Transitory callose envelope surrounding the generative cell in pollen grains (Communication)

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The character of the border zone separating the generative cell from the pollen vegetative cytoplasm remains obscure. The opinion dominating for many years that a plasmatic membrane surrounds the generative cells has been confirmed in observations under an electron microscope. Some investigators report that the generative cell is surrounded by a plasmatic membrane (Larson 1963, 1965), whereas others claim that a wall-like structure or cell wall occurs around it (Diers 1963; Sassen 1964).

The present report describes the transitory occurrence of callose around the newly developed generative cell of *Chlorophytum comosum*, *Chlorophytum elatum*, *Hyacinthus orientalis* *Tradescantia bracteata*, and *Impatiens Balsamina*.

Methods. The presence of callose was detected by two methods assumed as specific for this substance. The first consisted in staining with resorcinol blue and was employed either for non-fixed material or for the material fixed in 5 per cent glutaraldehyde. The second was the fluorescence method in which an aqueous solution of aniline blue was applied (Eschrich and Currier 1964). The results of both methods were consistent. Acid phosphatase was detected by the modified Gomori's method and by the azo-dye coupling method (Pearse 1961).

Results. In all the plant species investigated callose appeared around the generative cell at an early stage of its development. At this period, the lens-like generative cell is parietally situated, its cytoplasm is separated by a distinct arch from the remaining pollen cytoplasm, the vegetative nucleus is near the generative cell and is separated from the latter by a thin layer of cytoplasm. The process of callose formation starts in the central part of the arched border line delimiting both the differentiating cells. Later the callose gradually appears in further parts of this zone and reaches the walls of the pollen grain (Figs. 1, 2 and 4). For a certain period the callose envelope of the generative cell is absent on the side adjacent to the pollen wall (Figs. 2 and 4). The callose envelope closes around the generative cell in somewhat older pollen grains in which this cell shifts towards the grain centre and acquires a spherical shape (Fig. 5). Soon the callose disappears and the generative cell acquires a spindle-like shape.

The thickness of the callose layer surrounding the generative cell is difficult to determine owing to the spherical shape of the cell within the non-sectioned pollen

grains. Measurements, performed in non-fixed or fixed in 5 per cent glutaraldehyde grains stained with resorcinol blue indicate that the callose layer is 1.5–2.5 μ thick. The callose layer is thinner at the beginning as at the latest stage, and this seems in keeping with the process of its formation and disappearance. Most frequently, the callose forms a continuous, smooth layer, whereas sometimes a granular or scale-like structure was observed.

The callose envelope is present for only a very short period (probably over a dozen hours), therefore it is observed in few pollen grains in anthers in which the development of these grains is not synchronized. In the investigated plant material the "callose stage" was best distinguishable in the generative cells of *Chlorophytum*. In these anthers callose envelopes were sometimes observed in over 80 per cent of the pollen grains derived from one anther.

The activity of acid phosphatase particularly strong around the generative cell is associated with the occurrence of the callose envelope (Figs. 6 and 7). It is noteworthy that acid phosphatase occurs at the sites where callose is present. An increased activity of acid phosphatase before the callose plugs in the pollen tubes was observed also by Haeckel (1951) and Schlösser (1961). It seems that callose metabolism is connected with the activity of this enzyme.

The fact of periodical appearance and disappearance of callose in the developmental cycle of normal active cells is known in the literature. Here belongs the occurrence of callose in the pollen mother cells and in the microsporocytes (Eschrich 1964; Waterkeyn 1964) as well as in the developing phloem and pericyclic fibres, preceding the lignification process (Górská-Brylass and Smoliński 1966). The transitory occurrence of callose around the generative cells is one more example pointing to the physiological role of this substance in plant cells.

Two equivalent nuclei, which later differentiate otherwise, are formed after the first mitosis in the pollen. It is possible that the isolation of the parietal cell with

Explanation to plate

Figs. 1—2. Arch-like curved callose layer surrounding the generative cell of *Chlorophytum elatum* (Fig. 1) and *Chlorophytum comosum* (Fig. 2). Staining with resorcinol blue. Magn. ca 1250 $\times$.

Fig. 3. Fluorescence of callose envelopes surrounding generative cells of *Chlorophytum elatum* pollen. Fluorochrome-aniline blue. Magn. ca 450 $\times$.

Fig. 4. Arched callose layer surrounding one side of generative cell adjacent to the pollen wall seen in a fluorescence microscope. *Chlorophytum elatum*. Fluorochrome-aniline blue. Magn. ca 800 $\times$.

Fig. 5. Closed callose envelope around generative cell of *Chlorophytum elatum* seen in a fluorescence microscope. Fluorochrome-aniline blue. Magn. ca 800 $\times$.

Fig. 6. Acid phosphatase surrounding generative cell of *Hyacinthus orientalis* pollen. Azo-dye coupling method. Fast Blue RR. Magn. ca 1200 $\times$.

Fig. 7. Acid phosphatase surrounding generative cells of *Chlorophytum elatum* pollen. Azo-dye coupling method. Fast Blue RR. Arrow points to vegetative nucleus. Magn. ca 1250.

a small amount of cytoplasm by the callose wall from the vegetative cytoplasm rich in energetic substances is an important element conditioning the different course of development of these nuclei.

The varying development courses of the latter initiated at the early period of isolation remain different after the disappearance of callose and finally lead to the formation of two cells with differing functions.

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SUMMARY

The temporary short-lasting appearance of a callose envelope surrounding the newly developed generative cell was observed in pollen grains of *Chlorophytum comosum*, *Chlorophytum elatum*, *Hyacinthus orientalis*, *Impatiens Balsamina* and *Tradescantia bracteata* (Figs. 1—5). An increased activity of acid phosphatase around the generative cell was connected with the period of occurrence of the callose envelope (Figs. 6—7).

The supposition is advanced, that the callose temporarily isolating the generative cell from the vegetative cytoplasm, may play a certain role in the differentiation of the generative nucleus in pollen grains.

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STRESZCZENIE

W ziarnach pyłkowych *Chlorophytum comosum*, *Chlorophytum elatum*, *Hyacinthus orientalis* oraz *Tradescantia bracteata*, wykazano krótkotrwałe, przejściowe występowanie otoczki kalozonej wokół nowo powstałej komórki generatywnej (Fig. 1—5). Z okresem występowania otoczki kalozonej jest związana wzmożona aktywność kwaśnej fosfatazy wokół komórki generatywnej (Fig. 6—7).

Wysunięto przypuszczenie, że kaloza izolująca okresowo komórkę generatywną od cytoplazmy wegetatywnej, może odgrywać rolę w różnicowaniu się jądra generatywnego w ziarnach pyłkowych.