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Hydrolases in pollen grains and pollen tubes

by

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INTRODUCTION

In the present paper I have tried to localize cytologically the hydrolytic enzymes in pollen grains and pollen tubes. I was prompted to this attempt both by the results of Wałek-Czernecka's studies (1962, 1963) demonstrating the localization of some hydrolases in the spherosomes of onion scales epidermis and by the fact that up till now in the studies concerning pollen enzymes no attempt was made to associate the discovered enzymes with definite morphotic structures of the cells.

Another consideration suggesting the need of such studies is the lack of more precise data on pollen hydrolases. In order to interpret correctly the results of enzymic reactions, the studies on hydrolase localization were preceded by intravital observations of pollen grains and tubes with particular attention to the spherosomes of these cells.

MATERIAL AND METHODS

The pollen grains and pollen tubes of 34 plant species were used as material.

More precise intravital observations were carried out on pollen grains and tubes of *Bryonia dioica* Jacq., *Vinca minor* L., *Tradescantia bracteata* and eight species of *Campanula* and also for comparison, on all the below-mentioned plants which were the object of enzymatic studies.

The pollen tubes were cultured in vitro on a nutrient medium containing 1 per cent agar-agar and sucrose. The optimum concentration of sucrose was determined separately for each species and varied from 2 to 35 per cent.

Enzymic reactions were carried out both on fresh material and on specimens fixed with formalin vapours, formalin with phosphate buffer (pH 7) or with Baker's fixative.

Exceptionally some pollen tubes, e.g. of *Vinca minor*, were examined only after fixation because the living specimens were destroyed in the incubation medium.

Live or fixed pollen grains were examined in toto; only for determination of acid phosphatase in pollen grains of *Campanula* was it

necessary to use sections, as in this case the thick membrane of the pollen grain hindered the penetration of the incubation medium into the grain both in fixed or fresh pollen grains. The sections were prepared by the paraffin method. The anthers after fixation in Baker's solution at 4°C were very quickly passed through alcohols and benzene, embedded in paraffin and then cut with a microtome into c. 4 μ sections. Deparaffinized sections were immediately tested enzymically. The methods used in the enzymic tests are listed in the Table 1.

Table 1

Methods of enzymic investigation

Enzyme	Substrate azo dye	Control	Incubation time	Temperature °C
Acid phosphatase *	Sodium α-glycero-phosphate	incubated without substrate	4 hours	37°
	Sodium α-naphthyl-phosphate Fast Blue B Fast Blue RR	substrate NaF 10^{-2} M	10—60 min.	37° and room
Alkaline phosphatase *	Sodium α-naphthyl-phosphate Fast Blue RR	0.1 N HCl for one hour before incub.	10—60 min.	37° and room
Non-specific esterase	o-acetyl-5-bromo-indoxyl	before incub. 1) 5% aqueous phenol 2) Lugol solution	15 min.	room
	α-naphthylacetate	3) H_2O at 100°C 10—20 sec.	20 min.	room
Lipase *	Tween 20, 40, 60, 80	incubated without substrate 5% aqueous phenol before incubation	2—12 hours	37°
Deoxyribonuclease **	DNA Sigma	incubation without substrate	2—6 hours	37°
Arylsulphatase *	6-bromo-2-naphthyl sulphate	Substrate + Na_2SO_3 3×10^{-4} M	30 min.	room
β-glucuronidase *	8-hydroxy-glucoronidae quinoline		30—60 min.	room

* Methods used after Pearse (1961).
** Method of Aronson et al. modified by Vorbrodt (1961).

Acid phosphatase was found in the pollen grains and tubes of *Campanula persicifolia* L., and in the pollen tubes of *Antirrhinum maius* L., *Asparagus officinalis* L., *Begonia semperflorens* L., *Betonica officinalis* L.,

Bryonia dioica Jacq., *Dianthus* sp., *Gentiana pneumonathe* L., *Glaucium flavum* Cr., *Narcissus Jonquilla* L., *Haemanthus Katharinae* Bak., *Hyacinthus orientalis* L., *Tradescantia bracteata*, *Tropaeolum maius* L., *Vinca minor* L., *Viola tricolor* L. and *Zantedeschia aethiopica* Spr.

The activity of alkaline phosphatase was detected in pollen tubes of most of the same plants in which acid phosphatase was found and in a few other species. The pollen tubes of the following sixteen species have been examined: *Antirrhinum majus* L., *Gentiana pneumonanthe* L., *Glaucium flavum* Cr., *Papaver californicum* L., *Tradescantia bracteata*, *Tradescantia virginiana* L., *Viola tricolor* L., *Vinca minor* L., *Campanula persicifolia* L., *Bryonia dioica* Jacq., *Asparagus officinalis* L., *Dianthus* sp., *Betonica officinalis* L., *Chelidonium maius* L., *Tropaeolum maius* L. and *Begonia semperflorens* L.

For the presence of non-specific esterase mainly pollen grains and pollen tubes of *Campanula persicifolia* L. and also pollen tubes of *Bryonia dioica* Jacq., *Tradescantia bracteata*, *Tradescantia virginiana* L., *Vinca minor* L., *Hyacinthus orientalis* L., *Papaver californicum* L. and *Gentiana pneumonanthe* Cr. were examined.

The other hydrolytic enzymes; lipase, deoxyribonuclease, arylsulphatase and β-glucuronidase were preliminarily investigated only in pollen grains of *Campanula persicifolia* L., and pollen tubes of *Hyacinthus orientalis* L.

RESULTS

I. Spherosomes of pollen grains and tubes. Intravital observations

a. Morphology and optical properties of spherosomes.

The spherosomes occurring in pollen grains and pollen tubes correspond to the descriptions of all other spherosomes (Sorokin 1955, 1958; Perner 1958). Their size varies from 0.3 to 0.8 μ; the pollen tubes of some species are characterized by the distinct prevalence of larger spherosomes while in others smaller ones occur.

When the growth of the pollen tubes ended and sometimes earlier, a swelling of the spherosomes could be observed. Now and then their dimensions increased considerably. Parallelly to the swelling process a part of the spherosomes lost their regular shape, assuming the form of swollen rings, sickles, crescents or agglomerated nubs inside a delicate capsule (Fig. 1).

The dimensions of the spherosomes and their optical properties allow to distinguish them from other cellular organelles such as proplastids, leucoplasts and mitochondria.

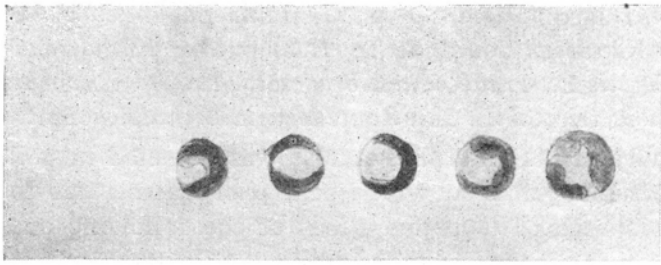


Fig. 1. Deformed spherosomes of pollen tubes of *Hyacinthus orientalis*. Magn. 5400 ×

It was possible to compare the morphologic and optical features of the sperosomes with those of proplastids in the stage of microspore during the period preceding the formation of the central vacuole. In this stage numerous proplastids occur beside spherosomes and are frequently concentrated round a central nucleus. In contrast to the globular spherosomes, proplastids are larger, their shape is irregular and they display amoeboidal movements. Seen in the light field of the microscope they are mat.

In phase contrast there is a clearly marked difference in their refractivity; proplastids as compared to the black spherosomes are slightly greyish.

Leucoplasts occurring in further developmental stages produce as a rule starch; owing to this they have a characteristic appearance and are easy to identify. The size of leucoplasts reaches 2—5 μ and sometimes even 13 μ, as well when they form single starch grains e.g. *Campanula multiflora* and *Viola tricolor* or when they form more numerous starch grains e.g. in the leucoplasts of *Crocus vernus*, *Allium sativum*, *Coronilla varia*, *Vinca minor*, *Ornithogalum* etc.

Thus the leucoplasts in the pollen grains and tubes examined (though they rarely occur in the latter) can easily be distinguished from spherosomes because of their different size, shape and optical properties.

Spherosomes differ by their size and shape from elongated mitochondria (clavate or thread-like). But it was difficult to distinguish in intravital observation the spherosomes from spherical mitochondria occurring in rather large numbers in the material examined and having approximate dimensions. The most convenient developmental stage for such comparative observations was that of the full-grown pollen tube.

In this period the accumulation of cytoplasm elements is lowest and the movement of the cytoplasm still intensive. In these conditions, the mitochondria were usually present in another, deeper layer of the cytoplasm.

A basic and exclusive feature by which spherosomes are distinguished from spherical mitochondria in a living, unstained pollen tube is the

difference in their refractivity. In the light field of the microscope, unlike the glittering spherosomes, the mat-grey mitochondria could hardly be detected in the cytoplasm.

The identification of the spherical mitochondria is still more reliable when intravital staining with Janus green is applied. As the result of such staining, after some 40 min. a blue-green colour appears in the mitochondria while the spherosomes remain colourless.

To distinguish the spherosomes from simple fat droplets postvital observation and in the first place cytochemical methods are required. The distinction of those two categories of cellular elements in living material, only on the basis of morphologic features and optical properties is essentially impossible (the large lipid spheres excepted).

But in a growing pollen tube some differences between spherosomes and fat droplets, consisting in their different behaviour, may be observed. During the whole period of intensive growth of the pollen tube, the spherosomes do not noticeably change their dimensions. They swell and are deformed only towards the end of growth or after it has ceased. The author never observed a coalescence of spherosomes, while simple fat droplets coalesce at every moment of growth forming large drops. The author has not been able to single out dictyosomes in the living pollen tube.

The number of spherosomes in pollen grains and pollen tubes is strikingly large as compared to that of other cells of the plants examined. In the examined material, pollen tubes of *Vinca minor* and *Bryonia dioica* contain the largest number of spherosomes which are much less numerous in *Hyacinthus orientalis*. Repeated observations of the development of pollen grains of the genus *Campanula* indicated consistently that the number of spherosomes increases during the period between the microspore stage and the formation of the binuclear grains and the appearance of spherosome agglomerations which form what is known as "elaioplasts".

In intravital observation it is noteworthy that in pollen and pollen tubes a population of very fine granules occurs. Their size lies at the limit of visibility or slightly exceeds it. They are characterized by strong refractivity; owing to this they give the impression of being quite black. They occur during the whole period of development of the pollen and the pollen tube, are particularly numerous in young tubes.

b. Movement of spherosomes.

During the early developmental stages as well as in the ripe grains, the cytoplasm of the pollen grains and pollen tubes seems motionless

to the observer. Also, the spherosomes in it are immobile since — unlike proplastids and leucoplasts — they possess no mobility of their own.

When the movement of the cytoplasm becomes visible during germination of the pollen grain and then during the growth of the pollen tube, the spherosomes carried by its currents become more visible. The characteristic "halo" effect appears repeatedly.

After termination of growth of the pollen tubes the displacement of spherosomes continues for a long time and is the best criterion of the vitality of the cell. The rapidity of motion of the cytoplasm depends on various external factors (K a m i y a 1959). However, in the pollen tubes examined when growing in similar thermic and humidity conditions, it was possible to establish that the displacement of spherosomes is fastest at the period of maximum elongation of the pollen tube which occurs two hours after the beginning of germination.

At the maximum rate of growth of the pollen tube the velocity of spherosome movement attains c. 3 μ per sec., Both the large spherosomes of *Vinca minor* (0.8 μ) and the small ones of *Narcissus Jonquilla* (0.3 μ) reach the same speed. No dependence could be noted between the size of spherosomes and the speed of their movement as has been suggested by D r a w e r t and M i x (1962).

Spherosomes shift in conformity with the motion of the cytoplasm. It is worth noting that during the growth of the pollen tube these bodies never reach its top. This top, characterized by a homogeneous structure, shows neither spherosomes nor any larger cellular elements. Spherosomes and all other cellular organelles reach the top of the tube only after the end of its growth period.

In some segments of the pollen tube the spherosomes exhibit an almost uniform flow, in others it slows down or they come to a standstill for a moment as if they encountered some invisible obstacle or cytoplasmic whirls. It is characteristic of the behaviour of spherosomes in the streaming cytoplasm that they join into groups of 2—3 grains, into short chainlets or form even irregular agglomerations. But their association is not durable. Unceasing separation and recombination of the flowing spherosomes are observed. Close observation excludes the division of spherosomes suggested by S t r u g g e r (1949).

The only instance of a more durable agglomeration of spherosomes which lasts for a longer time are the "elaioplasts". The elaioplasts in pollen grains of *Campanula* described by the author a few years ago, formed by "osmophile grains" (G ó r s k a - B r y l a s s 1962) are typical spherosomic elaioplasts. Further investigations have shown that they are formed by an accumulation of spherosomes. They keep the same morphologic and optical properties when agglomerated as when dispersed.

II. Results of enzymic reactions

Acid phosphatase

The efficiency of the two methods used for the detection of acid phosphatase proved to be unequal.

When the modified Gomori method was applied the control preparation frequently revealed the presence of black precipitate (this phenomenon and its causes have been described by many authors). When the azo dye coupling method was applied, the control preparations remain unstained. That is why the investigations were mainly based on results obtained by the latter method while the modified Gomori method was used only as an auxiliary procedure.

The presence of acid phosphatase in the pollen tubes of all 17 plant species examined and in the pollen grains of *Campanula* was established.

The data obtained prove that spherosomes are the main site of activity of this enzyme. It was found namely, that acid phosphatase activity occurs only in the small grains present in the usually colourless cytoplasm. The reactions applied to the investigated material gave no other precipitate and only in some sections there occured a diffuse staining of the cytoplasm. The grains correspond to spherosomes in dimensions, shape and distribution in the cytoplasm. Their size in the pollen tubes of each species corresponds to the specific size of the spherosomes (Plate I). In young pollen tubes they are entirely spherical (Plate I, Figs. 1, 3) while in older ones they have irregular shapes of sickles, crescents or rings; thus they correspond to the shapes of deformed spherosomes (Plate I, Fig. 9).

The distribution of stained grains indicating the site of activity of acid phosphatase in the various development stages of the *Campanula* pollen corresponds exactly to the distribution of spherosomes (compare Plate II, Figs. 1—3 with Figs. 4—6). Just like spherosomes, in young pollen, they are dispersed in the cytoplasm or joined in groups of two or three. In pollen grains containing agglomerations of spherosomes (elaioplasts) — the stained sites of activity of acid phosphatase reveal their shape and granular structure.

The tips of young pollen tubes which in intravital observation showed no presence of spherosomes were also devoid of acid phosphatase. The remaining cytoplasmic organelles, the vacuoles and the generative cell (Plate I, Fig. 10), remain colourless. Neither has any final product of the enzymic reaction been noticed in the larger lipid spherules. Beside spherosomes, only some unidentified minute granules reacted positively to acid phosphatase; their presence was also noted in living pollen tubes. In all the objects no precipitate occurred in the nutritive media on which the pollen tubes were growing.

Plate I

Acid phosphatase in spherosomes of pollen tubes
Azo-dye coupling method, Fast Blue RR Magn. *c.* 1350 ×
1. Hyacinthus orientalis; *2. Betonica officinalis*; *3. Haemanthus Katharinae*; *4. Vinca minor*; *5. Gentiana pneumonanthe*; *6. Viola tricolor*; *7. Tradescantia bracteata*; *8. Antirrhinum maius; 9. Bryonia dioica* (acid phosphatase localized in characteristically deformed spherosomes); *10. Asparagus officinalis.*

Plate II

Spherosomes of *Campanula* after treatment with osmium tetroxide and after enzymic reactions
a) — in young pollen grains, agglomerated in "elaioplasts" (column I); b) — in ripe pollen grains, after dissociation of elaioplasts (column II); c) — in pollen tubes (column III)
1—3 — pictures obtained after fixing in OsO_4; *4—6* — acid phosphatase, coupling method, Fast Blue RR; *7—9* — non-specific esterase, coupling method, Fast Blue RR; *10—12* — DNA-ase detected by method of Aronson et al. in. Vorbrodt's modification. Magn. 1300 ×

Plate III

Non-specific esterase in pollen tubes:
1. Bryonia dioica — indoxyl method, *2. Hyacinthus orientalis* — coupling, method, *3. Gentiana pneumananthe* — coupling azo dye method, *4. Papaver californicum* — coupling azo dye method. Magn. 1300 ×
Alkaline phosphatase in pollen tubes:
5. Campanula persicifolia. 6. Bryonia dioica. 7. Vinca minor 8. Tropaeolum majus. Magn. 1300 ×

Plate I

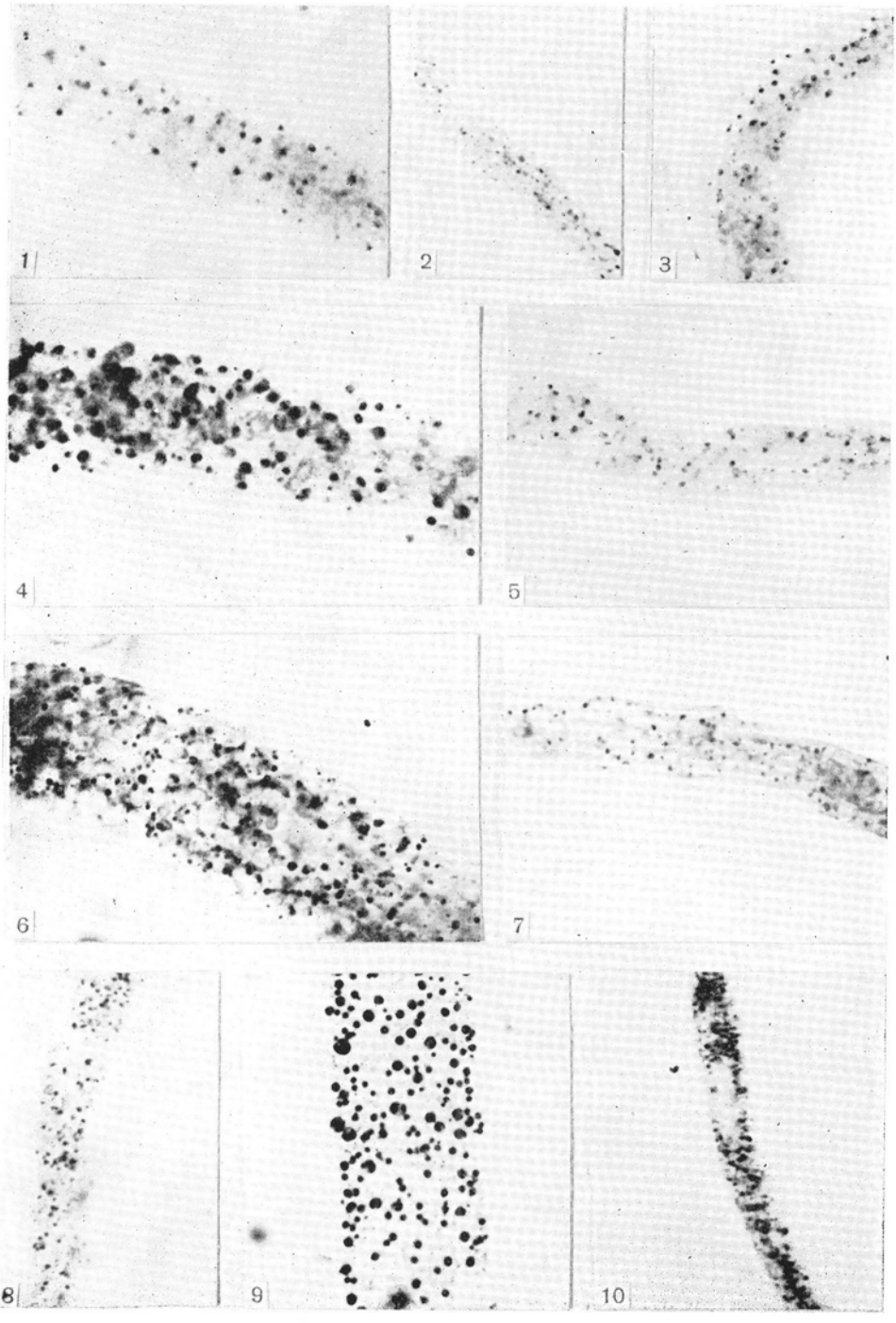


Plate II

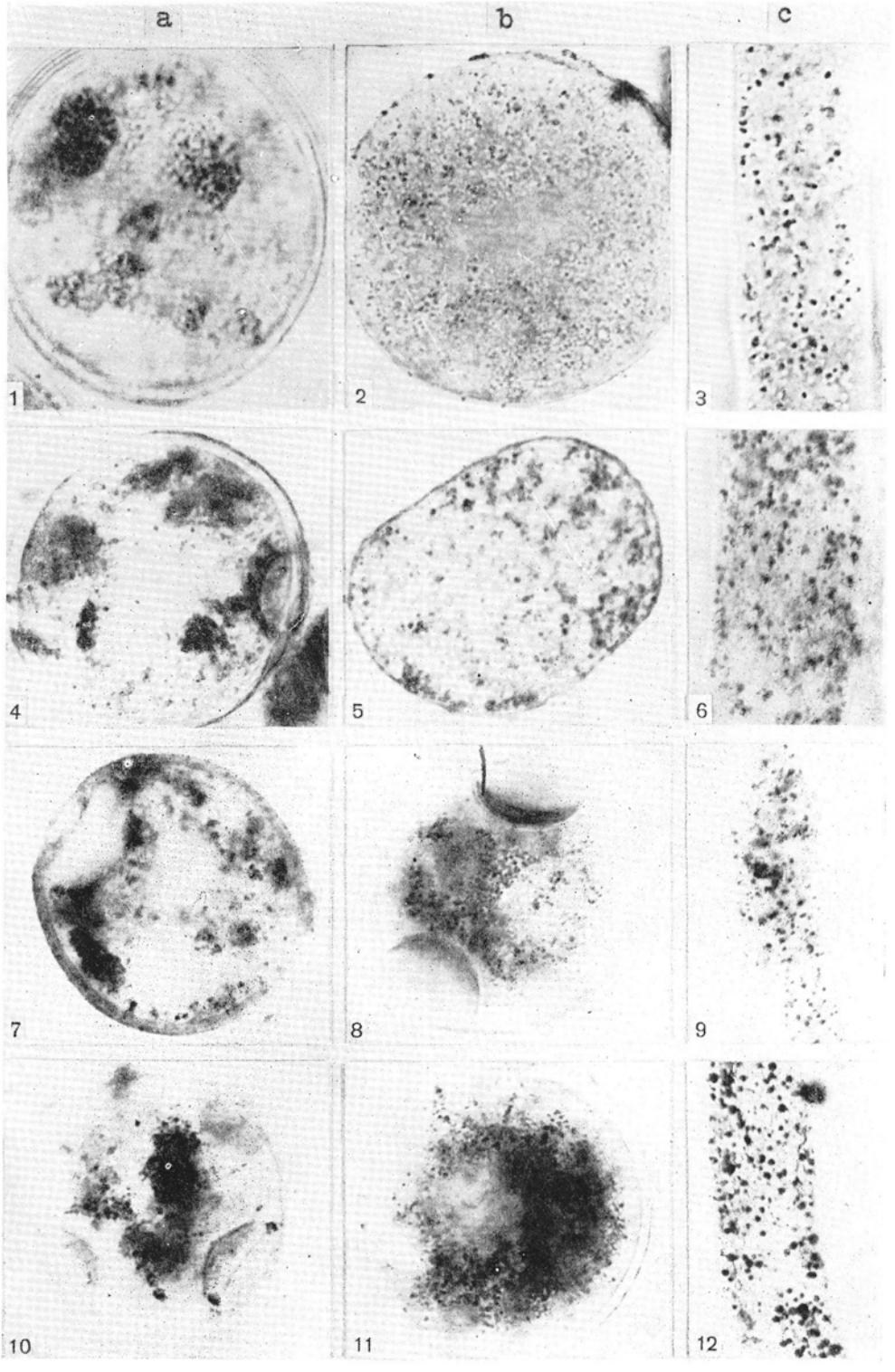
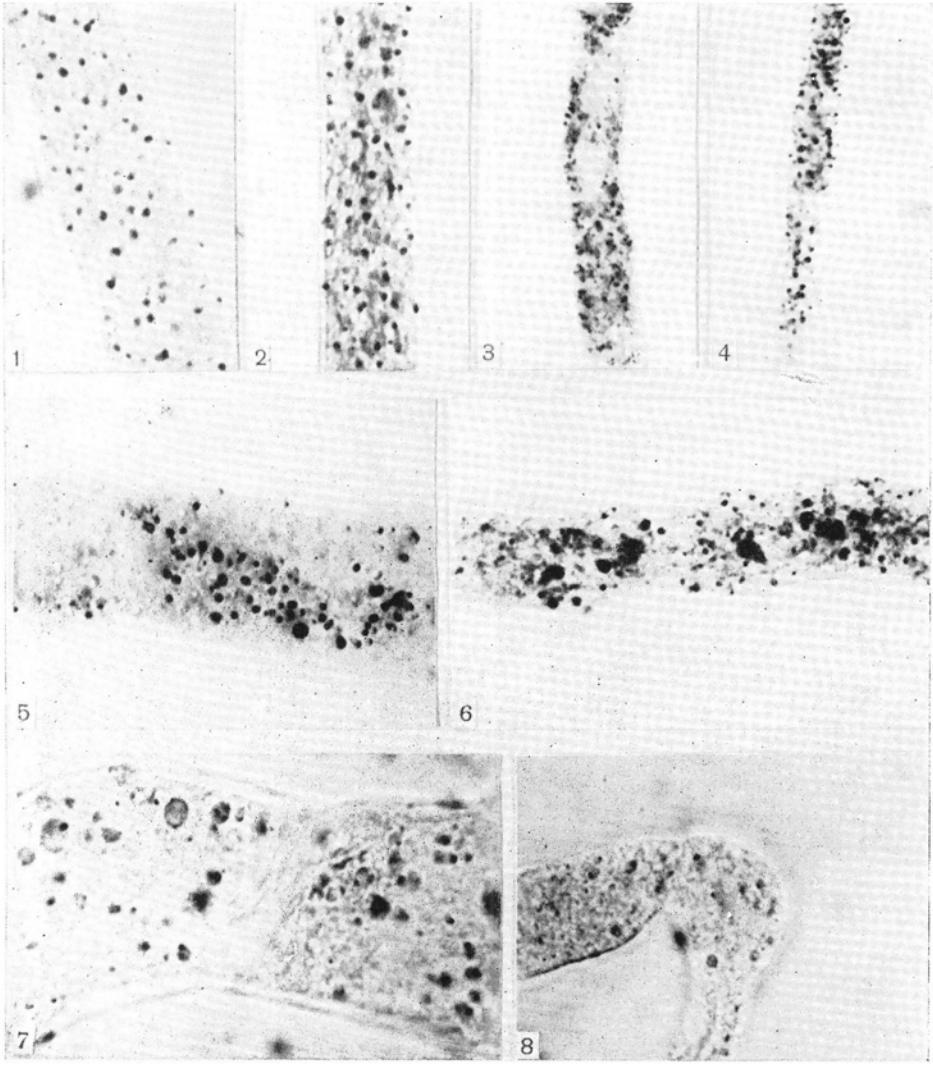


Plate III



Alkaline phosphatase

The occurrence of alkaline phosphatase in pollen tubes, in contrast to acid phosphatase, was not general. Alkaline phosphatase activity could be established in 13 plant species. In three of the investigated species, namely: *Viola tricolor*, *Tradescantia bracteata* and *Tradescantia virginiana* in which the activity of acid phosphatase had been previously established, no alkaline phosphatase was detected.

Alkaline phosphatase in pollen tubes was localized in a slightly different way than acid phosphatase.

Most of the spherosomes remained unstained and the site of alkaline phosphatase activity was found to be in the granules of various sizes, sometimes quite large, ellipsoidal in shape, dispersed in the cytoplasm or forming agglomerations (Plate III, Figs. 5 and 6). In some tubes the enzyme was localized characteristically in small granules inside the usually oval vacuoles (Plate III, Figs. 7 and 8).

In the control preparations no coloured granulations were visible in the pollen tubes.

Non-specific esterase

Both methods used to detect non-specific esterase gave the same results, but the indoxyl method proved less convenient in the case of the material examined. In some cases, beside the uniformly blue staining of the grains, due (according to the method's assumptions) to indigo particles, there appeared large crystals of indigo in the cytoplasm. The crystals, however, did not always form; they occurred regularly and in rather large numbers in the pollen tubes of *Vinca minor*; in *Bryonia* and *Campanula* they appeared now and then, while the pollen tubes of *Tradescantia* did not contain any. The presence of indigo crystals was most frequently observed in older tubes. It is difficult to establish for certain the cause of this phenomenon. It seems, however, that it is connected with the large amount of fat in the tube. The pollen tubes of *Vinca minor* contain numerous lipid droplets, those of *Bryonia* and *Campanula* are poorer in fats, while in the tubes of *Tradescantia*, larger lipid drops do not generally occur. The same relation might to true here as that observed in animal tissues tested by this method (P e a r s e 1961).

Preparations treated by simultaneous coupling azo dye method with the use of α-naphthyl acetate as substrate gave clear and unequivocal pictures.

The activity of non-specific esterase like that of acid phosphatase was localized in granules corresponding in size to spherosomes and in

minute unidentified granules. The number of the latter was considerable, particularly in young pollen tubes. The shape, size and distribution of the stained grains left no doubt that they correspond to spherosomes (Plate II, Figs. 7—9); (Plate III, Figs. 1—4).

Other hydrolytic enzymes

The results of investigations on lipase localization in pollen grains and tubes proved unsatisfactory. The repeatedly performed reactions resulted in staining of the control sections. It is true that a certain difference could be noticed in the colour intensity of the experimental preparations and the control sections but it was not so marked as to allow certain conclusions.

The detection of deoxyribonuclease by the method of Aronson et al. modified by Vorbrodt (1961) gave somewhat better results. Control sections incubated in a medium devoid of the DNA substrate showed a certain staining due, maybe, to the presence of free phosphate groups in the cells; but the difference in the colour intensity was distinct.

Thus it seems probable that deoxyribonuclease occurs in pollen grains and tubes.

The size and distribution of the black granules corresponding to the size and distribution of spherosomes may prove it (Plate II, Figs. 10—12).

Preliminary reactions carried out on a small quantity of material, for localization of arysulphatase and β-glucuronidase, have shown that the activities of those enzymes are also localized in the spherosomes of pollen tubes.

DISCUSSION

Though spherosomes occur in very large numbers in the pollen and particularly in pollen tubes of many plant species, they have not been investigated thoroughly. The only data concerning them are found in Steffen's observations on pollen tubes of *Galanthus nivalis* (1953).

The results of the author's present observations, obtained from extensive material have confirmed the opinion of numerous authors that spherosomes are a permanent component of plant cells. Spherosomes always occurred in pollen grains and tubes. Most spherosomes in young, growing pollen tubes exhibit a constant size, characteristic of the given species, what is in agreement with Perner's (1954) and Sorokin's (1955) data.

Beside spherosomes of sizes characteristic of the given species, and also slightly larger or smaller ones, in the cytoplasm of all the specimens

examined, minute (almost at the limit of visibility) granules were observed. In the living cell they refract light so strongly that sometimes they seem quite black. They give the same enzymic reactions as the spherosomes.

The occurrence of small granules was also reported by other authors (Strugger 1949; Perner 1952, 1953; Jarosch 1961; Wałek-Czernecka 1962) and others. Drawert (1953) referred this granulation to mitochondria. But in view of the present results of enzymic reactions, the opinion of Frey-Wyssling (1963) seems more plausible; like Jarosch (1961), he believes that these granules are either the initial stage of spherosomes or young spherosomes.

An interesting problem connected with spherosomes is their ability of agglomerating temporarily in bodies which I designate as spherosomal elaioplasts. The reason of the formation of spherosomal elaioplasts and of all the other bodies known as elaioplasts (Kwiatkowska 1962) is not known so far. Spherosomes in the agglomerates — elaioplasts — exhibit the same morphological features, the same optical properties and the same set of hydrolytic enzymes as the spherosomes dispersed in the cytoplasm. Acid hydrolases have previously been found in the elaioplasts of *Malvaceae* by Wałek-Czernecka (unpublished data) and recently by Kwiatkowska and Stęplewski (1965).

The results of enzymic investigations presented in this work have confirmed the observations of Wałek-Czernecka (1962, 1963, 1965) concerning the localization of hydrolytic enzymes in spherosomes. The spherosomes of pollen grains and pollen tubes similarly as those of the onion scale epidermis have proved to be the site of acid phosphatase, non-specific esterase, arylsulphatase, β-glucuronidase and probably deoxyribonuclease.

The occurrence of alkaline phosphatase in pollen and pollen tubes, in contrast to other hydrolases, was not general. In three of the sixteen investigated species the presence of this enzyme could not be demonstrated. The lack of alkaline phosphatase in pollen tubes of some species has also been described by Haeckel (1951) and Schlösser (1961).

It has not been established so far, whether the absence of alkaline phosphatase in pollen tubes is a permanent or temperary phenomenon. Neither has it been possible to determine the exact localization of this enzyme.

Alkaline phosphatase occurred only in some spherical bodies corresponding to spherosomes in size, it was also present outside the spherosomes in the form of irregular granules, disks and agglomerates. It is not excluded that the few spherical granulations exhibiting alkaline phos-

phatase activity are actually dictyosomes (Kuff and Dalton 1959; Kwiatkowska and Stęplewski 1965; Gabara 1965; Palczewska 1965; Olszewska et al. 1965).

In that case, among the granular bodies with acid phosphatase activity dictyosomes might also be found. For it is a well-known fact that acid phosphatase has been described in the structures of the Golgi apparatus (Nowikoff and Goldfisher 1961; Wachstein et al. 1961; Poux 1963; Olszewska et al. 1965).

Attempts to localize the activity of lipase in pollen grains and tubes have not been successful.

The unsatisfactory or unreliable results of the enzymic reactions are due to the fact that the methods based on the Gomori technique cannot always assure a correct interpretation of the pictures obtained. This is difficult, on the one hand, owing to the possibility of adsorption of lead ions from the incubation medium on various cellular structures, and on the other hand, on account of the presence of phosphate groups in many of the specimens examined. Owing to these facts a black precipitate of lead sulphide can frequently be observed in the control preparations. It is true that some authors advise in such cases to "read" the colour difference between the control and the experimental preparation (Petrovskaja-Baranova and Zinger 1962) or to interrupt the incubation at the "proper moment" (Jensen 1956). It seems, however, that in view of the individual differentiation of cells and tissues, such proceedings might be the source of numerous errors.

That is why I consider the results concerning the localization of deoxyribonuclease only as probable.

In the literature dealing with the occurrence of hydrolases in the plant cell, acid phosphatase occupies an important place. To date this enzyme has been found by histochemical methods in a number of tissues and cells, among them also in pollen grains and pollen tubes. It should be noted here that acid phosphatase is (beside alkaline phosphatase) the only described hydrolytic enzyme found in pollen grains and tubes by histochemical methods (Haeckel 1951, Schlosser 1961; Petrovskaya-Baranova and Zinger 1962). The few remaining hydrolases described in pollen, such as lipase (Paton 1921, for review see: Lundén 1954) were revealed exclusively by biochemical or physiological methods.

According to many authors, small spherical or irregular granules or sometimes rods have proved to be the most frequent site of acid phosphatase. But most authors have made no attempt of a more precise cytological localization of acid phosphatase and of relating it with definite organelles. Jensen (1956), Wilson and Cutter (1955)

and Avers and King (1960) associated the occurrence of acid phosphatase with mitochondria. Later investigations by Wałek-Czernecka (1962) have shown, however, in a conclusive way that mitochondria are devoid of this enzyme.

It would seem that the pictures of histochemical localization of acid phosphatase, reported by other authors do not contradict the conclusion that spherosomes are the main site of this enzyme. For instance the distribution, in the pollen tubes, of granules exhibiting acid phosphatase, described by Anges Haeckel (l.c.) and Schlösser (l.c.) is identical with the distribution of spherosomes. The pictures illustrating the localization of this enzyme in meristematic cells, presented by Dyar (1950), Beneš et al. (1961), Olszewska, et al. (1963, 1964) may correspond to the distribution of spherosomes.

The difficulty of identifying spherosomes in the demonstration of acid phosphatase is due — as it seems — to the fact of deformation of spherosomes, on the one hand, and on the other — if the Gomori method is exclusively used — to the localization of the enzyme (frequently difficult to interpret).

The present observations confirm and also explain some data of Haeckel (1951) concerning the localization of acid phosphatase in pollen tubes, severely criticized by Petrovskaya-Baranova and Zinger (1962). Haeckel emphasized the fact that acid phosphatase gives no reaction in the top segment of the pollen tube, but occurs in a more distant part where it penetrates from the pollen grain. This phenomenon became understandable when it was established that spherosomes are the carriers of acid phosphatase, when — transported by cytoplasmic currents — they pass from the pollen grain to the tube, never reaching its top during the growth of the tube. That is why in well fixed pollen tubes, the acid phosphatase is never present in their top segments. The enzyme together with the spherosomes reaches the top of the pollen tube only when its growth ceases or during its degeneration.

I have never observed any penetration of acid phosphatase into the environment of the pollen tube, as suggested by Petrovskaya-Baranova and Zinger (1962). This confirms Haeckel's opinion (1951) that acid phosphatase represents a "closed", intracellular enzyme.

I wish to express my deep gratitude to Professor Anna Wałek-Czernecka for her helpful guidance during the accomplishement of the present work.

SUMMARY

Intravital observations of pollen grains and pollen tubes of 34 plant species with particular consideration of spherosomes have been carried out.

In a part of the material the localization of the following hydrolases was

investigated: acid and alkaline phosphatase, non-specific esterase, lipase, deoxyribonuclease, β-glucuronidase and arylsulphatase.

The results indicate that these hydrolases are present in pollen grains and tubes of all the plant species investigated. The only exception is alkaline phosphatase, its occurrence not being general. The activity of acid phosphatase, non-specific esterase, arylsulphatase, β-glucuronidase and probably deoxyribonuclease is localized in the spherosomes of pollen grains and tubes. The localization of alkaline phosphatase has not been exactly established.

The author did not observe penetration of acid phosphatase into the environment of the tube as described in the literature.

Spherosomes occurring in agglomerates — elaioplasts — appear to have the same morphologic features and optical properties and the same set of hydrolytic enzymes.

The top zone of young, growing pollen tubes is devoid of spherosomes. Cytoenzymic investigations have shown no presence of acid phosphatase in this zone.

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Hydrolazy w ziarnach i łagiewkach pyłkowych

Streszczenie

Przeprowadzono obserwacje przyżyciowe ziaren i łagiewek pyłkowych 34 gatunków roślin ze szczególnym uwzględnieniem sferosomów.

W części materiału badano zlokalizowanie następujących hydrolaz: kwaśnej fosfatazy, zasadowej fosfatazy, esterazy niespecyficznej, lipazy, dezoksyrybonukleazy, β-glukuronidazy i arylsulfatazy.

Uzyskane wyniki wykazały, że hydrolazy te są obecne w ziarnach i łagiewkach pyłkowych wszystkich badanych gatunków roślin. Wyjątek stanowi jedynie zasadowa fosfataza, której występowanie nie okazało się powszechne.

Aktywność kwaśnej fosfatazy, esterazy niespecyficznej, arylsulfatazy, β-glukuronidazy i prawdopodobnie dezoksyrybonukleazy zlokalizowana jest w sferosomach ziaren i łagiewek pyłkowych. Lokalizacja fosfatazy zasadowej nie została dokładniej ustalona.

Nie obserwowano podawanego w literaturze zjawiska przenikania kwaśnej fosfatazy do środowiska otaczającego łagiewki.

Sferosomy występujące w aglomeratach — elajoplastach wykazały te same cechy morfologiczne, właściwości optyczne oraz ten sam zespół enzymów hydrolitycznych.

Wierzchołkowa strefa młodych, rosnących łagiewek pyłkowych pozbawiona jest sferosomów. Badania cyto-enzymatyczne nie wykazały również w tej strefie obecności kwaśnej fosfatazy.