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Histogenesis and Localization of Non-Specific Esterase in Root Tip

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Abstract. A procedure was developed for satisfactory freeze - sectioning of root tips. The use of Ca formol-fixed material kept and frozen in Holt's syrup is recommended. The existence and different localization of 2 fractions of non — specific esterase was verified in root tips of *Vicia faba*. The same results were revealed in fixed and unfixed material. The dynamics of *in situ* reaction was followed with respect to optimal incubation time. The results with substrates of different chain length support the existence of 2 fractions of the studied enzyme, none of which, concerning substrate specificity, is a lipase. It follows from the present studies in *Vicia faba* and other species (*Cucurbita pepo, Lupinus albus, Pisum sativum, Zea mays*), that non - specific esterase localization is not directly given by histogenesis.

A series of papers originating from this laboratory have given some findings concerning the relationship, if any, between the localization of enzymes in the root apical meristem and histogenesis proceeding there. Our early studies showed that it was not possible to understand readily the results obtained by the established cytochemical methods so that the localization of non — specific esterase was the theme of, or was mentioned in, several papers (BENEŠ *et al.* 1961, BENEŠ 1962a, b, 1966, SAHULKA and BENEŠ 1969).**

In the present paper 1. attention will be paid to the root tip of *Vicia faba*, the standard material, especially from the viewpoint of developmental anatomy; 2. a standard method for the handling of material will be described and 3. applied to the localization of non-specific esterase; 4. some problems of evaluating sections will be mentioned; 5. incubation time will be taken as an important factor in enzyme localization studies; 6. substrates of various chain length will be applied; 7. the reliability of the procedure and the variability of the material will be evaluated, the apical and basal parts of the root tip and the tips of young and old roots will be compared; whereas

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** It is the author's responsibility, that in some of his previous papers an improper term "primary core' was used instead of "cortex". In the common usage, accepted here, the term cortex comprises also the endodermis.

points 1. to 7. will concern *Vicia faba* root tips only 8. a comparative study of esterase localization in root tips of different species will also be performed.

Material and Methods

Standard Material

Seeds of *Vicia faba* cv. Chlumecký were soaked in water overnight and allowed to germinate for 3 days under controlled conditions (ADÁMKOVÁ and BENEŠ 1966). When the roots were 3 cm long, the segments comprising the 2nd and 3rd mm from the physical tip of the root were normally utilized.

Fixation and Sectioning

Free frozen sections were used both from fixed and unfixed objects.

Root tips were fixed for 1 hour in ice-cold Baker's Ca formol (after HOLT see DANIELLI 1958), but the formalin was neutralized with $CaCO_3$ beforehand. (The pH of the freshly prepared fixative, at room temperature, was measured using a glass electrode and shown to be pH 6·7 to 6·9.) After fixation, the material was transferred to cooled Holt's syrup (HOLT *et al.* 1960) and was kept at about 2 °C for no longer than 5 days. During the first day, the syrup was changed several times to wash out the fixative from the tissue.

Half and double concentration syrups (with regard to sucrose content) were tested, together with H_2O instead of the standard syrup.

Prior to sectioning, the root was removed from the syrup and the pertinent segments cur with the aid of a cutter made from razor blades and plastic (Novodur) plates. The objects were frozen and cut in the same medium as was used for washing and storing. After cutting, the sections were transferred to the same medium.

Unfixed material was processed in a similar way

In an attempt to improve the procedure, the use of gelatin-embedded material was tested. The fixed material, washed in Holt's syrup, was placed for 30 minutes at 37 ° in a medium made of 5 g gelatin + 5 ml glycerol + + 23 ml H_2O, stirred beforehand at 55 ° until clear. The gelatin blocks were kept in a refrigerator in well-sealed eprouvetts.

Transverse sections, 50 μm thick, of both fixed and unfixed material were cut using a semi-conductor freezing microtome, with the knife additionally colled with solid CO_2. Any material which thawed during the procedure was discarded.

The handling of sections was simplified by the use of small open glass eprouvetts provided with gauze at one end.

After rinsing in H_2O the sections were ready for the enzyme incubation.

Esterase Reaction

The following incubation medium was used (BENEŠ 1962b): 19·5 ml phosphate buffer pH 6·4 (10 g $Na_2HPO_4 . 12 H_2O$ and 10 g $NaH_2PO_4 . H_2O$ in 1000 ml H_2O) + 0·5 ml 1% substrate solution in dimethylformamide + + 20 mg diazonium salt; α naphthyl acetate Lachema, naphthol AS acetate

synthesized by Dr Vereš, Isotope laboratory, Czechoslovak Academy of Science, Praha; fast blue RR salt Lachema. (The substrates will be referred to as α acetate and AS acetate respectively.) As controls, sections were incubated similarly in the full medium but in the absence of the substrate. After incubation, the sections were washed with H_2O, placed on slides and mounted in Gama.

Evaluation of Slides

The reactions were evaluated throughout using a 160 x magnification and modified five — grade colour intensity scale: 1 — slight, 2 — weak, 3 — well, 4 — strong, 5 — intense, cf. BENEŠ 1968.

Incubation Time

To determine the optimal incubation times, the *Vicia* root tip sections were reacted with α-acetate for 15, 30 and 45 minutes, and with AS acetate for 2, 4 and 6 or 8 hours. For *Vicia*, the standard incubation time was 30 minutes with α-acetate and 2 hours with AS acetate.

Substrates of Various Chain Length

To further identify the enzyme under study the following substrates were applied: α-naphthyl propionate, α-naphthyl butyrate, α-naphthyl caprilate and α-naphthyl myristate; all from Lachema. (These substrates will be referred to as α-propionate, *etc.*) The procedure described above was used. In the case of the fluid substrates (α-butyrate and α-caprilate) a 1% vol./vol. solution of substrate in dimethylformamide was employed. Sections of *Vicia* root tips were incubated at 22 ° for 45 minutes with α-propionate, for 3 hours with α-butyrate, and for 5 hours with α-caprilate and α-myristate.

Comparative Studies

The above basic method of tissue preparation and incubation was used as a standard procedure to allow, in the case of *Vicia*, a comparison of enzyme activity between the apical (the 2nd and 3rd mm segment) and basal (the 5th and 6th mm segment) parts of the tip of 3 cm long root. The 2nd and 3rd mm segment was also studied in roots not more than 1 cm in length and in those about 6 cm long (2 days and 5 days of germination after overnight soaking). To determine the variability of the material and the reliability of the procedure, 20 fully independent runs with seeds from one harvest used during almost two years were evaluated.

Esterase Patterns in Different Species

Esterase activity was determined, with the standard procedure descíibed above, in root tips of *Cucurbita pepo* cv. Veltruská obrovská; *Lupinus albus* cv. Pflugs ultra; *Pisum sativum* cv. Raman; and *Zea mays* cv. Český bílý koňský zub. The seeds were soaked overnight and then allowed to germinate in the same way as *Vicia: Lupinus* and *Pisum* and *Zea* for 2 days, *Cucurbita*

for 3 days. The objects were handled in the standard way, the incubation time with α-acetate was 15 minutes for *Cucurbita*, for *Lupinus* 10 minutes, *Pisum* 20 minutes, *Zea* 20 minutes, with AS acetate 2 hours for *Cucurbita*, *Lupinus* and *Pisum*, *Zea* 3 hours.

Results

Standard Material

In Vicia root tip, notable differences can be seen between the basal and apical ends of the 2nd and 3rd mm segment. The two ends of the segment are distinguishable by the area of the section (both cell division and radial enlargement of cells are involved, see JENSEN 1961) and by some other characteristics: the sections from the apical end have several root cap layers, rhizodermis is not yet organized into one layer of radially extended cells, the differentiation of sieve tubes commences; those from the basal end reveal only one or two layered cap, typical rhizodermis, several sieve tubes within one pole, vessels are not observable (under the growing conditions employed). It is necessery to mention, that only lateral parts of root cap were touched within the segment used.

In our material, no characteristic core was identified within the central cylinder.

Concerning pericambium (root pericycle) it appears that, after a continuous pericambium was distinguishable, its cells started to enlarge, first within the phloem pole.

Fixation and Sectioning

The sectioning of unfixed material was laborious, with the best sections being prepared from tissues frozen in water. The use of Holt's syrup caused the sections to disintegrate.

Treatment of the fixed material in water was found to prevent shrinkage of the cytoplasm, but sectioning was difficult. Sectioning was quite easy after treatment in Holt's syrup in this case. Lowering the concentration of sucrose did not improve the preservation. Doubling the concentration made sectioning impossible.

Embedding the tissues in gelatin did not facilitate sectioning markedly, and did not prevent shrinkage or loss of protoplasts.

Evaluating our findings concerning esterase localization in *Vicia* at the tissue level, the same results were found with fixed objects using H_2O, ordinary Holt's syrup and that of half sucrose concentration. There were no differences in esterase localization between fixed and unfixed material.

Esterase Reaction

Completing the standard procedure with the sections of the 2nd and 3rd mm segment of 3 day-germinated roots of *Vicia*, the following results were observed. The whole section is positive (black or black brown colour) with α acetate, cortex being less coloured than root cap and central cylinder. With AS acetate, positive reaction (blue colour) appears in root cap, cortex

(except endodermis) is practically negative (weak carmine colour), central cylinder positive: phloem poles including appertaining parts of pericambium more highly coloured than future xylem ones. Central region of the central cylinder is coloured less than phloem poles but more than those of xylem. There is a strikingly polar distribution of carmine colour within the cells of rhizodermis: root cap adjacent side of cells is highly coloured but colour intensity disappears towards the opposite *i.e.* cortex adjacent side. Endodermis adjacent to phloem poles reveals blue colour: no polarity in colour distribution is observable within its cells. The parts of endodermis adjacent to xylem poles are weakly coloured, similarly as appertaining parts of pericambium. Differences in the colour intensity of the endodermis and especially of the pericambium adjacent to the xylem and phloem, and of the poles of the xylem and phloem were barely apparent when employing α acetate as substrate, depending upon the incubation time.

Non-crystalline azo-dye results with AS acetate, but crystals appear several days after mounting, most striking where the colour was of the "well" level. No coarse crystals — if any — appear of azo-dye resulting from α-acetate hydrolysis.

Evaluation of Slides

An important factor in evaluating the results concerns the selection of suitable sections since the colour intensity is likely to vary with section thickness. In addition, the loss of whole protoplasts from the sections and the shrinkage of the cytoplasm may also make in situ measurements problematic.

Incubation Time

Following the time course of the reaction with α acetate, a heavy colour appeared first in the root cap and the rhizodermis, followed by the phloem poles of the central cylinder. The reaction proceeds at a lower rate in the cortex, but if the incubation is prolonged, the colour intensity will almost reach that level achieved by shorter incubation times with the root cap. When AS acetate was employed as substrate, even after incubating for 4 times the standard procedure time (8 hours of incubation), the same enzyme pattern resulted, with the cortex remaining practically negative.

Substrates of Various Chain Lenth

When the material was incubated with α myristate or α caprilate, no colour was observed. However, α propionate yielded the same result as was observed with α acetate, and α butyrate yielded a result similar to that with AS acetate.

Comparative Studies

No differences were revealed on comparing the studied segments of 1 cm and 6 cm long roots. Even in the 5th and 6th mm segment of the standard (3 cm) root, the same localization pattern was seen, but there was no difference in the staining intensity of the endodermis and the pericambium in relation to the poles of the phloem and xylem in this case.

A comparison of 20 separate experiments under the standard procedure using α acetate as substrate, resulted in no apparent differences due either to insufficient processing of the material, or to the variability of the objects. Some fluctuation was observed in the root cap when using AS acetate. About 1 root in 10 revealed a weak reaction instead of the normally strong one.

Esterase Patterns in Different Species

The development — anatomical studies of the objects used besides *Vicia* in this work lead to the conclusion, that the 2nd and 3rd mm segment represents a part of root of ontogenetically different level and extent in different species. Moreover, although the differentiation of individual parts or root tip seems to follow a general order *e.g.* sieve tubes mature earlier than vessels, the time and space relations are not even relatively constant: whereas in *Vicia*, under the conditions of culture used here, there is a distance of several mm between the region of sieve tube and vessel maturation (the region of vessel maturation is not touched in our case) then, in *Zea*, both regions succeed one after the other closely or even pass through each other. Thus, it is highly difficult to compare the given parts of roots of corresponding maturity.

Unequal level of pericarbium differentiation in relation to xylem and phloem poles was also evident in *Lupinus* and *Zea*. In *Cucurbita* and *Lupinus*, no morphologically distinct rhizodermis was observable on transverse sections through the root segments under study.

In *Cucurbita* α acetate was hydrolysed quite evenly throughout the whole section, the particular parts being well to strongly coloured. With AS acetate, the root cap, rhizodermis and cortex were negative, whereas the central cylinder was positive: phloem poles were more coloured than those of xylem and middle region.

After 10 minutes incubation, the whole sections were highly positive with α acetate in *Lupinus*. With AS acetate the phloem poles and the parts of pericarbium passing through them were weakly positive. Phloem-adjacent parts of endodermis were also weakly coloured. The result in root cap, rhizodermis and cortex was regarded as very weak or negative: more or less pale carmine colour was seen there.

In root cap, rhizodermis and cortex, weak reaction appeared with α acetate in *Pisum*. Phloem-adjacent parts of endodermis were more coloured, through phloem poles passing parts of pericambium still more, as the phloem poles themselves. At xylem poles, a gradient of colour intensity was seen: an increase, centripetally. Middle region of central cylinder was definitely positive. With AS acetate, a positive reaction was seen in root cap. In rhizodermis a polar distribution of colour was revealed similar to that in *Vicia*. Cortex was negative. It is of interest, that xylem poles and corresponding parts of pericambium and endodermis were more intense than phloem poles in this case. The middle of central cylinder was also positive.

In *Zea*, the whole section was positive with both α acetate and AS acetate. The azo-dye resulting from the hydrolysis of AS acetate crystalized extremely easily here.

Discussion

Standard Material

A thorough knowledge of the anatomy of the material under study is conditio sine qua non of any histochemistry especially in objects where histogenesis, cell differentiation, growth and development are proceeding. Even if histogenetical relations are not taken into account, the description alone of such material is difficult (BENEŠ 1970). The core, i.e. the middle region of the central cylinder, is worth mentioning as an example.

It was observed that the formation of the pericambium is not equal in relation to the phloem and xylem poles, which corresponds to the histochemical findings. Similar examples are given by BÜNNING (1952) and by ESAU (1953).

Other factors which should be remembered when interpreting histochemical data, were discussed previously (BENEŠ 1962a, b) and include variation in the number of xylem and phloem poles, and the dependence of differentiation processes upon the type of seed culture *e.g.* germination in sand or in a moist chamber, *etc.*

Fixation and Sectioning

In spite of the possibility of making free-hand sections of plant material, different techniques of freeze-sectioning have been preferred recently, since the sections are of a more uniform thickness and available in larger numbers. Freeze-drying and freeze-subtitution are less used. Special studies have been devoted to the use of the cryostat for plant tissues (LÄUCHLI 1966a, b, GAHAN *et al.* 1967). In the present study, satisfactory results were obtained using a semi-conductor freezing microtome with additional knife cooling with solid CO_2, though only the tissue level of enzyme localization was considered.

Freezing and thawing are highly complicated events (*e.g.* in BENEŠ 1958, 1959). Some effects of freezing and thawing appear to be less acute in the fixed material. This was the 1st reason for using fixed tissue in the present study. The insolubilization of enzymes was the 2nd reason, and the 3rd was the more uniform penetration of the incubation media into the cells of the fixed tissues, in agreement with LOJDA (1965). Although enzyme inactivation, and, possibly, activation, may result from fixation, it may result equally well from the effects of freezing and thawing. The data from the present experiments and those of SAHULKA and BENEŠ (1969) show that, on comparing fixed and unfixed material, fixation is not the decisive factor in esterase localization at the tissue level in *Vicia*. In unfixed material, azo-dye is apparently gathered on section surface, which again is an argument in favour of fixation.

Calcium-containing formalin is a traditional fixative in enzyme histo- and cytochemistry. Without commenting on such problems as the presence of ions in the fixative, tonicity, fixation time and fixation temperatures (see in HOLT and HICKS 1961, LOJDA 1965, WOLMAN *et. al.* 1967, HOPWOOD 1969) it is worth noting that the mode of preparation of the fixative used here was reliable, and the variation of its pH rather small. BAKER (1958) mentioned large differences in the pH of calcium-containing formalin fixatives.

Problems such as cell compartmentalization, lyo- and desmoenzymes, and extracellular enzymes will not be considered here (see de JONG 1965, GAHAN 1965, GAHAN and MAPLE 1966, LÄUCHLI 1966a, MEANY *et al.* 1967 concerning acid phosphatases), since the intracellular localization is not assessed in these studies (but see WALEK-CZERNECKA 1965, LIVINGSTON *et al.* 1969)

Esterase Reaction

With the exception of the incubation time, the enzyme reaction and its conditions were not tested in the present study, since they were broadly analyzed by BENEŠ (1962b), who examined the current fixation procedure in comparison with Wolman's acidified ethanol — fixed and paraffin vacuum-embedded material (Wolman/paraffin). In addition to azo-coupling methods, indoxyl methods were used in the mentioned work successfully with material processed by either technique. The Tween and thiolacetic acid substrates employed with Gomori-type reactions worked well with the Wolman/paraffin material only.

The results of our previous work on *Vicia* were summarized briefly elsewhere (SAHULKA and BENEŠ 1969). Two esterase fractions (I and II) were distinguished according to 3 criteria, namely, substrate specificity (where fraction I preferentially hydrolysed α-acetate, and fraction II hydrolysed both α acetate and AS acetate), the response with different processing of the material and inhibition with E 600. In the present paper, only substrate specificity has been considered.

According to the present results, fraction II is in the root cap, which is positive with both substrates. Rhizodermis is occupied by fraction I: it is positive with α acetate; with AS acetate the polar distribution of a carmine reaction product was seen. No peculiarities in the behaviour of the middle part of central cylinder — the possible core — were revealed, nor in the 2nd and 3rd nor in the 5th and 6th mm segment. Even anatomically the question of core seems to be open for further studies, especially from the viewpoint of mutual correspondence of tissues in root and shoot.

The analysis of non-specific esterase distribution is more suitable for studies devoted to the relation of histogenesis and enzyme localization than is the histochemistry of acid phosphatase in *Vicia*, since the latter enzyme is ubiquitous and the whole section is reactive. The situation is similar with α acetate, but fortunately not with AS acetate. Cases of more selective localization are better suited for study of the problem outlined above. This view was expressed in the quotation by de JONG (1965) from our paper (BENEŠ and OPATRNÁ 1964).

By the first approach, fraction I of the non-specific esterase in present in the cortex whereas fraction II is present in the central cylinder. The question as to whether the initials of the cortex and of the central cylinder are the same (*i.e.* common) or different cannot be answered with any certainty. Thus, a histogenetical basis for answering the question raised is lacking.

Accepting that there is a common origin for the outer parts of the root cap and rhizodermis, then, in the parts of common origin there are different enzyme fractions, namely fraction I in the rhizodermis, and fraction II in the root cap.

The endodermis, especially its phloem-adjacent parts, is positive with AS acetate, but not the remainder of the cortex. Then, again, the enzyme localization is not directly given by histogenesis.

With AS acetate, the reaction in the endodermis that is adjacent to the phloem, and especially that part of the pericambium passing through the phloem pole, is much more intense than in the corresponding parts associated with the xylem. Thus, a histogenetically uniform part of the root reveals a varied histochemical reaction for non-specific esterase.

Assuming that the central cylinder originates from one group of initials, then the localization of esterase does not correspond with its histogenesis: xylem poles behave differently from the phloem poles. It is necessary to mention, however, that the differences under discussion, though striking, are quantitative only: there is a weak reaction at the xylem poles.

Although the conclusions derived from the results of the present work are often limited by some assumptions, and are not unequivocal in all cases, it seems possible to conclude that the enzyme localization is not

directly given by the origin of the part of the root tip, but by the more advanced steps in tissue differentiation, when function may play a role.

Evaluation of Slides

On viewing the endodermis, one cannot overlok that its cells are slightly vacuolated in comparison with the other cells of the cortex. Due to the different extents of the vacuolization of the cytoplasm, colour intensity need not correspond to the amount of or the activity of the enzyme (BENEŠ 1966), but in the present case, this factor does not seem decisive. The possibility of enzyme and/or primary reaction product diffusion from the central cylinder seems to be likely, together with the liposolubility of the endproduct. In comparison with rhizodermis, diffusion can be excluded in the case of the cells of the endodermis: no gradient in colour intensity was seen. Liposolubility of the resulting azo-dye can hardly cause errors in localization at the tissue level. Thus, high enzyme activity in parts of the endodermis adjacent to the phloem may be accepted.

The question remains on the cause of carmine colour obtained after the incubation with AS acetate in some parts of the sections. Since the controls are negative, it is not caused by phenolic substances. Liposolubility may be involved. Usually, the carmine colour is of low intensity, but it may be quite heavy, *e.g.* in rhizodermis. Therefore, it cannot be explained by the concentration effect. Even the formation of different dyes by coupling at different sites of the naphthol ring must be considered.

Incubation Time

Studies of enzyme kinetics *in situ* are useful *e.g.* for the characteristics of the particular enzyme (HOPSU and GLENNER 1963, HOPSU and MCMILLAN 1964, FOLGENHAUER and GLENNER 1966, HALL and BUTT 1968). But it is necessary to take this into account also in the current histochemical practice (GAHAN 1965), particularly in connection with optimal incubation time (v. DEIMLING *et al.* 1967). Following the time course of enzyme reaction in different parts of the section, it appears regularly, that different parts reach maximum colour intensity at different times. When the maximum is reached, the prolongation of incubation remains without effect in this respect. Theoretically, such a period could be regarded as the optimal incubation time in histochemical studies when the most active part of the section reaches optimal colour intensity whereas the place of lowest activity reveals at least some colour. In this case it is expected, that the patterns of enzyme activity within the section will be optimal for observation. In most cases both conditions are not fulfilled and a compromise must be made. As a means of evaluating the practically optimal incubation time *i.e.* the standard incubation time, the period necessary for obtaining maximum colour intensity at the most active site could be considered: in the present work, it was about twice the mentioned period of incubation.

Substrates of Various Chain Length

The application of substrates of different chain length in *Vicia* lead to the conclusion that with respect to substrate specificity, neither of the two fractions is a lipase. Further studies would be useful here (see ERÄNKO *et al.* 1962, ABE *et al.* 1964). Different results with α-propionate and α-butyrate represent a further criterion for distinguishing fraction I from fraction II; fraction I hydrolyses α-propionate preferentially, and fraction II hydrolyses both substrates equally well.

A question could be raised about the proper meaning of the term non-specific esterase, concerning its use in the present and similar works. This problem will be taken into account (BEDRNÍKOVÁ and BENEŠ, in preparation). Some inhibition tests were performed in a previous work (BENEŠ 1962b).

Comparatives Studies

The possibility of functioning of different initials in different periods of ontogenesis (von GUTTENBERG, see BENEŠ 1970) was the reason for comparison of tips of younger and older roots. However, no differences were seen in tips of roots of different length.

Raising the question on the uniformity of the material and the reliability of procedure resulted from our previous experience: the characteristic "star" in *Vicia* root tip incubated to reveal

acid phosphatase (high colour intensity at xylem poles) did not appear in all cases (BENEŠ and OPATRNÁ 1964). In the present work, with 20 independent materials run separately, practically the same results were obtained, which proves that both the material and the procedure are standard. It was bred matérial from one harvest used for almost 2 years. If some differences in non-specific esterase localization were previously observed (see *e.g.* Fig. 5 and 6 in BENEŠ 1962a) it is assumed that different level of tissue differentiation is responsible.

Esterase Patterns in Different Species

The aim of the last part of the present work was to select objects of different types of root histogenesis and to perform a comparative study study of non-specific esterase localization on them. It was necessary to use large roots which can be handled in the standard way. The chosen plants were those which are bred and commonly cultivated, and often used pro experimentis. It is an advantage if the cells of the material are large, for enzyme localization can be obserbed and evaluated more precisely and easily.

On the basis of substrate specificity, different localizations of fractions I and II were revealed in the roots from all species with the exception of *Zea mays* where fraction II occupied the whole section. All of the other species were similar to *Vicia* in that fraction I was localized in the cortex and fraction II in the central cylinder.

From the finding that the reaction or the colour intensity is not the same for a histogenetically uniform part of the given root, it can be deduced, in a way similar to that in *Vicia*, that the localization of non-specific esterase activity does not correspond to the histogenesis patterns.

A similar conclusion follows from the comparison of esterase localization in root tips of different types of histogenesis.

The present state of the classification of root histogenesis types which is the basis for such an evaluation of results has been reviewed recently (BENEŠ 1970). Kroll attempted to summarise the different classifications published mostly during the last third of the nineteenth century. If it is admitted, that it is possible to deduce the way of formation of a tissue from the cell arrangement in that tissue, the question remains as to whether or not the results in material handled with the old fashioned microtechnique are reliable and whether or not it is possible to compare findings in the main and lateral roots, primary and adventitious roots etc. Kroll's paper together with the works on root tip "geometry" was the basis for the work of Schüepp who proclaimed the Körper-Kappe (K.-K.) theory. Romberger regarded it as worthwhile in spite of the fact that some of the findings of Clowes are not in agreement with it. Popham included the classification of Janczewski and of Engard in his book. Although more recent concepts of root histogenesis have appeared (von Guttenberg) the K.-K. theory seems to be useful for the purpose of the present work. Undoubtedly it is a theory of histogenesis, not of root tip organization only, but there are some problems open: are only two groups of initials present in the root tips, is the existence of different groups of initials within e.g. the Körper excluded? Both Kroll and Schüepp are mindful of the relation of the root cap to the "body" of the root primarily, leaving aside the genesis of the cortex and of the central cylinder, which is in the focus of our interest.

Using the K.-K. theory as a basis for evaluating our results, in spite of the above mentioned objections, then *Lupinus* and *Zea* represent two extreme histogenetical types. The Körper is limited to the central cylinder in *Lupinus* whereas in *Zea* it comprises the central cylinder, cortex and rhizodermis. With α acetate as substrate, the results are practically the same in both of these species, but with AS acetate, the positive reaction corresponds to the K.-K. boundary. In plants bearing transversal meristems, to which belong *Cucurbita*, *Vicia* and *Pisum* (according to Kroll), the positive reaction to AS acetate in the central cylinder and the cortex is to be expected, if fraction II follows the K.-K. boundary. However, the findings are not in agreement with such an idea.

Generally, the results of the present paper lead to the conclusion that the non-specific esterase localization does not correspond to the histogenesis of tissues. It seems to be connected with the more advanced phases of differentiation when function can plays its role. It is a handicap for solving the question raised initially, that reliable histogenetical background is often lacking for the evaluation of the histochemical findings. In our future work, it will be necessary to take into account the regulatory mechanisms of enzyme activity and synthesis. Moreover, it is possible to ask a more

general question, namely, what, if any, is the order or regularity of enzyme localization in the root tip (cf. Thielke 1966)? Staying within the limits of histogenesis, an approach contary to that of the present work might be useful, namely, to interpret histogenesis from the enzyme localization pattern. Then, enzyme localization would be used as a marker of the origin of the particular part of root tip. However, the results of the present work are not in favour of this idea.

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V této práci byl vypracován postup pro získávání dobrých zmrazených řezů ze špiček klíčních kořenů *Vicia faba* i jiných druhů rostlin *Cucurbita pepo*, *Lupinus albus*, *Pisum sativum*, *Zea mays*). Spolehlivě lze použít Ca formolem fixovaného materiálu praného a řezaného v Holtově sirupu, lze však použít i materiálu nefixovaného, řezaného v H_2O. U *Vicia* byla ověřena existence a odlišná lokalisace 2 frakcí nespecifické esterasy. Výsledky u fixovaných i nefixovaných objektů byly stejné. Používaný postup se ukázal spolehlivý a nebyla zjištěna větší variabilita objektu. Rovněž u *Vicia* byla sledována dynamika reakce in situ v souvislosti s optimální dobou inkubace. Existence 2 různých frakcí nespecifické esterasy byla zde ověřena použitím substrátů o různé délce řetězce. Z hlediska substrátové specificity není u *Vicia* prokazovaný enzym lipasa. Z nálezů frakcí nespecifické esterasy u *Vicia* i ostatních sledovaných objektů (*Cucurbita*, *Lupinus*, *Pisum*, *Zea*) plyne, že jejich lokalisace není jednoznačně dána histogenesí. Důsledně respektovat vývojově anatomické hledisko je conditio sine qua non jakékoliv srovnávací studie lokalisace enzymů.