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Mitochondria during androgenesis in *Hordeum vulgare*

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Abstract

Different number of mitochondria of varying structure was observed in particular stages of the development of barley *(Hordeum vulgare)* microspores, stimulated by the *in vitro* culture to form embryoids. This variability was reflected in different shape of sections, different ratio between total area of mitochondria profiles and area of cytoplasm sections, varying number of cristae, and different density of the matrix. Within the cristae of some mitochondria crystalline inclusions were observed. Mitochondria divided by a contraction. In the matrix of some mitochondria spheric bodies were formed. They were surrounded by one or two membranes. It is suggested that the bi-membrane forms constituted promitochondria, whereas unimembrane forms could constitute promicrobodies.

INTRODUCTION

Barley microspores in an *in vitro* culture are able to develop into the embryoids (Clapham 1973, Wilson et al. 1978, Zenkteler 1976). Several stages may be distinguished in this process: from the first abnormal mitoses (Idzikowska et al. 1979) most frequently leading to the formation of multinucleate pollen grains, through the stage of cell wall formation by their centripetal growth from the intine side (Idzikowska and Młodzianowski 1979), to multicellular embryoids and calluses from which plants regenerate (Zenkteler 1976). Although the plants originate from a haploidal microspore, only part of them retains the n number of the chromosomes. It is very difficult to obtain green haploidal plants this way because in most cases we deal with albinotic individuals which are of no real value for further experiments and cultures.

It seemed that it would be interesting to present the behaviour of plastides and mitochondria during various stages of androgenesis, as in

normal embryo development the cytoplasm of the egg cell is the main donator of these organella. In the present work changes of mitochondria are discussed from the stage of microspore to the stage of a multicellular embryoid.

MATERIAL AND METHODS

Anthers of *Hordeum vulgare* var. Alsa originated from plants grown in green-houses. Some anthers containing pollen in the stage of uninucleate microspore were preserved as an initial material, other were cultured on the medium of M u r a s h i g e and S k o o g (1962), with an addition of 8% saccharose which induces the process of androgenesis. After 3 days of culture the anthers contained pollen in the form of a 1- or 2-cellular microspore. At longer culturing pollen developed into some- or several-cellular embryoids. Fragments of the middle part of anthers were fixed in a 6% glutaraldehyde buffered with 0.1 M cacodylate (pH 6.8) for 18 h in 4°C, washed for 1 h in 4 baths of cacodylate buffer, and fixed again in 2% OsO_4 in 0.1 M cacodylate buffer for 2 h. Staining was performed with a 2% solution of uranyl acetate for 1 h, dehydrated in a series of ethanol, acetone, and propylene oxide, and immersed in Epon 812.

Preparations were cut on an ultramicrotome LKB, and the sections were stained again with a solution of uranyl acetate and lead citrate (R e y n o l d s 1963, V e n a b l e and C o g g e s h a l l 1965). Observations were carried out using an electron microscope (Jeolco, type 7A).

Number of the mitochondria and surface of their profiles were assessed on the basis of 10 different individuals in each stage. Analyses were made of 5 different sections originating from each of the 10 individuals. Measurement error was 3-5%.

RESULTS AND DISCUSSION

In the microspores constituting the initial material, mitochondria were rather numerous (Table 1) and possessed very dense matrix (Figs. 1, 15). On the background of dark matrix swollen hollows were visible in the internal mitochondrial membrane.

After 3 days of culture some pollen grains in the anther were still in a 1-nucleate stage, but most were already 2-nucleate or 2-cellular. In the uninucleate pollen, just prior to the first mitotic division, mitochondria possessed less dense matrix, and hollowings of the internal membrane were less swollen. Minute osmophilic globules were present in the matrix (Fig. 2).

In the 2-cellular pollen, structure of the mitochondria did not differ, being similar to the initial material, independently of the fact whether the cells resulted from an asymmetric division (generative cell and vegetative cell) or from an abnormal symmetric division (Fig. 9). In the generative cell there were significantly less mitochondria than in the vegetative cell (Fig. 9, Table 1).

Table 1

Number of mitochondria and their profiles area in different stages of androgenesis

Stage of microspore development	Average number of mitochondria per 100 μm² of cytoplasm section area	Average section area of 1 mitochondrium, μm²	Area of mitochondria profiles on the section of cytoplasm, %
Unicellular microspore, initial material	70 (34-161)*	0.08 (0.01-0.22)	5.6
Vegetative cell after 3 days of culture	48 (12-123)	0.19 (0.03-0.75)	9.1
Generative cell after 3 days of culture	11 (3-20)	0.28 (0.06-0.78)	3.1
Embryoids (stages 4-20 cellular)	43 (15-115)	1.07 (0.29-2.20)	46.0

* In brackets: the highest and the lowest values noted.

In the multicellular pollen (embryoids) number of mitochondria per unit of area was lower than in the microspore (Table 1). In young, a few-cellular embryoids, mitochondria profiles were usually circular (Fig. 10), in the older, 20- and more cellular ones, mitochondria profiles were usually ring-like (Fig. 8) and lobe-like (Fig. 11). Small number or even total lack of any cristae was a most characteristic feature of mitochondria sections at this stage (Figs. 7, 8, 10, 11). Matrix of these mitochondria was very loose, with visible nucleoid-like areas. Mitochondria had the largest (on the average) area of profiles compared to mitochondria at other stages, and the ratio between area of mitochondria profiles and cytoplasm area amounted to 46% (Table 1).

Assuming that the number of the mitochondrial cristae is directly proportional to active mitochondria (intensity of ATP synthesis) it may

Fig. 1. Mitochondria profiles of 1-nucleate microspore — initial material. 42000 ×
Fig. 2. Mitochondria profiles of 1-nucleate microspore after 3 days of *in vitro* culture. 17500 ×
Fig. 3-4. Spherical bodies with electron-dense matrix, formed within mitochondria. When the outer membrane brakes they pass into the cytoplasm. Fig. 3 — 62000 ×, Fig. 4 — 54500 ×
Fig. 5-6. Bodies with electron-dense matrix within the cytoplasm. Some of them had single membranes (Fig. 5), some — double (Fig. 6). Fig. 5 — 60000 ×, Fig. 6 — 42000 ×
Fig. 7. Mitochondrium of multicellular embryoid dividing by a contraction 15000 ×
Fig. 8. Ring-like mitochondrium profile (M) of a multicellular embryoid. 21000 ×

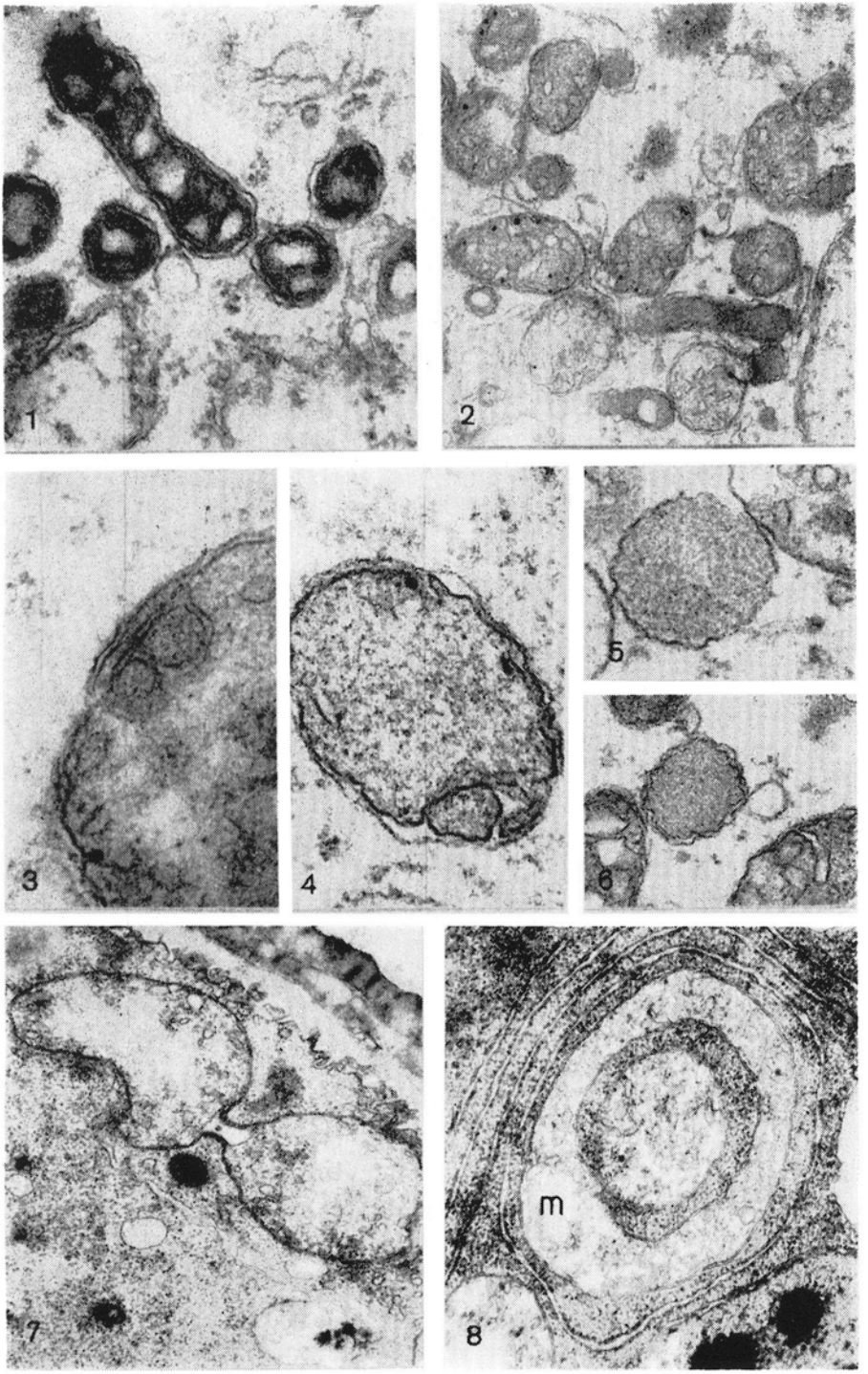
be stated that the mitochondria of multicellular embryoids are of low activity. On the other hand, large area of mitochondria sections in the cells at this stage, and large total area occupied by mitochondria sections compared to cytoplasm sections (Table 1) must result in large surface area of internal membranes which determines the respiratory process.

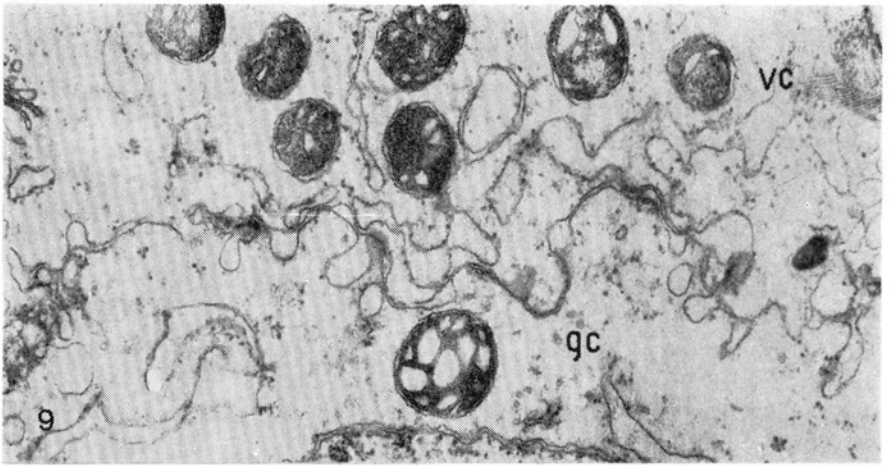
According to a hypothesis based on experimental data, dealing with configuration changes of the mitochondria (Hackenbrock et al. 1971), mitochondria of early stages of pollen development would correspond to the condensed form (dense matrix, swollen cristae). Such condensed configuration reflects the state of low energy, occuring in the presence of ADP. Only in some stages, just before the microspore division (Fig. 15) mitochondria of the orthodox type were present (light matrix, unswollen cristae), characterized by the state of high energy at low ADP level. Morphological similarity between isolated mitochondria in the experiment by Hackenbrock et al. (1971) and some mitochondria in *Hordeum* is quite significant. Nevertheless, its definition as a function may be an oversimplification. Kwiatkowska et al. (1980) have recently observed condensed type of mitochondria in the spermatogenic filaments of *Chara* after the divisions had stoped in prolonged darkness. These authors suggest that such picture of mitochondria may result from starvation caused by low level of substrates at high ADP level, relatively high mitochondrial activity in the process of oxidative phosphorylation and/or as a result of ion transport modifications. Innis et al. (1976) explain condensed state of mitochondria before fertilization of red urchin eggs by an increase of ADP/ATP.

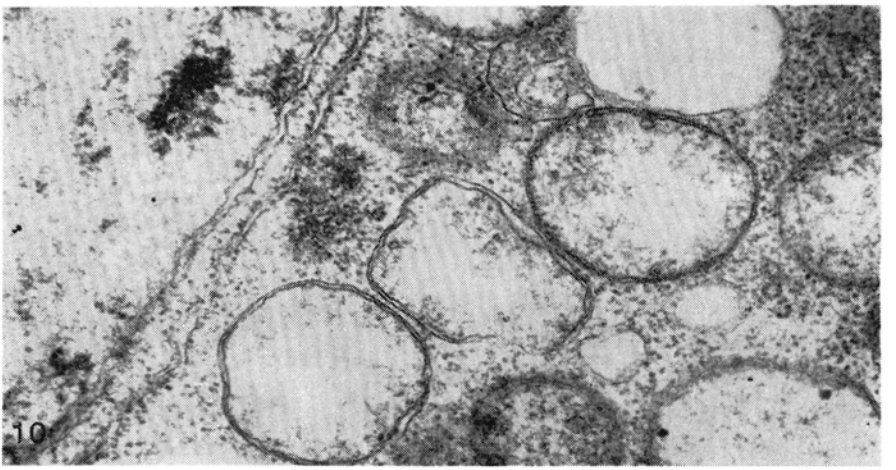
In the dividing cells of the embryoids, i.e. in the systems characterized by very high metabolism, only large mitochondria were observed, with light matrix and no cristae. Swelling of these mitochondria is similar to the IV stage of isolated mitochondria according to Chance and Williams (1955).

Although the structure of mitochondria observed during androgenesis in barley is certainly somehow adequate to their function, without

Plate I







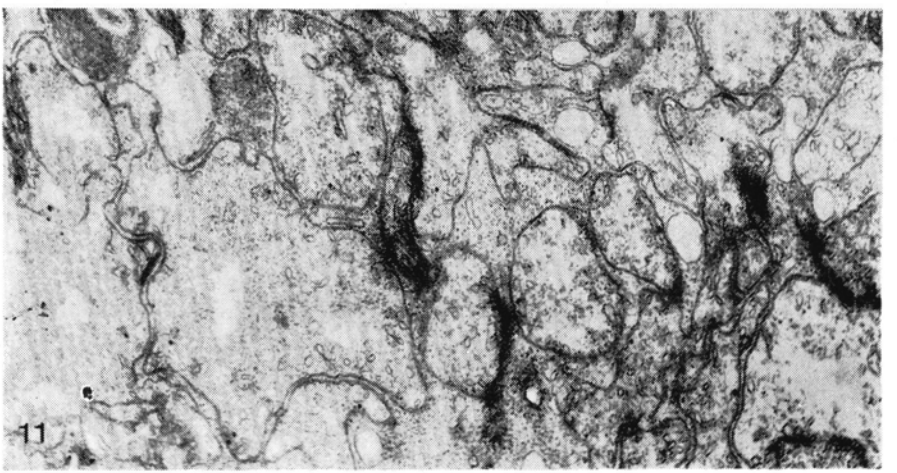


Fig. 9. Mitochondria of vegetative (VC) and generative (GC) cell. 23000 ×
Fig. 10. Mitochondria of a few-cellular embryoid. 30500 ×
Fig. 11. Lobe-like profiles of mitochondria of multicellular embryoid. 13500 ×

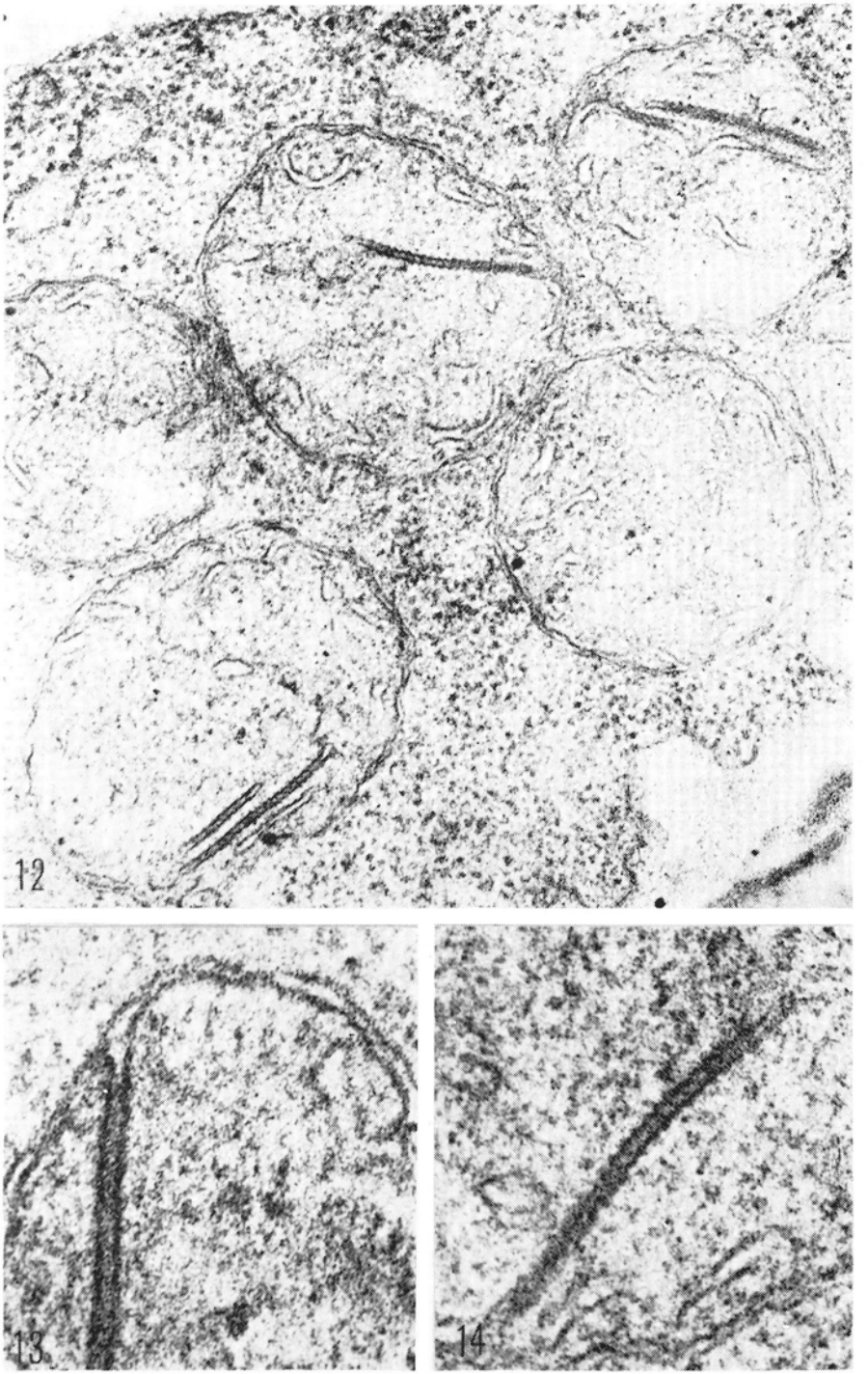


Fig. 12-14. Crystalline inclusions within the mitochondrial cristae. Fig. 12 — 53000 ×, Fig. 13 — 135000 ×, Fig. 14 — 104000 ×

Plate IV

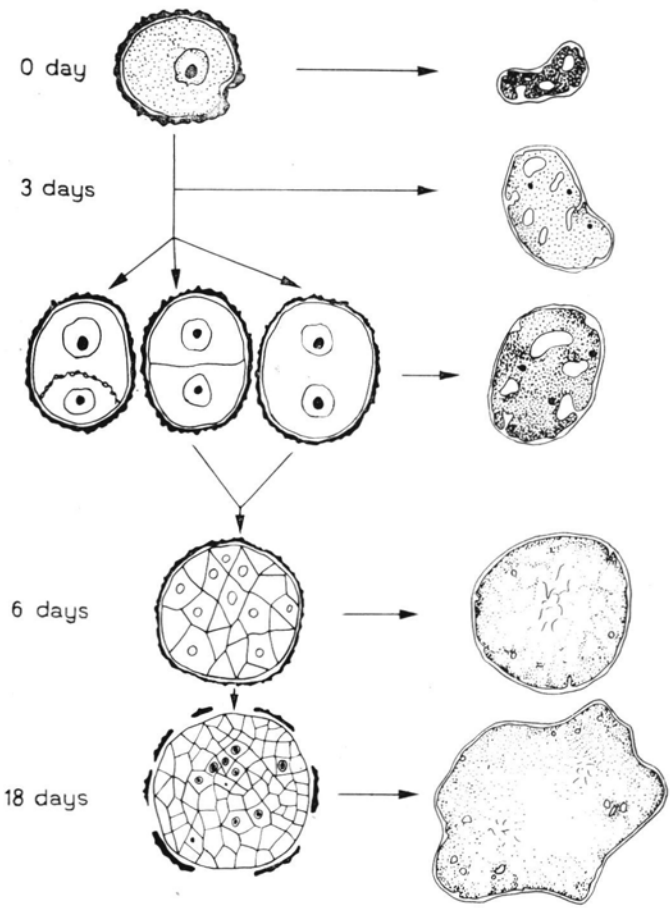


Fig. 15. Morphology of mitochondria in various stages of androgenesis

further biochemical studies it is difficult to ascribe any clear functional dependences to the morphology of these mitochondria. However, it is known beforehand that due to several reasons it is much more difficult to relate mitochondria structure to their functional state *in situ* than it is *in vitro* (Michejda 1973).

So far, ultrastructure of embryogenic pollen was studied only in *Nicotiana tabacum* (Vazart 1971, 1973, Dunwell and Sunderland 1974a, 1974b) and *Datura innoxia* (Norrel 1973, 1975, Dunwell and Sunderland 1976a, 1976b, 1976c, Sangwan-Norrel 1978). Comparison of the ultrastructure of mitochondria of androgenic pollen in *Hordeum* with the two *Solanaceae* (*Nicotiana* and *Datura*) shows that some similarities can be found, especially between *Hordeum* and *Nicotiana*. In both mitochondria possessed well developed cristae before and after the first haploidal mitosis, and in both they were similar in the generative and vegetative cell. On the other hand, in *Datura* number of mitochondrial cristae rapidly decreased after the first mitosis, and mitochondria in the vegetative cell were bigger. However, in *Datura*, similarly as in *Hordeum*, number of mitochondria was higher in the embryoids than in 2-cellular pollen (Sangwan-Norrel 1978). Divisions of mitochondria by a contraction (Fig. 7) were observed in all stages. Especially numerous divisions were noted before the first mitosis. In this stage some sections suggested possibility of a formation within the mitochondria of spherical bodies, enveloped in a membrane and with dense matrix (Figs. 3, 4). These bodies passed into the cytoplasm when the outer membrane broke (Figs. 5, 6). Further fate of these bodies is not known. Some of them had an envelope (two membranes) and it is possible that they were the promitochondria. Similar formation of promitochondria was suggested by van Steveninck and van Steveninck (1969). Unimembrane spherical bodies, with uniform matrix could — in my opinion — be some form of microbodies. In this case this suggestion was supported only by structural similarity. Nevertheless, since some enzymes are common for mitochondria and microbodies (Chollet and Ogren 1975, Beevers 1977), this possibility cannot be excluded, the more so that some further modifications of these forms are possible in a dynamic and abnormal system represented by embryogenic pollen grains.

Similar formation of both uni- and bimembrane bodies was observed in the plastides of cotyledons after the treatment with growth substances — cytokinins (Młodzianowski and Gazela 1974). Since the medium used for the culture of anthers contained also cytokinins, it can be supposed that there is some dependence between cytokinins and the formation of similar bodies in the plastides and mitochondria. However, it is difficult to define this dependence more precisely.

Formation of characteristic, spiral crystalline inclusions within the mitochondrial cristae was observed in various pollen stages, beginning from the third day of culture (Figs. 12-14). Formation of the inclusions was preceded by an elongation of the cristae and increase of membrane contrast (Figs. 12, 13). One mitochondrial profile never contained more than three cristae with the inclusions, and they were always placed parallely, close to each other. Similar crystalline inclusions were described in animal and plant mitochondria (Behnke 1965, Williams 1967, Chaly et al. 1974, Bonilla et al. 1975, Hanzlikova and Schiaffino 1977, Suzuki and Mostofil 1967). Modified cristae membranes and enclosed area are taken as being responsible for the formation of these inclusions (Newcomb et al. 1968, Tani et al. 1971, Nathaniel 1976). According to Hanzlikova and Schiaffino (1977) crystalline inclusions in the mitochondria of rat skeletal muscles are formed as a result of ischaemia. They are of protein, most probably enzymatic origin. Cytochemical studies showed that they are not cytochromes, but adenyline and creatine kinases which in case of local anemia can accumulate and polymerize in the cristae. It is not possible to define as yet what is the nature of these inclusions and causes of their formation in the mitochondria of androgenically developed barley embryoids.

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Mitochondria w czasie androgenezy u Hordeum vulgare

Streszczenie

W różnych stadiach rozwoju mikrospor jęczmienia *(Hordeum vulgare)* stymulowanych warunkami hodowli *in vitro* w kierunku tworzenia embrioidów obserwowano różną liczbę mitochondriów oraz ich zmienną ultrastrukturę. Zmienność ta wyrażała się różnym kształtem przekrojów, różnym stosunkiem ogólnej powierzchni przekrojów mitochondriów do powierzchni przekroju cytoplazmy, różną liczbą grzebieni i różną gęstością matriks. W obrębie grzebieni niektórych mitochondriów obserwowano krystaliczne inkluzje. Mitochondria dzieliły się przez przewężenie. W matriks niektórych mitochondriów tworzyły się obłonione, kuliste ciała. Były one otoczone jedną lub dwiema błonami. Przypuszcza się, że formy dwubłoniaste były promitochondriami, natomiast formy jednobłoniaste mogły stanowić promikrociała.