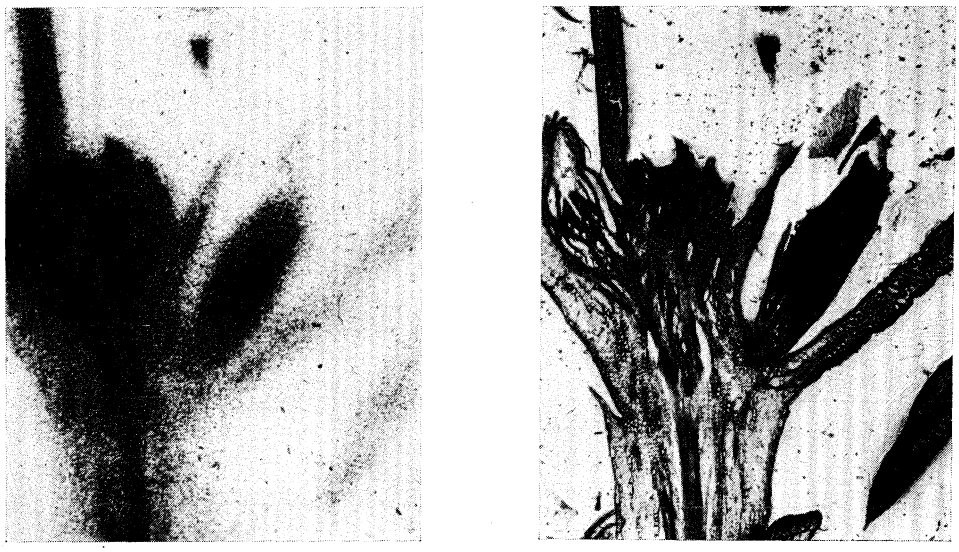

Figure 7 a. Autoradiogram of a section of a shoot of *Pinus silvestris*, and b. corresponding section, showing the distribution of absorbed radiophosphate in the apical part of the shoot. 20 ×.

It is clear that the largest accumulation of phosphorus — 32 was found in tissue which is known or supposed to have high metabolic activity.

## *Summary*

This report describes an application to plant tissue of an autoradiographic method previously worked out for animal tissue by Ullberg. The following notable results are mentioned. With sulphur — 35 as sulphate it was shown that a difference exists in the pattern of ion accumulation between young rapidly elongating lateral roots of *Vicia Faba* and roots with arrested elongation. This variation must be referred to an altered capacity of ion accumulation owing to the difference in age of the tissue of the two types of roots.

In the stele the sieve tubes were the most active accumulating tissue. The concentration in the xylem elements could not be distinguished from that of the surrounding ground tissue. A falling concentration gradient for radiosulphate was noted in a tap root fragment with an attached actively absorbing lateral root.

In shoots of pine which absorbed phosphorus — 32 via the root the strongest accumulation was located in meristematic tissue in the needles, in the buds and cambium as well as in the phloem. The concentration in the xylem was lower. In the cortex it was lowest.

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