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ON THE FUNCTION OF THE CASPARIAN STRIPS IN ROOTS

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SUMMARY

The dyes safranin and basic fuchsine as well as ferric salts enter the stele when applied as dilute solutions to intact root parts where Casparian strips are well developed. As the entrance is not hampered when the protoplasm has been killed or its activity and permeability diminished by low temperature the most likely way of entrance is through the cell walls.

In the Casparian strips no fatty substances could be detected. As soon as traces of fatty substances are found in the ageing exodermis all entrance into cortex and stele of the substances used is blocked.

1. INTRODUCTION

On the structure of the Casparian strips many contradictory statements can be found in the literature. It is often stated that the Casparian strips contain cutin, suberin, or similar fatty substances. ESAU (1965), SCOTT-RUSSELL & BARBER (1960), BROUWER (1954, 1965), PRIESTLEY & NORTH (1922), PRIESTLEY & RHODES (1926) and VAN WISSELINGH (1926) have stated that unsaturated fatty-acids are found in the strips. FREY-WYSSLING (1959) doubted the function of these acids in the Casparian strips and suggested that the substances might be precursors of the fatty substance which at a later stage covers the whole inner surface of the endodermal cells. KROEMER (1903) and later MYLIUS (1913) and ZIEGENSPECK (1921) doubted the occurrence of fatty substances in the Casparian strips as the results of attemps to stain the strips with fat-colouring substances were negative. WARDEN (1935) has reported negative results with Sudan III and Sudan IV in *Senecio vulgaris*.

Electron micrographs did not reveal any abundance of fatty substances in the Casparian strips (FALK & SITTE 1960).

Other substances occurring in the strips are pectin, cellulose and lignin, all of which are readily recognized.

Differences of opinion about the function of the Casparian strips in roots also occur. KRAMER (1949) hardly recognized them as a functional structure. VAN FLEET (1961) (p. 204) stated: "It is apparent that the radial walls of the endodermis are impermeable to water..." FREY-WYSSLING (1959) regarded the strips as a barrier for ions. He based his statement on the work of RUFZ DE LAVISON (1910) who stated that ferrous ions, taken up from very dilute solutions, do not pass the endodermis while more concentrated solutions do. It is, however, not clear from de Lavison's paper how he prevented the oxidation

of the ferrous-sulphate solution and consequently the precipitation of basic ferri salts. A consequence of the impermeability of the Casparian strips would be the separation of the free space of the cortex and the stele, which physiologically might be very important. The differences of opinion enticed us to try to investigate the permeability of the Casparian strips.

2. MATERIALS AND METHODS

The plant material used was cultivated on Hoagland solution in water culture. Species used were *Vicia faba*, *Limnobium stoloniferum*, *Pisum sativum*, and *Aesculus hippocastanum*. In all species the main roots were used. For special purposes some cultures of *Vicia faba* on vermiculite and on cultivating earth were used. Detection of fatty substances was done with Sudan III, Sudan IV and Fettrot; the latter was used most of the time as it proved to be the most sensitive reagent. Dye solutions were made up of tapwater with safranin, basic fuchsine and Mohrs salt in the concentrations mentioned in the text. Ferric-ions were detected with a slightly acidified solution of $K_4Fe(CN)_6$.

3. RESULTS

In the first experiments we placed the roots of intact plants of *Vicia faba* in a solution of safranin 0.1 g/l in water. After about 48 hours hand sections revealed safranin in the xylem which was brilliantly red and in phloem fibres which were only very slightly tinged. The cortex was coloured in the different experiments with different intensities, which proved to be caused by different degrees of suberisation of the exodermis. The endodermis had the intensity of the cortex at the outer side of the Casparian strips; the inner cell wall was not tinged or only very slightly, like the pericycle.

Experiments were also done with *Limnobium*. Safranin and basic fuchsine gave the same results as the experiments with *Vicia*. The results indicated that indeed the Casparian strips block the entrance of the dye solution. The question remained, however, as to how the safranin reached the xylem and to a lesser extent the phloem fibres. We first thought that safranin could have leaked into the xylem by way of the very young parts of the roots where the Casparian strips are not yet developed.

Experiments to exclude this way of entrance were done by inserting various lengths of the tips of the roots into plastic tubes filled with lanolin. After immersing them in the safranin solution we found Casparian strips in the zone just inside the tubes. Inside the tubes no colouration was observed. In the part of the roots that had been in contact with the solution the xylem was just as red as in the former experiments. Experiments with roots of *Pisum sativum* gave essentially the same results, except that in *Pisum* all the walls of the endodermis were stained.

Another way of entrance of the solution might have been the lateral roots (DUMBROFF & PEIRSON 1971).

To exclude entrance by way of the latetal roots or root initials plants were placed with their root systems in water-saturated air. In a number of places along the roots we placed a drop of basic fuchsine or safranin 80 ppm in water to which 12% of gelatine was added; the gelatine was applied at its solidifying temperature. After 24 hours the roots were sectioned and we observed that the dyes had entered the stele and coloured the whole xylem, the other stelar tissues being tinged only very slightly or not at all. A similar experiment was performed with a diffusion time of only half an hour. Even in this short time the dyes were observed in the xylem at the side of the root where the drop of gelatine was applied. The Casparian strips did not stain with Fettrot. The experiment with a diffusion time of 24 hours and basic fuchsine was repeated with roots of *Aesculus hippocastanum*, with identical results. Clearly fuchsine and safranin pass the endodermis. The pattern of colouration of the root sections appeared always the same.

To elucidate this point sections of untreated plants were placed in solutions of very low concentrations of basic fuchsine. The pattern of colour intensities was the same as in our previous experiments, being caused by the different affinities of the walls for the dye.

Now there remain two possible ways of entrance of the dye solutions into the stele; either by way of the cell wall or by way of the living protoplasm of the endodermal cells. To exclude the last-mentioned pathway, roots were freeze-dried or fixed the night over in alcohol 96% and air dried, so that all cells only contained air. Application of basic fuchsine in gelatine and subsequent sectioning after a few hours of the dried material showed that the dye and of course water had reached the xylem.

In order to exclude that the dye entered the stele owing to metabolic activity, we performed an experiment in which two groups of living plants were treated in the same way with coloured gelatine. The only difference between the two groups was that one group was kept at 23 °C and the other at 2 °C. Four hours after application of the gelatine the results for both groups were the same, viz. the xylems were stained. If at 2 °C the metabolism and the permeability of the plasma membranes of roots of *Vicia faba* are reduced to negligable proportions, the only remaining way of entrance should be by way of the cell walls.

To check if the same results would be found with more physiological substances such as ions, the experiments with dried roots were repeated with 5% gelatine to which $Fe(NH_4)_2\ (SO_4)_2$ 3,4% had been added. The gelatine was stored overnight at 4 °C which caused the oxydation of ferro to ferri. After sectioning the dry material 4 hours after the application of the gelatine the ferric ion was detected by addition of a slightly acidified solution of $K_4Fe\,(CN)_6$. The reaction revealed iron in the stele.

In all experiments we looked for fatty substances in the visible Casparian strips with fat colouring dyes, mostly using Fettrot, apparently the best reagent in this case. Also Sudan III and IV were used.

Staining of the Casparian strips could never be observed. To ascertain that the lack of fatty substances in the Casparian strips could not be due to the fact

that the plants were cultivated on water culture, plants were also grown on vermiculite and potting earth at somewhat lower temperatures, 22 °C at day-time and 15 °C at night. Under these conditions no fatty substances could be detected, either.

If, however, the slightest stain with Fettrot was obtained in the *exodermis* there was an absolute blockade for the dyes used.

In the developing roots the ageing endodermis develops a film of fatty substances over all cell walls that was very clearly detectable with Fettrot. This process starts opposite the phloems. The results with roots in this stage of development showed entrance of the dyes only through the gaps in the secondary endodermis.

4. DISCUSSION

From the experimental observations described in the preceding section it seems evident that the Casparian strips do not completely prevent transport through the endodermal cell walls. The possibility remains that incrustration with lignin reduces the velocity of transport through the endodermal cell walls to some extent. Our experiments were not designed to warrant any conclusion on that topic.

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