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Ultrastructural and metabolic transformations of differentiating *Hyacinthus orientalis* L. pollen grain cells. I. RNA and protein synthesis

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Abstract

RNA and protein synthesis were investigated in generative and vegetative cells during maturation of pollen grains. The rate of RNA and protein synthesis was analysed in reference to the successive interphase periods of the life cycle of pollen cells as well as against the background of the growth dynamics of the cell volume. The results of studies demonstrated that the pollen grain increases in size owing to the growth of the vegetative cell. The generative one does not grow. RNA synthesis and that of proteins in differentiating pollen cells has a different course. In the growing vegetative cell it lasts longer and is more intensive than in the generative cell which does not grow. RNA and protein synthesis in the vegetative cell take place in the period from the callose stage to the stage of lemon-shaped generative cell, that is in the period of phases G_1, S and G_2. This synthesis is positively correlated with the growth of the pollen grain. RNA and protein synthesis in the generative cell comprises the period from the callose-less lenticular stage to the stage of spherical generative cell, that is the phases S and early phase G_2. These results suggest that in the vegetative cell RNA and protein synthesis is utilised above all to increase of its cell, instead in non growing generative cell protein synthese is probably limited mostly to a histones and enzymatic proteins serving for the DNA replication process.

Key words: pollen cells differentiation, RNA-protein synthesis.

INTRODUCTION

The development of the pollen grain consisting of two cells with different biological functions — a generative one destined for fulfilling the reproductive function, and the vegetative one fulfilling a somatic function — creates a model situation for investigations on cell differentia-

tion. Pollen grain maturation is composed, namely of a series of metabolic and structural transformations of both pollen cells, which take place in an almost closed system formed by the thick sporodermis layer. Metabolic studies on the differentiation of pollen grain were focussed on the finding of differences in the DNA amount in the nuclei of sister pollen cells and differences in the course of RNA and protein synthesis in these cells. The results of these investigations showed that pollen cells may differ in the amount of nuclear DNA (among others: Bryan 1951, Taylor 1953, Rodkiewicz 1960, Charzyńska and Maleszka 1978, Thieboud and Ruch 1978), the appearance of these differences is not, however, general (D'Amato et al. 1965).

RNA and protein synthesis in pollen grain undergoes changes during maturation of the latter. According to the results of biochemical studies of *Tradescantia paludosa* (Mascarenhas and Bell 1970, Peddada and Mascarenhas 1972, 1975, Mascarenhas 1975) RNA synthesis is most intensive in the pollen grain in the period following immediately microspore division. In the further stages of pollen grain maturation the rate of this synthesis decreases up to its complete cessation a short time before anthesis. The cytochemical investigations on *Lilium* and *Crocus* (Jalouzot 1969 a, b) and *Peonia* (Sauter and Marquardt 1967) showed an increase of the general quantity of RNA during pollen grain maturation. The results of cytochemical, cytophotometric and autoradiographic studies of *Lilium* (Jalouzot 1969a) cytophotometric studies of *Tradescantia* (Thieboud and Ruch 1978), cytochemical and autoradiographic studies of *Paeonia* (Sauter and Marquardt 1967, Sauter 1969) indicate that RNA and protein synthesis are much higher in the vegetative than in the generative cell.

The results of up-to-date investigations have so far not been analysed in reference to the successive interphase periods of the life cycle of pollen cells and the dynamics of their growth.

The aim of the present investigations was to gain a knowledge of the course of RNA and protein synthesis in the successive morphological developmental stages of pollen grain against the background of the interphase periods and dynamics of growth of the differentiating pollen grains. For these studies pollen grain of *Hyacinthus orientalis* was chosen, in which the morphological stages were earlier established and DNA replication occurs in both the pollen cells (Bednarska 1981).

MATERIAL AND METHODS

The material for study consisted of *Hyacinthus orientalis* L. pollen grains of the diploidal variety Pink Pearl. The investigations were performed in seven successive morphologically defined stages shown sche-

matically in Fig. 1. In each successive development stage the volume of the pollen grain and pollen cells was measured. These measurements were performed on living material placed in 2 per cent isotonic sucrose solution. For calculating the pollen grain volume the formula for the sphere was applied, with mean length and breadth of pollen grain as diametre of the sphere:

$$V=\frac{1}{6}\pi\left(\frac{d_1+d_2}{2}\right)3,$$

where: d_1 — grain length, d_2 — grain breadth.

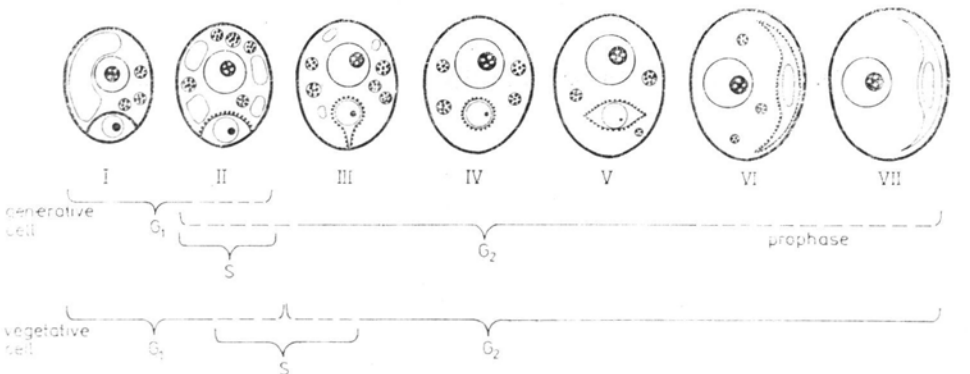

Fig. 1. Development stages of *Hyacinthus orientalis* L. pollen grains, marked interphase periods of pollen cells (after Bednarska 1981)
Stage I — lenticular generative cell surrounded with callose wall (callose stage), stage II — lenticular generative cell deprived of callose wall (lenticular callose-less stage), stage III — balloon-shaped generative cell, stage IV — spherical generative cell, stage V — lemon-shaped generative cell, stage VI — fusiform generative cell surrounded by granules, stage VII — fusiform generative cell without wreath of granules

For calculating the pollen cell volume a spatial model of each stage was established, the shape of which was taken into account in the calculations. The volume of the lenticular generative cell in stages I and II (Fig. 2) situated beside the wall was calculated as the sum of two spherical bowls according to the formula:

$$V=\frac{1}{2}\pi r^2 n_1+\frac{1}{6}\pi h^3+\frac{1}{2}\pi r^2 h_2+\frac{1}{6}\pi h_2^2.$$

The volume of the belloon-like generative cell in stage III (Fig. 3) was calculated as the sum of volumes of a sphere and a cone according to the formula:

$$V=\frac{3}{4}\pi r_1^3+\frac{1}{3}\pi r_2^2 h.$$

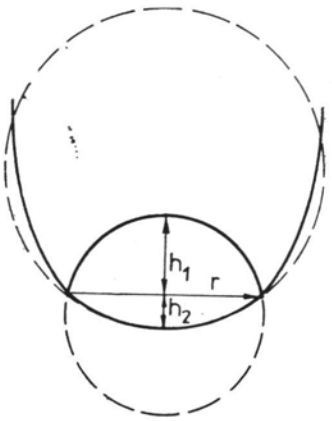

Fig. 2. Model of lenticular generative cell situated beside the wall

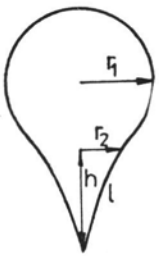

Fig. 3. Model of balloon-shaped generative cell

The volume of the spherical generative cell in stage IV was calculated according to the formula for the sphere:

$$V=\frac{4}{3}\pi r^3.$$

In the lemon-shaped generative cell of stage V (Fig. 4) three geometric figures were distinguished: a barrel and two cones. The cell volume was calculated from the formula:

$$V=\frac{2}{5}\pi h_1(r_2^2+2r_1^2)+\frac{2}{3}\pi r_2^2 h.$$

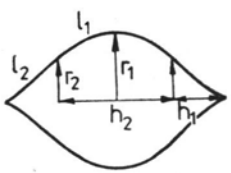

Fig. 4. Model of lemon-shaped generative cell

The volume of the generative cell in stages VI and VII was calculated as the sum of five geometric figures: a barrel, two truncated cones and two cones (Fig. 5) by the formula:

$$V=\frac{\pi}{3}h_1(r_2^2+2r_1^2)+2\frac{\pi h_2}{3}(r_2+r_2r_3+r_3^2)+2\left(\frac{1}{3}\pi r_3^2h_3\right).$$

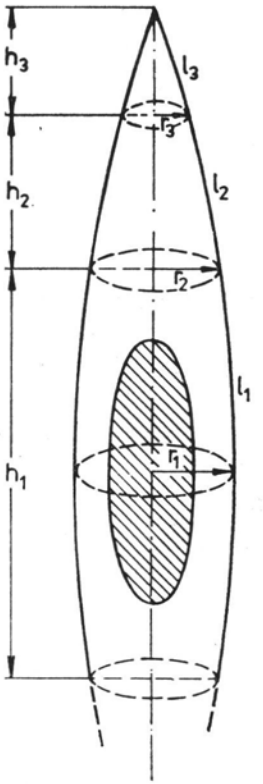

Fig. 5. Model of fusiform generative cell

The vegetative cell volume consisted of the difference between the pollen grain volume and that of the generative cell.

For following the RNA and protein synthesis in pollen cells the autoradiographic method was used with the application of RNA and protein synthesis precursors (^{3}H-uridine) and (^{3}H-leucine) respectively. The isolated anthers in successive development phases were placed in an incubation mixture containing ^{3}H-uridine (spec. act. 20 000 mCi.mM^{-1}) or ^{3}H-leucine (spec. act. 20 000 mCi.mM^{-1}) in a 50 μCi.cm^{-3} concentration in a 2 per cent sucrose solution. The incubation time was 2 h and was shorter than the duration of the successive morphological developmental stages of the pollen grains under natural conditions. The material was fixed in 10 per cent formalin in phosphate buffer, pH 7.0 at +4°C. The

paraffin preparations were coated with Ilfort L-4 emulsion. The exposure time was for ^{3}H-uridine 65 days and for ^{3}H-leucine 62 days.

After developing and fixing the autoriadiograms were stained with methyl green and pyronin (UNNA) after Brachet (1953). The number of traces over 30 nonserial cross sections of pollen grains was calculated in the successive developmental stages, taking into account the cytoplasm, nucleus and nucleolus in both pollen cells. The results were statistically elaborated by Student's t test.

The presence of a callose wall was tested by the fluorescence method according to Eschrich and Currier (1964).

RESULTS

CHANGES IN POLLEN GRAIN AND POLLEN CELL VOLUMES DURING POLLEN RIPENING

In the period of pollen grain maturation that is in the period from the division of the microspore to anthesis, the volume of the pollen grain increases twofold, from about 25 000 μm^3 to about 58 000 μm^3 (Fig. 6). Growth is most intensive between stages I and IV, in further stages it is less conspicuous.

The generative cell volume does not undergo significant changes throughout the whole period of pollen grain differentiation, its volume

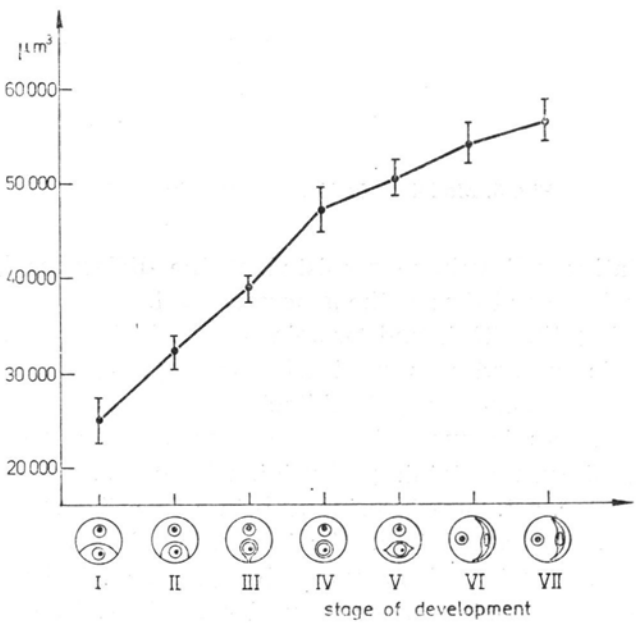

Fig. 6. Volume of pollen grain in successive stages of its differentiation

amounts to about 1600 μm^3 (Fig. 7). The increase in pollen grain volume occurs owing to the increase in size of the vegetative cell. The volume of the latter increases from about 24 000 μm^3 in stage I to about 56 000 μm^3 in the stage of anthesis, thus the increase is more than twofold.

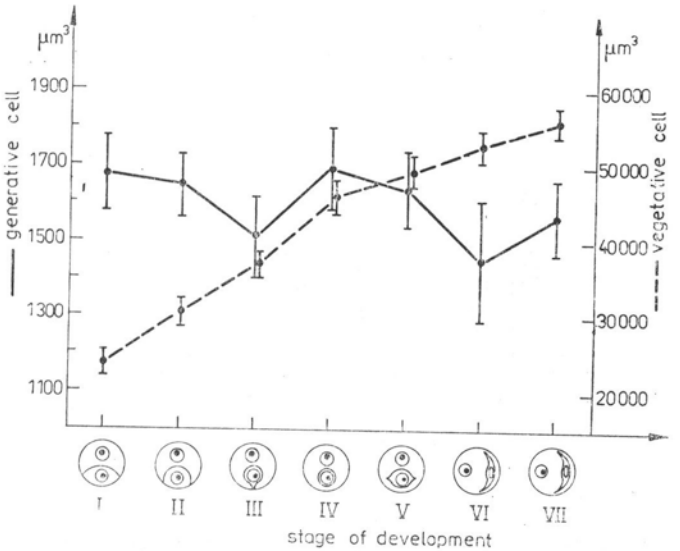

Fig. 7. Generative and vegetative cell volumes in successive stages of pollen grain differentiation

^{3}H-URIDINE INCORPORATION

Generative cell

Traces of ^{3}H-uridine appear over the nucleus and cytoplasm in the generative cell in stage II of development, that is after disintegration of the callose wall and they are visible for three successive stages up to the spherical generative cell inclusively (Fig. 8). The largest number of traces of ^{3}H-uridine over the nucleus and cytoplasm appear in stage II. In further stages of development a decrease of the number of traces of radioactive uridine was observed. ^{3}H-uridine incorporation into the nucleolus of the generative cell was not noted.

Vegetative cell

The number of ^{3}H-uridine traces over the vegetative cell is much larger than that over the generative one (Fig. 11). Traces of radioactive uridine are visible over the nucleus, nucleolus and cytoplasm of the vegetative cell for the first five developmental stages that is from the callose stage to that of lemon-shaped generative cell stage inclusively

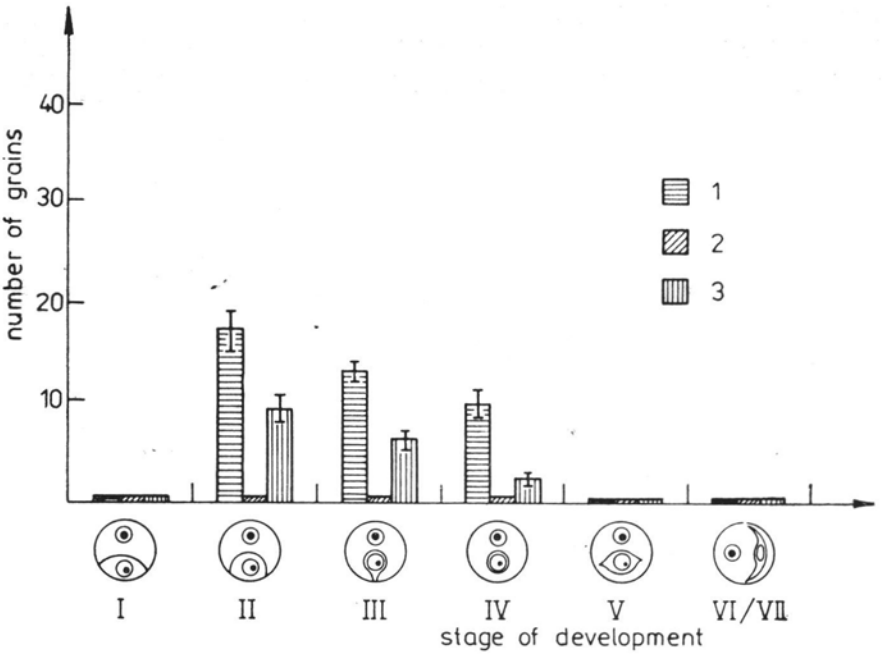

Fig. 8. ^{3}H-uridine incorporation into generative cell in successive stages of pollen grain differentiation, 1 — nucleus, 2 — nucleolus, 3 — cytoplasm

(Fig. 9). An increase of the ^{3}H-uridine incorporation rate was observed in the nucleus from stage I to II. The number of ^{3}H-uridine traces in stage III was similar, while in further stages a decrease of intensity of this precursor incorporation was noted. Over the nucleus of the vegetative cell in stage VI/VII traces of ^{3}H-uridine were very scarce. Labelling of the vegetative cell nucleolus was very intensive. A maximal number of traces over this structure was noted in stages II and III of development. The vegetative cell nucleolus in stage VI/VII did not show traces of ^{3}H-uridine. Labelling of the vegetative cell cytoplasm increases from stage I to II and remains at a similar level through stage III. In further stages the number of ^{3}H-uridine traces diminishes. In the pollen grain with a fusiform generative cell no traces of radioactive uridine were observed over the vegetative cell cytoplasm.

^{3}H-LEUCINE INCORPORATION

Generative cell

^{3}H-leucine incorporation into the generative cell cytoplasm takes place as late as stage II of development (Fig. 10). In stage I, that is in the period when the callose wall is present, radioactive leucin is not incorpora-

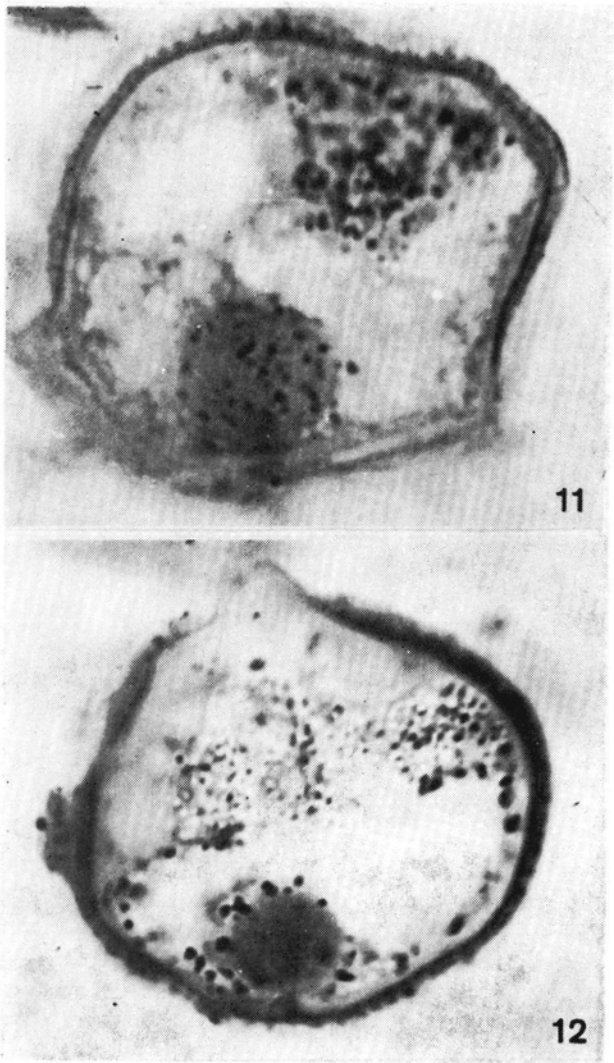

Fig. 11. ^{3}H-uridine incorporation into *Hyacinthus orientalis* pollen grain in lenticular callose-less stage, × 1200

Fig. 12. ^{3}H-leucine incorporation into cytoplasm of generative cell in lenticular callose-less stage, × 1200

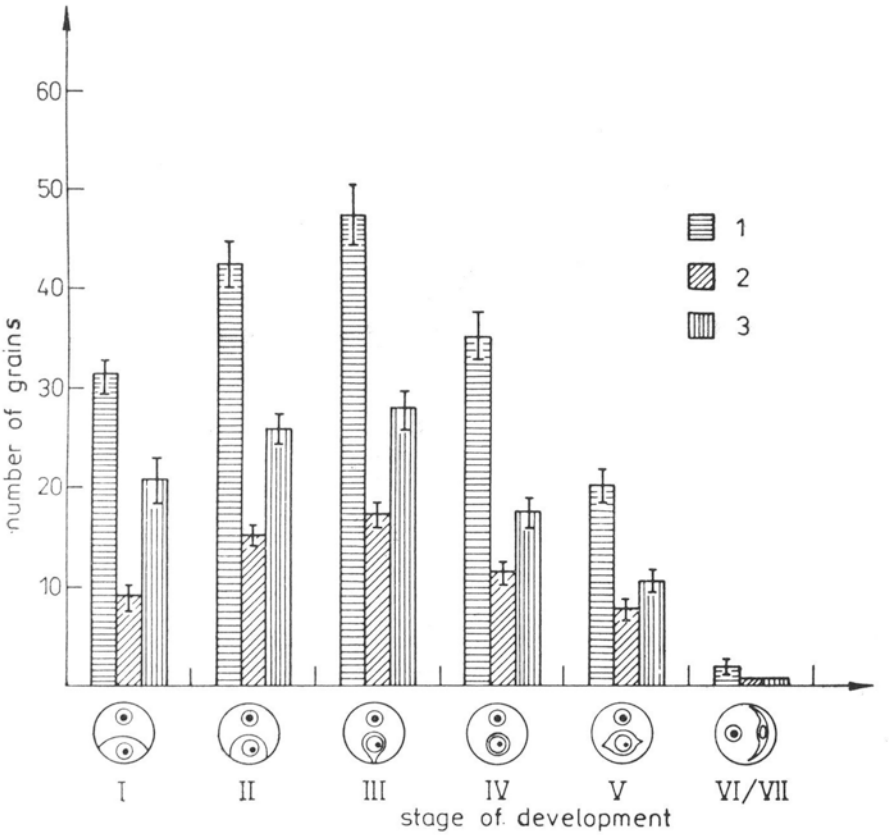

Fig. 9. ^{3}H-uridine incorporation into vegetative cell in successive stages of pollen grain differentiation, 1 — nucleus, 2 — nucleolus, 3 — cytoplasm

ted. Traces of ^{3}H-leucine over the cytoplasm and nucleus were observed in the period from stage II to stage IV of development, that is in the period when the generative cell assumes a spherical shape. The maximal number of ^{3}H-leucine traces was noted over the cytoplasm and nucleus in developmental stage II (Fig. 12). At later times of development the number of traces over the generative cell decreases to complete disparition over the lemon-shaped and fusiform cells.

The vegetative cell

Traces of ^{3}H-leucine were visible over the nucleus and cytoplasm of the vegetative cell in the first five stages of development from the callose to the lemon-shaped cell stage inclusively (Fig. 13). The number of radioactive leucine traces over the vegetative cell was markedly higher than that over the generative one. Maximal ^{3}H-leucine incorporation into the vegetative cell cytoplasm was noted in the period from stage II

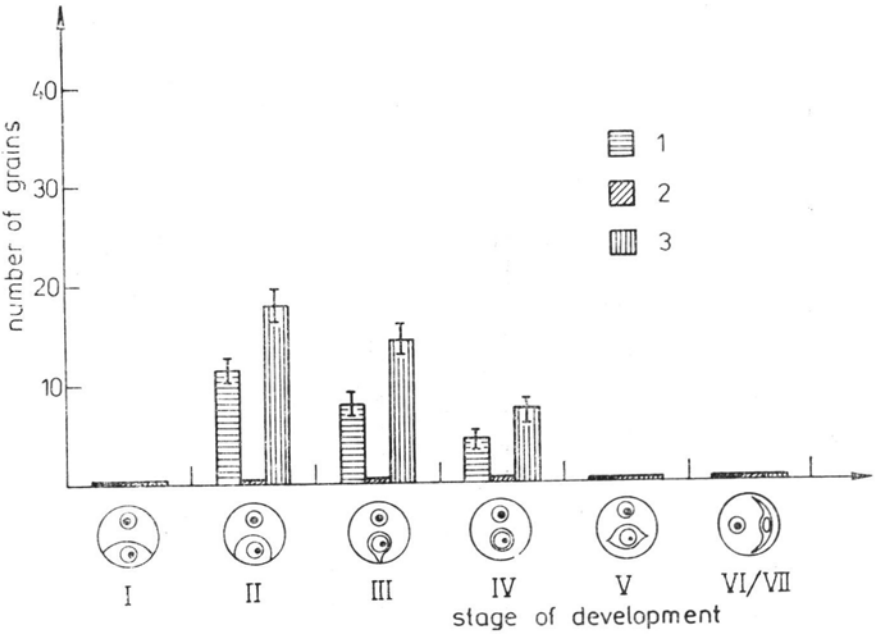

Fig. 10. ^{3}H-leucine incorporation into generative cell in successive stages of pollen grain development, 1 — nucleus, 2 — nucleolus, 3 — cytoplasm

to stage IV of development, whereas in further stages ^{3}H-leucine incorporation decreased. In the pollen grains with the fusiform generative cell there were no traces of ^{3}H-leucine over the cytoplasm and nucleus of the vegetative cell.

DISCUSSION

Autoradiographic studies with the use of ^{3}H-uridine and ^{3}H-leucine demonstrated that in the pollen grain of *Hyacinthus orientalis* RNA and protein synthesis is much intensive in the initial stages its maturation. In further stages its synthesis decreased to complete decline in the mature pollen grain. The observed dynamics of RNA and protein synthesis in the pollen grain of *Hyacinthus orientalis* is in agreement with the biochemical studies on *Tradescantia paludosa* (Mascarenhas and Bell 1970, Peddada and Mascarenhas 1972, 1975, Mascarenhas 1975) and also with the results of cytochemical, cytophotometric and autoradiographic investigations on *Lilium candidum* and *Crocus longiflorus* (Jalouzot 1969a, b) and *Paeonia tenuifolia* (Sauter 1969, Sauter and Marquardt 1967) which indicated an increase of the total amount of RNA during pollen grain maturation.

In the vegetative *Hyacinthus orientalis* cell intensive RNA and protein synthesis last from the callose stage to that of the lemon-shaped

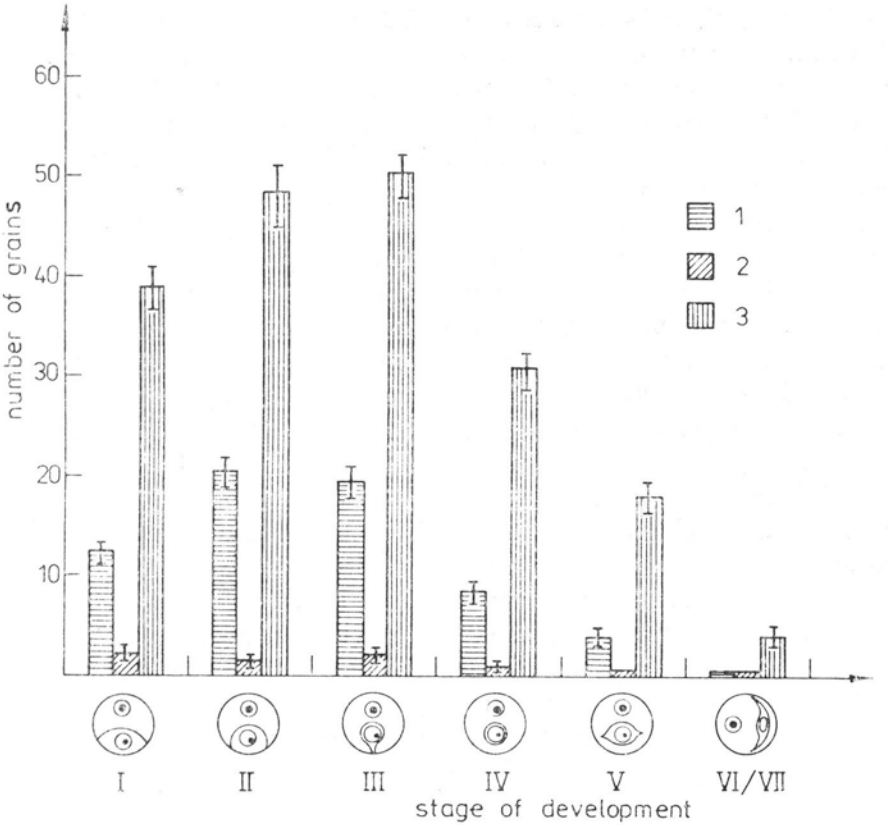

Fig. 13. ^{3}H-leucine incorporation into vegetative cell in successive development stages of pollen grain: 1 — nucleus, 2 — nucleolus, 3 — cytoplasm

generative cell and are inhibited only a short time before anthesis, thus synthesis occurs in phases G_1, S and G_2 of the interphase (B e d n a r s k a 1981). In the generative cell, however, noticeable processes of RNA and protein synthesis appear only as late as the lenticular generative cell is devoid of callose wall last only to the stage of spherical generative cell inclusively, they are, therefore, limited mainly to phase S and early phase G_2. Peak activity in RNA and protein synthesis in the generative cell falls to the lenticular callose-less stage, thus to the period when DNA replication occurs (B e d n a r s k a 1981).

The differences concerning the period of RNA and protein synthesis and intensity of this synthesis in *Hyacinthus orientalis* pollen cells, when compared with the analysis of their increase in volume reflect the developmental pathways of the generative and vegetative cells. It results from this confrontation that in the vegetative cell RNA and protein synthesis are not only a preparation of this cell to the development of the pollen tube, as demonstrated on *Tradescantia paludosa* (M a s c a-

renhas et al. 1974, Frankis and Mascarenhas 1980), but it is also utilised to the growth of the vegetative cell which contributes to the increase of the pollen cell in volume (cf. Figs. 6 and 7). In this cell there is a positive correlation between the protein and RNA synthesis rate and the volume of the pollen grain. The most intensive pollen grain growth from the callose stage to that of spherical generative cell coincides with the high rate of RNA and protein synthesis in the vegetative cell. In the final developmental stages, where RNA and protein synthesis decrease, the growth of the pollen grain is slower (cf. Fig. 6 and Figs. 9 and 11). The above mentioned correlation seems to indicate that in the vegetative cell constitutive protein prevails among those synthesised, which are utilised for the growth of the vegetative cell.

RNA and protein synthesis were found to be less intensive in the generative than in the vegetative cell. In the former it is limited above all to phase S and early phase G_2. Confrontation of these data with the lack of growth of the generative cell (cf. Fig. 7 and Figs. 8 and 10) may be an indication that among the proteins synthesised in this cell a large part consists above all of histon proteins (Bednarska 1981) and also enzymatic ones serving for the DNA replication process.

A closer knowledge of the sequence of changes in the protein and RNA synthesis rate in the pollen grain of *Hyacinthus orientalis* may facilitate the understanding of the structural transformations which the differentiating pollen cells undergo.

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Ultrastrukturalne i metaboliczne transformacje różnicujących się komórek ziarna pyłkowego Hyacinthus orientalis L. I. Synteza RNA i białek

Streszczenie

Badano syntezę RNA i białek w komórce generatywnej i wegetatywnej *Hyacinthus orientalis* L. podczas kolejnych morfologicznych stadiów jego dojrzewania. Poziom syntezy RNA i białek analizowano w odniesieniu do kolejnych okresów interfazy cyklu życiowego komórek pyłkowych oraz na tle dynamiki wzrostu ich objętości. Wyniki badań wykazały, że ziarno pyłkowe rośnie dzięki wzrostowi komórki wegetatywnej. Komórka generatywna nie rośnie. Synteza RNA i białek w różnicujących się komórkach pyłkowych przebiega odmiennie. W rosnącej komórce wegetatywnej trwa ona dłużej i przebiega z większą intensywnością aniżeli w nierosnącej komórce generatywnej. Synteza RNA i białek w komórce wegeta-

tywnej odbywa się w okresie od stadium kalozowego do stadium cytrynokształtnej komórki generatywnej, tj. w okresie fazy G_1, S i G_2. Synteza ta jest pozytywnie skorelowana ze wzrostem ziarna pyłkowego. Synteza RNA i białek w komórce generatywnej obejmuje okres od stadium przyściennego bezkalozowego do stadium kulistej komórki generatywnej tj. okres S i wczesną fazę G_2. Na podstawie uzyskanych wyników wyraża się pogląd, że synteza RNA i białek w komórce wegetatywnej wykorzystywana jest przede wszystkim do wzrostu tej komórki, natomiast w nierosnącej komórce generatywnej służy głównie przygotowaniu aparatu genetycznego do wytworzenia gamet.