Histochemical demonstration of some hydrolytic enzymes in the spherosomes of plant cells

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INTRODUCTION

In previous investigations, the presence of the hydrolases: acid phosphatase and non-specific esterase was demonstrated in onion scales epidermis (Wałek-Czernacka 1962, 1963). It was found that the activity of these enzymes is localized in the spherosomes. The supposition was expressed that spherosomes play a role in intracellular hydrolytic processes. They were, therefore, compared with de Duve lysosomes in the animal cell.

The present paper is a continuation of the earlier investigations. Its object was the confirmation of the previously advanced view by demonstrating the activity of further hydrolases in the spherosomes. Particular attention was directed in the first place to enzymes characteristic of lysosomes. Further support was sought from histological evidence.

MATERIAL AND METHODS

The upper epidermis at the base of inner onion bulb scale-leaves was cut with a razor blade into small squares which were lifted carefully with a sectioning needle. In this zone the cells are thin-walled and of small dimensions, what facilitates uniform penetration of substrates into the cell (Holt and Hicks 1961). The living tissues (cytology in the cells) were either placed directly in the incubation media or they were previously fixed. As fixative Baker's Formol-Calcium (Fo-Ca) and 4 per cent formalin buffered with phosphate were used in both cases with addition of 7 per cent sucrose (Holt 1959; Holt and Hicks 1961). Sometimes also acetone was used. The material was fixed at 0—2°C for 12—24 h. Good results were obtained several times by fixing the material in formalin vapour for 15 min.

Anthers of Larix decidua Mill. developed on branches kept in water and small pieces of shortened stems (stem plates) at the base of onion bulbs producing in water adventitious roots were subjected to enzymic reactions in fresh state and after fixation in Fo-Ca with sucrose.
-hand sections were used. Only the sections from the tips of adventitious roots were cut with a freezing microtome.

β-glucuronidase activity was demonstrated using the method of Seligman et al. (1954). The substrate employed was 6-bromo-2-naphtyl-β-D-glucopyruronoside, incubation times 4 to 6 hours; 8-hydroxyquinoline coupling azo dye method was also applied (Pearse 1961), the substrate 8-hydroxyquinoline glucuronide, incubation times 2–12 hrs at room temperature.

β-galactosidase was demonstrated after Rutenberg et al. 1958 (as described by Pearse 1961), using 6-bromo-2-naphtyl-β-D-galactopyranoside. Incubation 4–6 hours at 37°C.

A similar method was employed for β-glucosidase (according to Pearse 1961) as substrate 6-bromo-2-naphtyl-β-D-glucoside. Incubation times c. 4 hours at 37°C.

α-glucosidase was demonstrated after Rutenberg et al. (Pearse 1961), as substrate 6-bromo-2-naphtyl-α-D-glucopyranoside. The period of incubation 1–4 hours at 37°C.

Arylsulphatase was demonstrated by the method of Rutenberg et al. (1952), the substrate potassium-6-bromo-2-naphtyl sulphate. Incubation at 37°C and at room temperature for 1–4 hours.

In the coupling azo dye methods the diazonium salts Fast blue B and Fast blue RR, or only the former salt, were employed.

For deoxyribonuclease Vorbrodt’s modification (1961) of Aronsen and al. method (1958) was used. The substrate DNA Sigma. Incubation times 2–24 hours at 37°C. The best results were obtained after 2–4 hours.

The technique used to demonstrate lipase was the standard Gomori Tween method (as described by Gomori 1952, and Pearse 1961) using Tween 40, 60 and Tween 80 (for “true” lipase). The best results were obtained when incubation times were 2–4 hours.

Thiamine pyrophosphatase was demonstrated according to Novikoff and Goldfischer (1961), the substrate — thiamine pyrophosphate, incubation 30 to 60 minutes at room temperature.

To obtain double staining of spherosomes Holts indoxyl method for esterase (the substrates O-acetyl-5-bromoindoxylin and Burstone’s Naphtol AS methods for esterase and acid phosphatase (the substrates Naphtol AS-D acetate and Naphtol AS-BI phosphate, the diazonium Fast red violet LB salt) were used (Burstone 1966, 1962).

A detailed description of the double staining techniques applied is given in the text.

Control experiments were performed by omitting the substrates from the incubation media and by using heat-inactivated tissues (10 minutes' in boiling water). In the controls for lipase the inactivation by 5% phenol (15–30 minutes) and by Lugol’s iodine (2–3 minutes) were also used.

0.01 M NaF was employed as inhibitor of acid phosphatase and esterase, 0.1 mM CuSO4 as inhibitor of β-glucuronidase (Reakallio 1963), 3×10−4 M Na2SO3 for arylsulphatase (Roy 1954), 10−5 M E-600 * as inhibitor for organophosphorus sensitive esterases (Pearse 1961).

For fat staining the mixture of Sudan III and Sudan IV in 70% alcohol and Oil red O in 60% triethylphosphate were employed. For proteins the mercury-bromphenol blue method was used (Mazia et al. 1953, and Pearse 1961).

* Diethyl-p-nitrophenyl phosphate.
RESULTS

1. Hydrolases in the spherosomes of onion-scale epidermal cells

Histochemical methods allowed to demonstrate the following hydrolases in onion epidermis: acid phosphatase, non-specific esterase, E 600-resistant esterase (cathepsine C?), β-glucuronidase, β-galactosidase, β-glucosidase, lipase, also Gomori's "true" lipase detected by Tween 80, and acid deoxyribonuclease (Plates I and II).

The test for α-glucosidase gave negative results. Thiamine pyrophosphatase was not found in the spherosomes.

The localization of all the above-mentioned enzymes in the cells was identical. Spherosomes always stained selectively. Enzymic reactions were negative in mitochondria and plastids.

In the coupling azo dye techniques and in the indoxyl method the cell walls never stained, neither did the nuclei which were sometimes brownish when methods based on Gomori's lead sulphide procedure were used.

Diffuse staining of the cytoplasm was frequently observed in the cells. It varied, in dependence on the method of cytoenzymic staining, on the azo dyes used and on the incubation time. This problem was not studied closer at present and no attempt was made to establish, in which cases the staining of the cytoplasm could be considered as proof of reaction in situ, and when it was due to diffusion of the enzyme or of the products of enzymic reactions from the spherosomes.

In all the preparations, the spherosomes stained most intensely and were the first to stain in the cell. This was ascertained by microscopic control of the course of enzymic staining, and in several cases the reactions were performed directly under the microscope in hanging drops or between slide and coverslip. The pattern of stain diffusion from the spherosomes into the cytoplasm was also observed. The coloured streams take rise in the spherosomes.

Thus the view expressed in earlier works that spherosomes are the chief sites of the hydrolase activities found its confirmation in all the tests performed.

The diverse techniques of enzyme detection proved of unequal value. The coupling azo dye methods revealed in general the enzymes consistently in all cells. For β-glucuronidase demonstration only the method of Seligman et al. gave highly reproducible results. The 8-hydroxyquinoline method gave positive results when the material fixed in formalin vapour for 15 min. was incubated overnight at room temperature in substrate medium. In this case selective orange staining of the spherosomes was obtained in all the cells of the preparations examined. The
Plate I

Localization of the activities of lipase and arylsulphatase in epidermal cells of *Allium cepa* bulb scales.

Fig. 1. Lipase activity localized in spherosomes of cells fixed with acetone. Tween 80. Fig. 2. Lipase activity in spherosomes. Tween 40. Unfixed cells.

Fig. 3. Lipase activity in spherosomes. Tween 80. Fig. 3a — epidermis fixed with acetone, plasmolyzed cell. Fig. 3b — fixed in 4 per cent formalin, with addition of sucrose.

Figs. 4, 5, 6. Arylsulphatase activity; fixed Fo-Ca with sucrose. In Fig. 4 several spherosomes are vesicle-like and show signs of denaturation; in Fig. 5 large spherosomes are visible in very young epidermis cells.

Figs. 1—6 magnification 1200 ×

Plate II

Localization of the activities of several acid hydrolases in spherosomes. Epidermal cells of onion bulb scales.

Figs. 1, 2. β-glucuronidase. Fig. 1 — fix. Fo-Ca with sucrose, Seligman et al. method. Fig. 2 — fix. in formalin vapour, 8-hydroxyquinoline method. Beside enzymically stained spherosomes, colourless mitochondria are seen.

Figs. 3 and 4. β-galactosidase. Fix.: Fo-Ca with sucrose.

Figs. 5, and 6. Acid deoxyribonuclease. Fix.: Formalin vapours.

Figs. 1, 3, 4 magnification 1500 ×; 2, 5, 6 1200 ×.

Plate III

Indoxyl esterase activity in anthers of *Larix decidua* Mill. Fo-Ca with sucrose.

Figs. 1, 3—5: tetrad stage; Figs. 2 and 6: young microspore stage.

Figs. 1, 2 show intensive enzymic activity in tapetum and in inner middle layers of anther walls, in Fig. 1 tapetal cells protrude in some places into the anther. (280 ×).


Fig. 6. Plasmoidal masses between young microspores are intensely stained (arrows). (250 ×).
staining disappeared quickly, when the epidermis was transferred to water.

The results obtained by methods based on Gomori's lead sulphide procedure, i.e. the methods of lipase and deoxyribonuclease demonstration did not give consistent results.

The controls (incubation in solutions devoid of substrate) were difficult to interpret. The method of deoxyribonuclease demonstration should probably be adapted to the plant material. Nevertheless, the results obtained by these methods may be evaluated as positive, the reactions were replicated many times, and staining was successful, if not in all, still in many instances. When for detection of acid phosphatase Gomori's method was used parallelly with the coupling azo dye method (Walczyk-Czernecka 1962), the results were in close agreement.

By the method of Rutenberg et al. for arylsulphatase, a dark blue or purple staining of spherosomes was obtained. This colour after several days or sometimes on the next day changed to red. Small spherosomes stained uniformly, whereas in the larger ones the centre sometimes seemed reddish. This may have been due to the dissolution of the final reaction product in the lipids accumulated in the centre of the spherosomes.

The results obtained with fresh were worse than with fixed material, the spherosomes were often damaged.

After treatment with $3 \times 10^{-4} M \text{Na}_2\text{SO}_4$ as inhibitor, the spherosomes stained red. This would indicate that the enzymic activity was partially suppressed. Pears (1961) evaluates this method as unsatisfactory. His final reaction products were not blue but red, and since they are liposoluble, he considers the localization as unreliable. Avers (1961) reports that she obtained by this method a positive reaction in the primary roots of germinating grass seeds, and that like in my preparations, a deep-blue colour appeared.

According to Roy (1962) it is not possible to distinguish A, B, and C arylsulphatases by histochemical methods.

In the epidermal cells after treatment with $10^{-5} M \text{E}-600$ for one hour prior to incubation in the standard indoxyl esterase medium, inhibition was incomplete. The activity of E 600-resistant indoxyl esterase is attributed, at least in part, to the activity of cathepsin C (Pepler and Pears 1956; Hess and Pears 1958; Pears 1961).

Attempts of inhibitors and activators application were unsuccessful. The only exception were: E-600, NaF, CuSO$_4$ and Na$_2$SO$_4$ inhibiting non-specific esterase (partial inhibition) and acid phosphatase (complete inhibition), $\beta$-glucuronidase (quite colourless preparations) and arylsulphatase, respectively.
In onion epidermis the number of spherosomes in the cells, their size and intensity of staining differ so widely even in neighbouring cells that the evaluation of partial activation or inhibition on the basis of staining results was unreliable (e.g. effect of PCMB* in the test for cathepsin, and of Na₂SO₄ as inhibitor of deoxyribonuclease). Therefore further trials were given up.

In the epidermal cells, beside large spherosomes about 0.8—1 μ in diameter, smaller ones occur up to the limit of the microscope resolution power. The proportion of the small spherosomes to the large ones varies widely. In some cells there is a clear-cut difference between the populations of large and of small spherosomes, as has been observed by U r l (1964) (Plate V, Fig. 10), in others (Plate V, Fig. 11) transitional forms exist (compare J a r o s c h 1961).

Sometimes in the epidermis treated by various enzymic techniques the spherosomes were deformed, particularly the large ones. There may occur cells or cell complexes containing spherosomes increased in size up to a 2 μ diameter or even more, often flattened to a disc shape, whereas the spherosomes of neighbouring cells may have normal dimensions. In other cases the denaturation caused probably by dissociation of the lipoprotein complexes leads to the appearance of vesicle-like spherosomes (Plate I, Fig. 4) in which the protein moiety seems to have retreated to the peripheral zone of the vesicle and in optical section appears as rings "caps" or crescents, staining with mercuric bromophenol blue (M a z i a et al.). Here too are deposited the products of the enzymic reactions applied. The lipids after "Entmischung" tend to accumulate in the remaining part of the vesicle which becomes highly refractive.

By the use of the indoxyl method for esterase demonstration and by staining subsequently the same preparations with Oil red O or with red Sudans it was possible to obtain simultaneously a red staining of the lipid spherule and blue staining of the adjacent cap or crescent (Plate V, Figs. 6—8). In the spherosomes, particularly after application of formalin fixatives, lipid(?) crystals of various shapes, birefringent when examined in polarized light were formed. Sometimes, in glycerine jelly preparations they had the form of angular platelets and in lateral view of rods. After heating the preparation in hot water, they disappeared (Plate V, Figs. 1—5).

The changes occurring gradually in the appearance of the spherosomes could be observed under microscope in the dying cells. Similarly as in the fixed material, only part of the spherosomes underwent deformation.

In the living cells the spherosome content seems to be homogeneous, and the whole spherosome is highly refractive.

* p-chloromercuribenzoate.
The spherosome population in onion epidermis cells proved to be in some extent enzymically heterogeneous. This already became manifest when E-600 was used as inhibitor of indoxyl esterase activity. After treatment with the inhibitor, the large spherosomes stained only weakly, indicating the occurrence in them of two types of esterase: one E 600-resistant (cathepsinlike) and the other sensitive to E-600.

In the population of small spherosomes, only a part gave a positive enzymic reaction, those, namely, which contained the E 600-resistant enzyme activity, the other ones remaining colourless. Further evidence of heterogeneity of the spherosomes was found in the pictures obtained by a modification of the double staining technique, developed by Shinitzky and Seligman (1961) for simultaneous demonstration of inhibitor-sensitive and -resistant esterases.

Free-floating fragments of onion epidermis were first incubated for 40—60 min. in Holt's 5-bromo-indoxyl acetate medium containing 10⁻³ M sodium fluoride as reversible inhibitor, a blue staining of NaF-resistant esterase was obtained. The tissues were washed in 8.5 per cent sucrose for 2 h. to remove all traces of the inhibitor and then reincubated for 60 min. at 37°C in Naphthol AS-D acetate substrate medium, containing Fast red violet LB salt capable of yielding a red azo dye. In the preparation, indoxyl NaF-resistant esterase and AS-D esterase sensitive to the inhibitor appeared simultaneously in contrasting blue and red colours.

By a similar method without using NaF, simultaneous staining of indoxyl esterase (blue) and acid phosphatase (red) was achieved in the cell by applying successively the specific methods for their detection: Holt's indoxyl and Burstone's — Naphthol AS, diazonium — Fast red violet LB salt. Incubation time for each reaction was about 1 h.

In double-stained preparation three types of spherosomes could often be distinguished: larger two-coloured, with separate blue and red zones or mixed-coloured ones exhibiting two kinds of enzymic activity, the other two types were small spherosomes red or blue exhibiting only one kind of activity.

Hydrolases in spherosomes of other plant tissues

An orientational attempt was made to demonstrate hydrolases in the spherosomes of short-lived tissues. These were: the rootcap in Allium cepa, the tapetum and middle layers of the anther in Larix decidua Mill. at the tetrad and young microspore stage and the parenchyma cells in the immediate vicinity of injured tissue in the onion stem plate.

In the anthers of Larix intensive activity of indoxyl esterase was demonstrated in the tapetal cells filled with very large spherosomes
Plate IV

Fig. 1. Cross-section through vascular bundle in onion stem plate. Fix.: Fo-Ca with sucrose. Intensive indoxyl esterase activity in phloem. Stained spherosomes visible (1000 ×).

Fig. 2. Parenchyma cells in vicinity of injured tissue in onion stem plate. Indoxyl esterase activity particularly strong in apical part of cells adjacent to dead tissue (arrows). (270 ×).

Fig. 3. Longitudinal section of root apex of Allium cepa. Fix.: Fo-Ca with sucrose. Acid phosphatase activity demonstrated by Burstone's Naphthol phosphate AS method. The rootcap exhibits intensive enzymic activity (60 ×).

Fig. 4. Fragment of Fig. 3 in high magnification. Rootcap cells. Spherosomes are seen to be the sites of enzymic activity (1500 ×). (Photo courtesy of Dr Gabara).

Plate V

Spherosomes in epidermal cells of onion bulb scales.

Figs. 1—8, 10, 11: Fo-Ca with sucrose; Fig. 9. Fo-Ca without sucrose.

Figs. 1, 2, 3, 4, 5. Acid phosphatase activity demonstrated by Burstone's method (Naphthol phosphate AS-BI, Fast red violet L.B. salt). Lipid(?) crystals with adjacent enzymically stained remaining parts of the spherosomes (arrows). Fig. 1. Magnification 2200 ×, other figures 3200 ×. Most spherosomes unchanged (Fig. 1).

Figs. 6, 7, 8. Vesicle-like spherosomes double stained by Holt's indoxyl acetate method and red Sudans (arrows). Lighter zone in spherosomes is pink in the preparations (staining of lipids), the darker zone — blue (enzymic staining) (2200 ×).

Fig. 9. β-glucuronidase. Spherosomes deformed. Enzymic staining visible in „caps“ and crescents (2200 ×).

Fig. 10. Acid phosphatase. Sodium-α-naphthyl phosphate, Fast red RC salt. Larger spherosomes mostly deformed. Distinct difference between large and small spherosome populations (1800 ×).

Fig. 11. Naphthylacetic esterase. Intermediate forms between large and smallest spherosomes visible (1800 ×).
stained blue with indigo (Plate III, Figs. 3—5). Diffuse staining of the cytoplasm was also observed. The deep-blue tapetum presents a striking contrast with the adjacent tissues (Plate III, Fig. 1). In some places, the tapetal cells stained by the reaction product protruding into the pollen sac assume the appearance of plasmodial masses (periplasmodium — Luxemburgowa 1922) (Plate III, Fig. 6). In the tapetum cells of the anther at tetrad stage shown at high magnification numerous large spherosomes partly destroyed are visible (Plate III, Figs. 3—5). The middle layers of microsporangium adjacent to the tapetum are flattened or partly crushed and also strongly stained (Plate III, Fig. 2).

In the tip of the adventitious root acid phosphatase activity is particularly pronounced in the rootcap. Here too the enzyme is localized in the spherosomes (Plate IV, Fig. 4). At first the lateral layers of the root cap and several of its layers at the root top exhibit intensive enzymic reaction (Plate IV, Fig. 3) which gradually subsides in the external layers. Similar staining patterns were obtained by the indoxyl method for esterase.

In the vascular bundles of the onion stem plate, indoxyl esterase activity was detected in the phloem, particularly in the companion cells (Plate IV, Fig. 1). The epidermis of the roots piercing through the stem plate parenchyma and the cells of the latter surrounding the root apex were also strongly coloured.

The onions were placed on Erlenmayer flasks filled with water. In order to speed up the appearance of adventitious roots, the lower surface of the stem plate was incised. On the cut surface dead tissue appeared. The underlying cells were elongated perpendicularly to the exposed surface and the spherosomes in them were accumulated in their distal part on the side of the lesion (Plate IV, Fig. 2). In this region, the cells showed a deep indigo-blue staining. Blue deposits indicating non-specific esterase activity appeared even on the cell walls and were visible in the intercellular spaces adjacent to the dead tissue. Penetration of the enzymes from the spherosomes into the whole cell contents and even its environment must have occurred here.

The cells of the stem plate parenchyma contained but few spherosomes.

DISCUSSION

By means of histochemical methods commonly used in investigation of animal tissues, the presence of a number of hydrolases was established in cells of the epidermis of onion scale-leaves. It was demonstrated that the enzymes are localized in the spherosomes. The results of enzymic reactions were reproducible. The staining due to enzymic reaction appea-
red in almost all the tests and in all the cells of the tissues investigated. This is particularly true of hydrolases, for the demonstration of which, the indoxyl method (esterase) or the coupling azo dye methods could be applied.

The latter methods, although continuously improved, do not so far ensure complete insolubility of the enzymic reaction products in lipids (Wolfgram 1961; Burstone 1962). Thus there might be a danger of false localization caused by the presence of lipids in the spherosomes. The following arguments, however, testify against this supposition:

1) the enzymic staining in the spherosomes is always specific, that is such that, under the method applied it may be considered as a positive reaction (cytoenzymic methods specify as a rule how lipids stain in each case);

2) in the spherosomes exhibiting symptoms of the above described denaturation, the final reaction products deposit only in the peripheral zone where the protein substances are accumulated; in optical section rings or crescents surrounding on one side vesicles of lipid character are stained;

3) in other plant material than onion epidermis, where fat droplets occur beside spherosomes, e.g. pollen tubes (Góriska-Bryl and elaioplasts (Walek-Czernicka — unpublished data, Kwiatkowska — in press), it was found that, in the methods for enzyme demonstration employed, the lipids remained unstained or showed different colour than the spherosomes.

Direct observation under the microscope of the course of enzymic reaction showed that the final reaction product first appears in the spherosomes. Staining of the cytoplasm is always secondary and less intense. The same seems to be indicated by the pattern of diffusion of the coloured reaction products from the spherosomes to the cytoplasm. The mitochondria and plastids are always colourless.

Histochemical investigations on plant hydrolases (for review see van Fleet 1952, 1962) supply not only scarce but controversial information about the intracellular distribution of enzymes. Of the hydrolase activities investigated in the present study, that of acid phosphatase has been attributed to undefined cytoplasmic particles (Dyar 1950; Avers and Grimm 1959), or to the mitochondria (Wilson and Cutter 1955; Jensen 1956) or else the enzymic activity was supposed to be associated with more than one organelle: the mitochondria, proplastids and lysosomes (Avers and King 1960; Avers 1961). Beneš et al. (1961) identified the bodies occurring in the rhizodermis and containing acid phosphatase as lysosomes. Avers (1961) gives a photomicrograph of arylsulphatase distribution in the root meristem of Phleum without specifying in which cellular component it was detected. Similarly Be-
neš et al. (1961) do not give a more detailed interpretation of the pattern of indoxyl esterase distribution presented in their paper. Glucosidase was found by Wardrop in cell walls (quoted after van Fleet 1962).

As seen from the above given review, the conclusions of these investigators do not conform with mine. Nevertheless I believe that the identification of the plant cell components containing hydrolases as spherosomes is correct. My studies were made on particularly convenient material. Onion epidermal cells have been the object of many exhaustive investigations. These cells at the base of inner onion bulb scales contain a small number of large spherosomes which, on account of their high refractivity and characteristic rapid displacement in cytoplasmic currents, are easily distinguishable from other cell components. The effects of enzymic reaction could in each case and at any stage be compared with living material. It is also significant that the application of various methods (based on different principles) for hydrolases detection always led to the same conclusion.

The results obtained found further confirmation in investigations carried out in our laboratory on different material. In the endosperm (Olszewska, Gabara 1964), in pollen tubes (Górskak-Bryliss 1965), in the epidermis of plants containing elaioplasts and in the elaioplasts themselves (Kwiatkowska — in press), in the root meristem of Malvaceae (Kwiatkowska and Stępielewski — in press) in fungal hyphae (Palczewska — unpublished data) and in algae Gabara 1965 — unpublished), spherosomes proved to be the sites of hydrolases activities.

The hydrolases demonstrated in the spherosomes are considered in animal tissues as lysosomal enzymes (de Duve 1959; Novikoff 1961; Holt 1956; see also de Duve 1960), lipase being an exception. De Duve (1961) makes the reservation that lysosome hydrolases form a digestive system capable of decomposing most natural compounds with the exception of lipids. Lipase (esterase detected by the Tween 80 method) activity, however, has been ascertained by Ogava et al. (1961) in the lysosomes of fibroblasts and astrocytes grown in culture of cerebellar cortices obtained from puppies*.

In the onion spherosome population a certain heterogeneity was observed. The spherosomes of one and the same cell differ in the degree of enzymic activity, they also seem to differ in enzyme equipment.

* The lysosome concept has lately been revised and is widely discussed (Novikoff et al. 1964; de Duve 1964; Hirsch and Cohn 1964). Therefore I do not try to establish further analogies between spherosomes and various categories of lysosomes, and I wish to stress that, according to the suggestions of de Duve (1964), this name is used here only as a "generic" term including both what is considered to be primary as well as secondary lysosomes.
Analogous investigations carried out simultaneously in this laboratory on different material gave the same results (Olszewska, Walek-Czernacka, Kwiatkowska 1965; Olszewska and Gabora — unpublished data; Kwiatkowska — unpublished data). In those studies more tests were applied that is more pairs of enzymes and more methods of their demonstration were used. Further investigations will prove whether the differences between the spherosomes are connected with the stage of their development — the minute ones might be juvenile forms not possessing yet the whole set of enzymes (Jarošch 1961; Frey-Wyssling 1963; Url 1964) — or whether there exist also qualitative differences between the full-grown spherosomes of the same cell. In the epidermis of Ornithogalum umbellatum the heterogeneous distribution of the enzymes occurred also within a group of full-grown spherosomes (Kwiatkowska — in press); in the epidermis of Dahlia variabilis two categories of spherosomes were found: one PAS-positive, the other negative (Kwiatkowska — unpublished data).

The function of spherosomes was unknown until quite lately. The view of Perner (1952, 1958) that they play a role in respiratory processes found no confirmation in subsequent investigations (Drawerk 1953, Straus 1956).

In 1962 and 1963 I published the first results of my studies on hydrolyses in which I demonstrated acid phosphatase and non-specific esterase in the spherosomes of onion epidermis. The suggestion was advanced that by their function in the cell, spherosomes are related to de Duve's lysosomes.

Recently Frey-Wyssling, Grieshaber, Mühlethalder (1963) supplied proof based on electron microscope studies that spherosomes of fat-storing tissues (oil seeds) transform into oil droplets. These authors, referring to the acid phosphatase demonstrated in spherosomes in my investigations, suggest that „at least the final step in fat synthesis, viz. the transesterification of glycerol by exchanging phosphoric acid with fatty acids, can be attributed to the spherosomes“. In the paper by Frey-Wyssling, López-Sáez, Mühlethalder (1964), the opinion concerning the function of spherosomes is more categorically formulated, however, without supplying additional evidence: „in contrast to lysosomes, which contain highly active digestive enzymes capable of degrading nucleic acids, proteins and glucosides, the spherosomes catalyze synthesis processes (e.g. fat synthesis)“.

The results of the present study do not fully confirm this last opinion. Evidence has been obtained that spherosomes exhibit a number of enzymic activities characteristic of lysosomes. If biochemical investigations confirm these findings, it may be admitted that spherosomes are involved in some way in the processes of intracellular hydrolysis.

As confirmation of the presence of acid phosphatase in spherosomes
may be considered the results of the biochemical investigations of Harrington and Altschul (1963). Acid phosphatase activity was measured by these authors in the particulate fraction obtained from embryos and endosperm of germinating onion seeds. The major part of enzymic activity (up to 89%) was bound in lysosome-like particles which judging by the material used must have been spherosomes.

Further support for my conclusions comes from histological evidence. Numerous spherosomes with high enzymic activity occur in tissues of varying character and role, the common feature of which is that they subsist for a short time only and undergo desorganization within life of plant organs. Degradation of organic material is known to occur in them, leading to complete destruction of protoplasts and even of the whole cells. As example have been quoted here: the tapetum with the adjoining middle layers in the larch anther wall, the external layers of the root cap in Allium cepa, the parenchyma cells in the immediate vicinity of injured tissue and themselves doomed to gradual destruction and finally the parenchyma of the stem plate surrounding the apex of the piercing adventitious root.

Further investigations should demonstrate the role of spherosome hydrolases in various processes and in various physiological states of the cell.

The existence of spherosomes producing or accumulating fats in certain conditions might supply one more proof of the heterogeneity of these cell components.

Almost 40 years ago I advanced the suggestion that the elaioplasts in the anthers of Malvaceae arise through agglomeration of osmiophilic bodies which I then considered as a special category of fat-producing plastids (Luxenburgowa 1927). In a subsequent work (Walek-Czerniecka and Kwiatkowska 1961) it was established that the osmiophilic granules forming elaioplasts are spherosomes. It could not, however, be ascertained whether spherosomes are involved in the production of the fats appearing in the elaioplasts substratum and what role they play in it.

In onion epidermis, the separation of lipids in the spherosomes occurs in already dying cells or after treatment with fixatives or incubation media. In living cells, the contents of spherosomes as seen in a light microscope are homogeneous. No transformation of spherosomes into oil droplets was observed.

**SUMMARY**

In the spherosomes of onion scale epidermal cells the activity of the following hydrolases has been demonstrated by histochemical methods: acid phosphatase, non-specific esterase, E600-resistant esterase (cathepsin-like), β-glucuronidase, β-galactosidase, β-glucosidase, lipase, arylsulphatase and acid deoxyribonuclease.
High esterase and acid phosphatase activities were demonstrated in numerous spherosomes of several tissues of only temporary functional character which are completely destroyed in the life-time of plant organs.

Examples of certain biochemical heterogeneity within the spherosome population of one and the same cell are described.

The possible role played by spherosomes in plant cells is discussed.

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Hydrolytic enzymes in the spherosomes


Badania cytochemiczne nad występowaniem hydrolaz w sferosomach

Streszczenie

W sferosomach skórki łusek cebuli ujawniono metodami histochemicznymi obecność następujących hydrolaz: kwaśnej fosfatazy, esterazy niespecyficznej, esterazy odpornej na E-600 (katepsyny C?), β-glukuronidazy, β-galaktozydazy, β-glukozydazy, lipazy, arylsulfatazy i kwaśnej dezoksyrybonukleazy.

Wykazano intensywną aktywność hydrolaz (kwaśnej fosfatazy i esterazy) w licznych sferosomach kilku tkanek o charakterze tranzytorycznym, które ulegają zniszczeniu jeszcze za życia organizmu roślinnego.

W oparciu o uzyskane wyniki wypowiedziano pogląd, że sferosomy biorą udział w procesach hydrolytycznych i autolitycznych komórek.

Podano przykłady pewnej heterogenności biochemicznej w obrębie populacji sferosomów tej samej komórki.

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