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Inorganic polyphosphates in pollen tubes

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Until quite recently inorganic polyphosphates were known only in some groups of lower plants like bacteria (Wiame 1947; Ebel 1952a; Sall et al. 1955), algae (Ebel 1952a, Ebel et al. 1958b; Albaum et al. 1950; Stich 1953; Keck and Stich 1957; Epply 1962), fungi (Mann 1945; Wiame 1946; Ebel et al. 1958c, Palczewska 1964) and mosses (Keck and Stich 1957).

The first data of the appearance of polyphosphates in higher plants were reported by Eschrich (1962, 1963), and Tewari and Singh (1964).

In the course of my studies on callose the presence of inorganic polyphosphates in the pollen tubes of *Hyacinthus orientalis* L., *Begonia semperflorens* L. and *Aquilegia vulgaris* L. has been revealed.

MATERIAL AND METHODS

The pollen tubes were grown in vitro in a medium of agar and sucrose.

The inorganic polyphosphates were detected by methods based on such properties of these compounds as their precipitation by lead salt at low pH (PbS reaction according to Ebel et. al. 1958 b) and their faculty of metachromatic staining with toluidine blue (TB reaction according to Keck and Stich 1957).

Both types of reaction were checked by treating the pollen tubes: a) with 1 N HCl resulting in hydrolysis of polymerized inorganic polyphosphates to orthophosphates, and b) with 10% cold trichloroacetic acid giving total extraction of these compounds.

The PbS reactions, as well as the TB reaction were always negative in control preparations.

Positive PbS reaction in conjunction with the metachromatic reaction allow, according to Ebel et al. (1958 b), to state with certainty the presence of inorganic polyphosphates.

Parallel tests were made with yeast cells (*Saccharomyces cerevisie* var. *elipsoideus*), because owing to the extensive investigations of Wiame (1947, 1948, 1949), Ebel (1952, 1958) and many other investigators, yeast cells represent a classical comparative material for checking the reactions for polyphosphates.

RESULTS

In the material under examination inorganic polyphosphates appeared as a rule in older pollen tubes, in the second or third hour of their growth. Only in a few cases their presence in younger pollen tubes, or in mature pollen grains could be notice.

At first inorganic polyphosphates appear exclusively in the cytoplasm; toluidine blue, when used as vital dye, visualizes them in the form of loosely dispersed or agglomerated pink granules carried away by cytoplasm currents. Identical structures located in the cytoplasm are seen after the PbS reaction, which stains them brown or black (Fig. 1.).

Simultaneously with the process of formation of callose, inorganic polyphosphates appear within the callose structures of pollen tubes.

Now and again I have noted inorganic polyphosphates, of the appearance of homogeneously stained zones, in some callose spheres in the cytoplasm. In the callose layers lining the inner wall of pollen tubes, inorganic polyphosphates can be observed as granulated or diffusely coloured streaks parallel to the callose layers (Fig. 2).

In constituted callose plugs, the inorganic polyphosphates form as a rule, a homogeneously stained centre (Fig. 4, 5), but the arising callose plugs show generally granular agglomerations of polyphosphates (Fig. 3).

Thus the inorganic polyphosphates were revealed in two ways by the PbS and the TB reactions: as homogeneous staining and as agglomerated granules. There were also rather distinct differences in the degree of staining: from brown to black in the PbS reaction and from light pink to red when stained with toluidine blue.

It seems that the differences in the degree of staining, as well as the varying shapes of the polyphosphates, also observed in animal material (Przełęcka 1960), may be due to a different degree of polymerization of the polyphosphates (vide Ebel and Müller 1958 a).

Since the polyphosphates are highly energetic compounds, they are believed to store phosphorus and energy which is used in so important process as RNA synthesis in yeast cells and bacteria (Wiane 1949; Sall et al. 1956; Harold 1963; Harold and Sylvan 1963) or in the photosynthesis of autothrophic plants (Keck and Stich 1957; Vagabov and Serenkov 1963).

Polyphosphates abounding in the cytoplasm of pollen tubes in the period of its maximum elongation would presumably be the source of the energy used in the process of growth.

The part played by polyphosphates enclosed in the insoluble callose layers of pollen tubes is difficult to understand and requires further study.

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Plate I

Hyacinthus orientalis

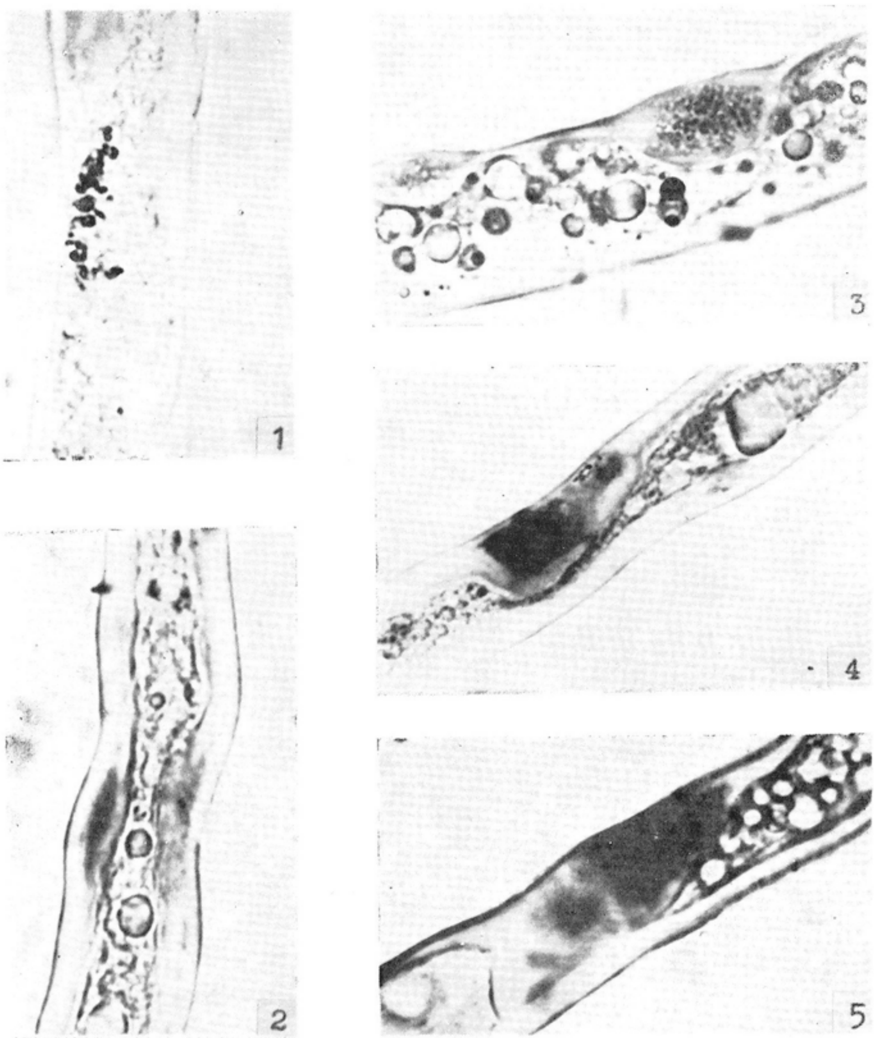

Fig. 1. Inorganic polyphosphates in the cytoplasm of young pollen tube. PbS reaction. Magnific. × ± 1400

Fig. 2. Diffusely coloured polyphosphates in the callose layers which line the inner wall of pollen tube. PbS reaction. Magnific. × ± 1300

Fig. 3. Granular agglomerations of polyphosphates in the arising callose plugs. TB reaction. Magnific. × ± 1400

Fig. 4, 5. Inorganic polyphosphates in the callose plugs of older pollen tubes. PbS reaction. Magnific. × ± 1400

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Polifosforany nieorganiczne w łagiewkach pyłkowych

Streszczenie

Stwierdzono występowanie polifosforanów nieorganicznych w łagiewkach pyłkowych *Hyacinthus orientalis* L., *Begonia semperflores* L. i *Aquilegia vulgaris* L. W pierwszym okresie wzrostu łagiewki pyłkowej polifosforany nieorganiczne występują wyłącznie na terenie cytoplazmy (Fig. 1). W miarę postępowania procesu tworzenia się kalozy, związki te zjawiają się również wewnątrz utworów kalozowych łagiewki (Fig. 2—5).