ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XXXIX – nr 1
1970

The effect of chloramphenicol on the ultrastructure of chloroplasts in the protonema of *Funaria hygrometrica*

F. MŁODZIANOWSKI, A. SZWEYKOWSKA AND J. SCHNEIDER

Chloramphenicol is an inhibitor of protein synthesis in bacterial systems. In higher plants, it has been found to interfere primarily with structure and functions of chloroplasts (Drawert and Mix 1961, Döbel 1963, Margulies 1966, Wrischer 1967). Bergfeld (1968) investigated the effect of chloramphenicol on young fern gametophytes and found an inhibition of chloroplast development, but no inhibition of morphogenesis and differentiation. In mosses, on the contrary, chloramphenicol produces a reversible inhibition of the morphogenetic process of bud induction in the protonema (Szweykowska and Schneider 1967, Szweykowska, Ratajczak and Schneider 1968). Therefore the effect of chloramphenicol on the fine structure of chloroplasts and other organelles in this material seemed to be of interest and a study on this problem was undertaken.

MATERIAL AND METHODS

The protonema of *Funaria hygrometrica* Hedw. was grown from spores in a liquid mineral medium with the addition of glucose at 0.25%, in continuous fluorescent white light of about 1000 lux and at a temp. of ca. 24°C. After 10 days, the protonema was filtered, washed and transferred to a mineral medium containing chloramphenicol at 0.5 mM/l. This concentration of chloramphenicol is not toxic, because its inhibiting effect may be reversed by transferring the protonema back to an inhibitor–free medium (Szweykowska, Ratajczak and Schneider 1968). As controls served cultures transferred to a mineral medium withouth any additions. After six days, the material was fixed in $KMnO_4$ according to Luft (1956). It was dehydrated in ethanol and propylene oxide, and embedded in Epon 812. Some sections were stained with uranyl acetace and lead citrate according to Reynolds (1963). The micrographs were taken in the JEM 7A electron microscope.

RESULTS

In the control material examined in the light microscope, the chloroplasts were very numerous and uniformly distributed in the protonema cells (Fig. 1). In the protenema treated for six days with chloramphenicol most chloroplasts accumulated

PLATE I

Fig. 1. Distribution of chloroplasts in the untreated protonema cells
Rozmieszczenie chloroplastów w komórkach splątka w pożywce bez chloramfenikolu
Fig. 2. Distribution of chloroplasts in the treated protonema cells
Rozmieszczenie chloroplastów w komórkach splątka w pożywce z chloramfenikolem
Fig. 3. Cross-section of chloroplast fragment (*Ch*), mitochondrion (*M*) and a microbody (*Mi*) in an untreated protonema cell. 30 000×
Przekrój przez fragment chloroplastu (*Ch*), mitochondrium (*M*) i mikrociało (*Mi*) w komórce splątka nie traktowanego chloramfenikolem. 30 000×

PLATE II

Fig. 4. Compact arrangement of chloroplasts in the treated protonema cell. 12 000×
Skupienie chloroplastów (*Ch*) wywołane działaniem chloramfenikolu. 12 000×
Fig. 5. Disintegration of chloroplast. Giant grana (GG) and numerous vesicle-like structures (*Vs*) in the stroma. 15 000×
Rozkład struktury chloroplastu. Grana olbrzymie (GG) i liczne pęcherzykowate utwory (*Vs*) w stromie. 15 000×

PLATE III

Fig. 6. Early stage in chloroplast disintegration. The stroma thylakoids disintegrated into linearly arranged bead-like structures (arrows). *St* — starch. 22 000×
Wczesne stadium rozkładu chloroplastu. Tylakoidy stromy rozpadają się na liczne pęcherzyki ułożone w charakterystyczne paciorkowate struktury (strzałki). *St* — skrobia. 22 000×

PLATE IV.

Fig. 7. Fragment of a treated protonema cell. Chloroplasts with giant grana (*GG*) and numerous vesicle-like structures (*Vs*). Mitochondria (*M*) with reduced tubules. *N*-nucleus. 22 000×.
Fragment komórki traktowanej chloramfenikolem. Chloroplasty z granami olbrzymimi (*GG*) i licznymi pęcherzykowatymi strukturami (*Vs*). Mitochondria (*M*) ze zredukowanymi tubulami. *N* — jądro. 22 000×

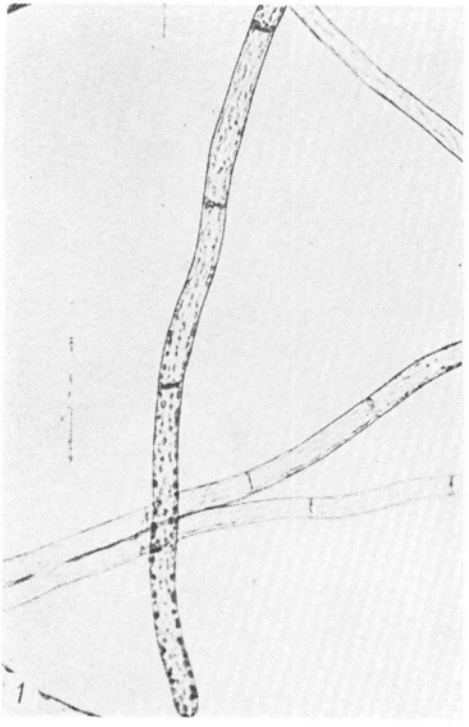
PLATE V

Fig. 8. Later stage in plastid disintegration. Strong swelling of chloroplast with numerous vesicle structures and starch grains (*St*) in the stroma. Numerous bodies (*Bd*) between the chloroplast membranes. 22 000×
Późniejsze stadium rozkładu chloroplastu. Silnie spęczniały chloroplast z licznymi pęcherzykowatymi strukturami i ziarnami skrobi (*St*) w stromie. Liczne ciałka (*Bd*) pomiędzy błonami otoczki plastydu. 22 000×

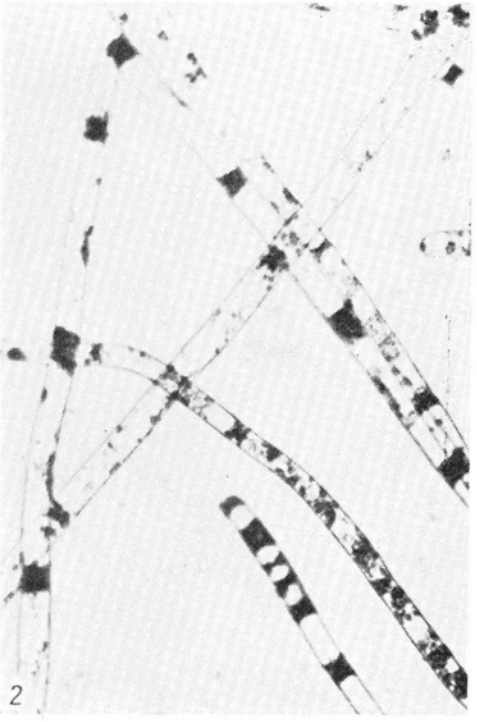
PLATE VI

Fig. 9. Nucleus (*N*) with vesicles separating from evaginations of its outer membrane, and a chloroplast (*Ch*) with disrupted envelope and numerous vesicles discharged to the cytoplasm. 22 000×
Jądro komórkowe (*N*) z pęcherzykami oddzielającymi się od zewnętrznej błony i chloroplast (*Ch*) z pękniętą otoczką i licznymi pęcherzykami wydostającymi się do cytoplazmy. 22 000×

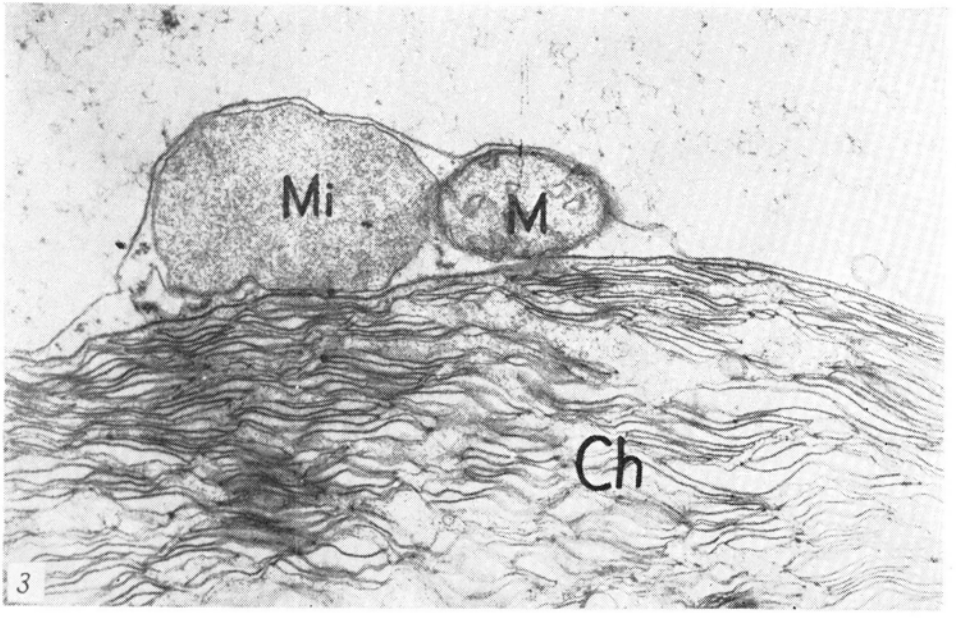
Plate I



1

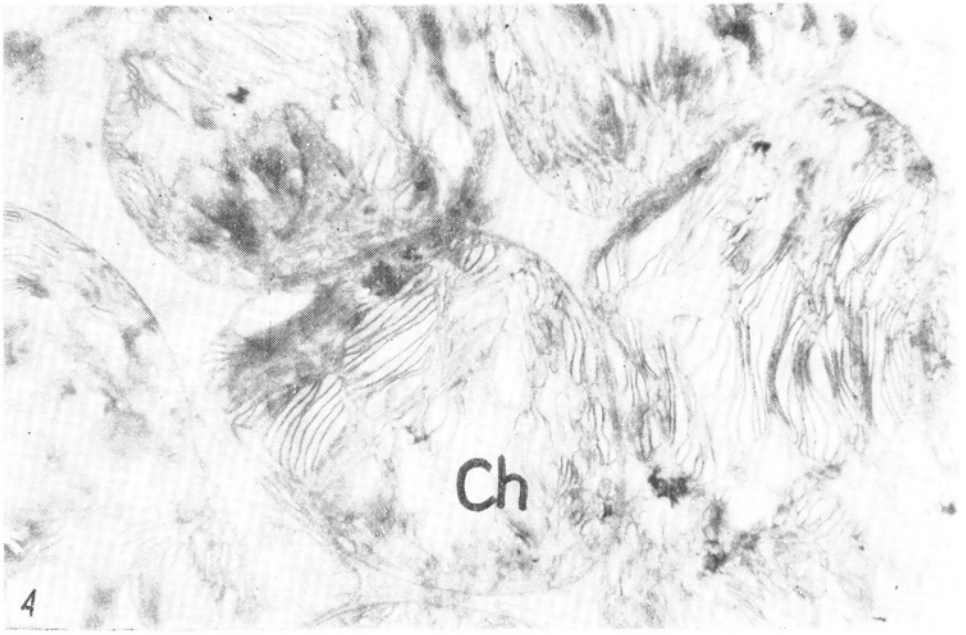


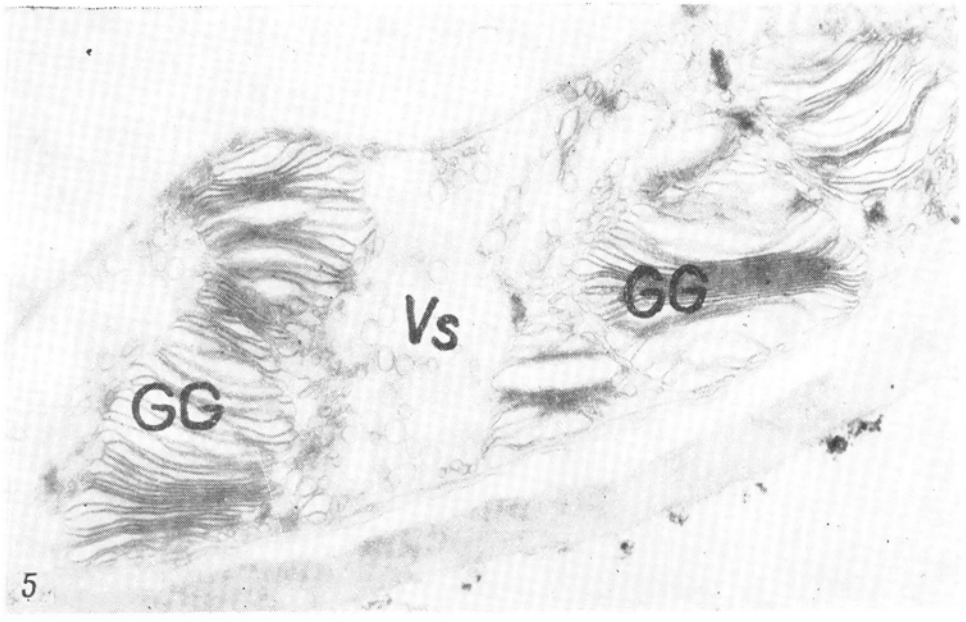
2

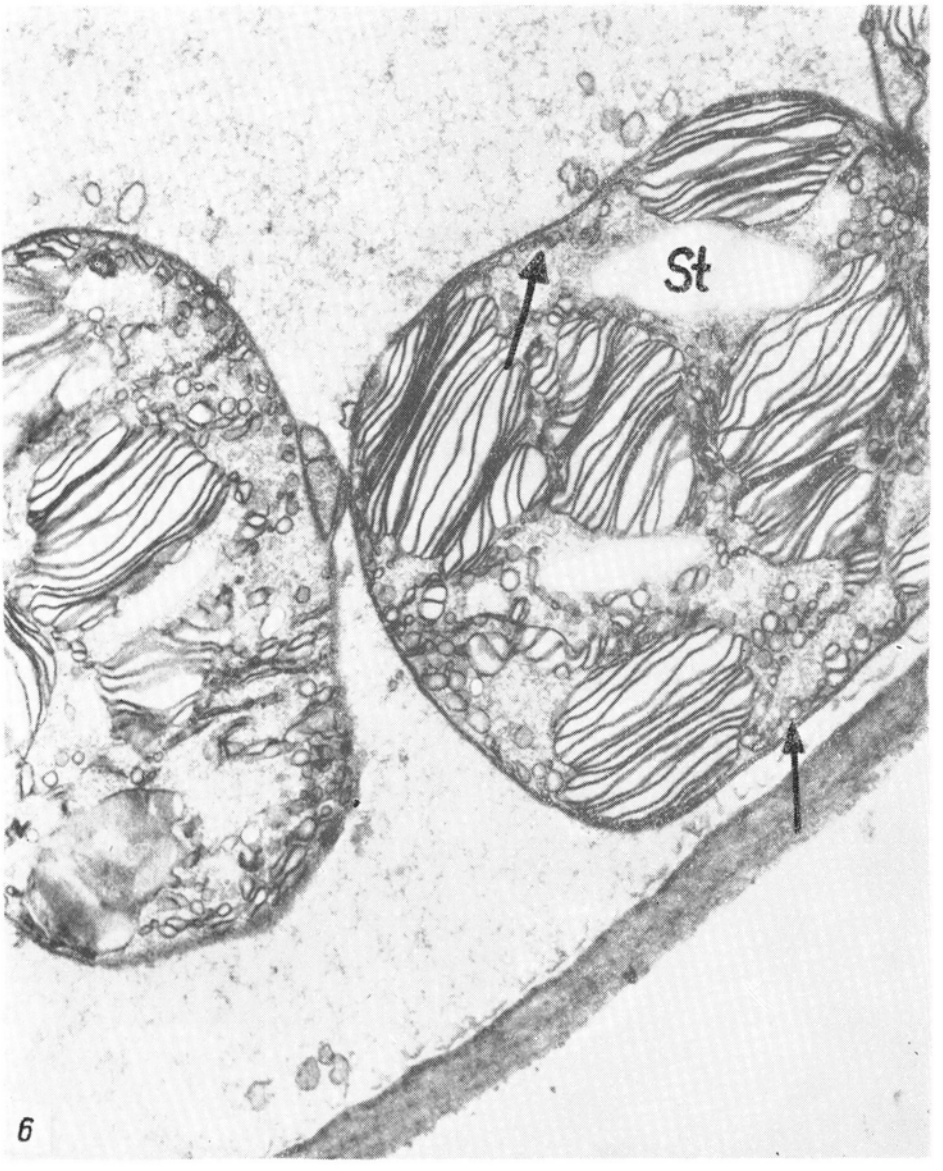


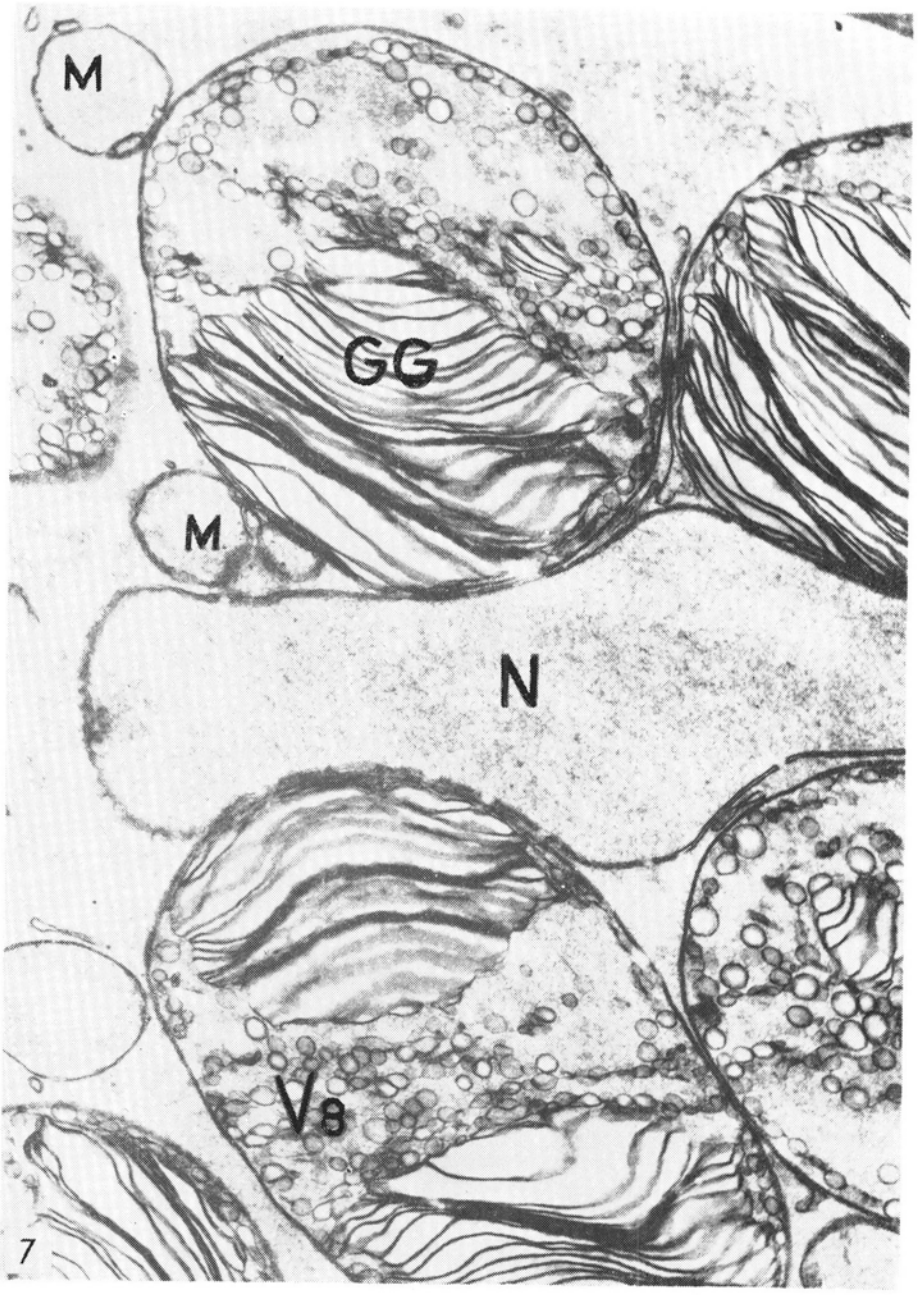
3

Plate II






St
6


M
GG
M
N
V8
7

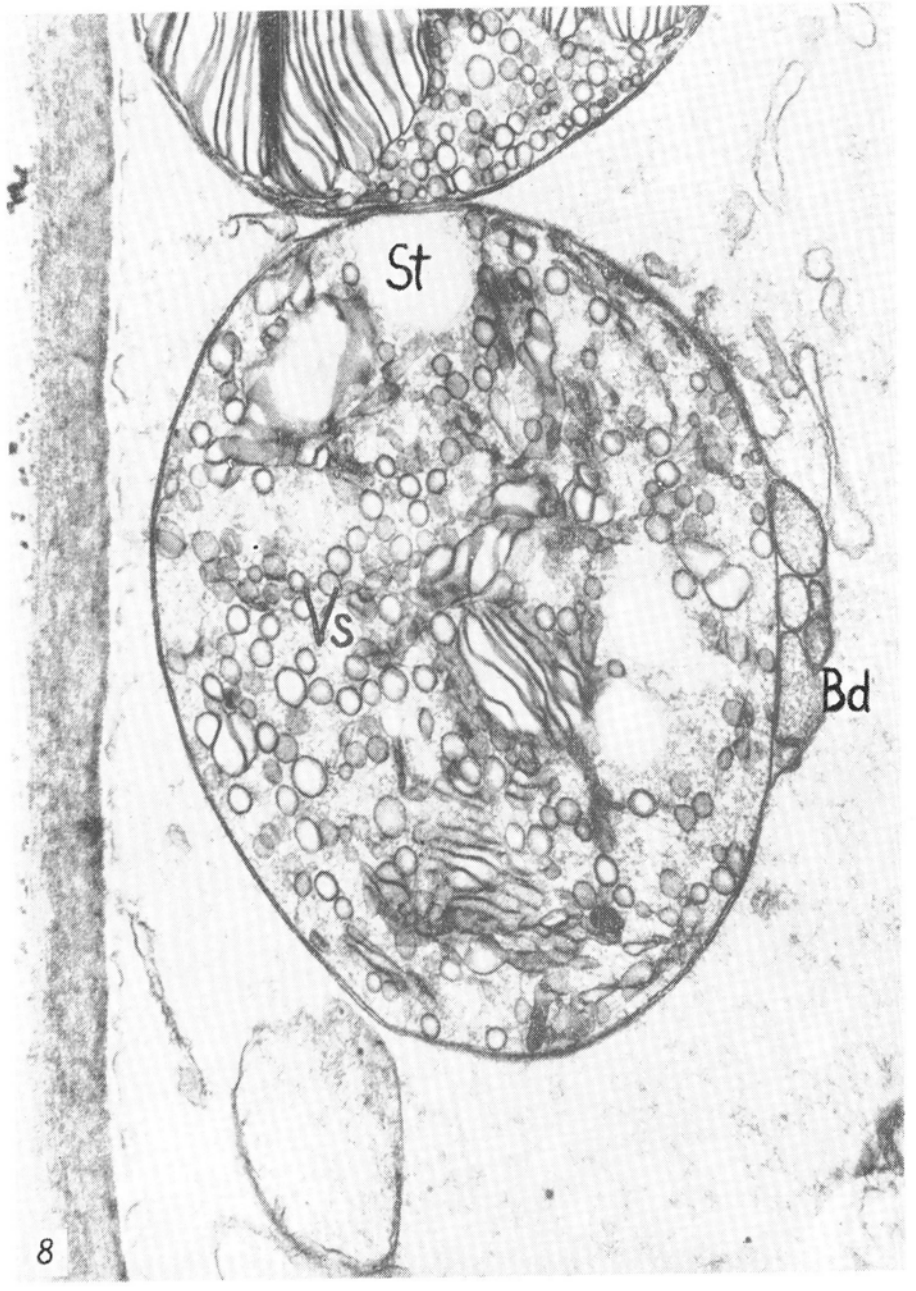
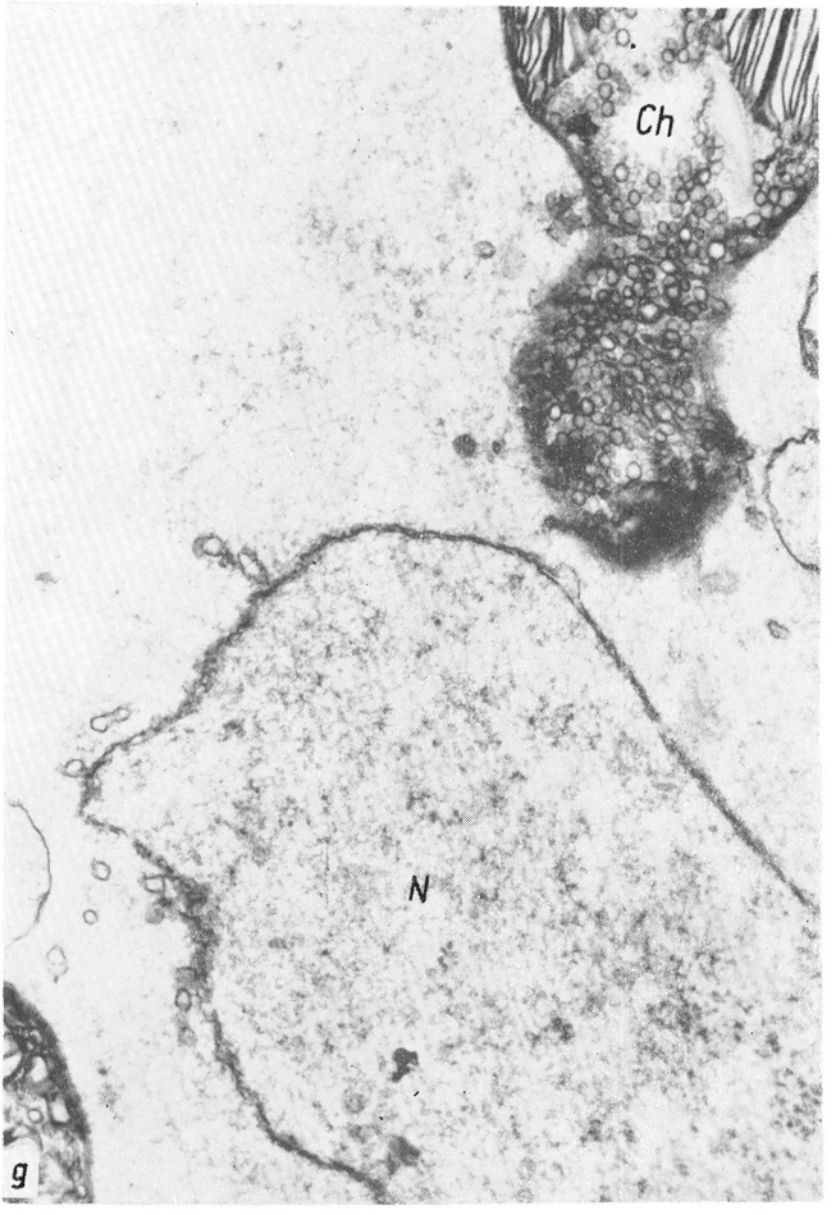

St
Vs
Bd
8

Plate VI



near the transverse cell walls forming there compact aggregates (Figs. 2 and 4). Some linse-shaped structures could be distinguished in the chloroplasts, which resembled „linsenförmige Einschlüsse" described by Döbel (1963) and which in the electron microscope appeared to be large grana. After a prolonged treatment with chloramphenicol (12 days), the protonema became chlorotic and the chloroplasts underwent fragmentation.

In controls examined in the electron microscope, the chloroplasts had a distinct lamellar structure with relatively poor grana differentiation (Fig. 3). The ultrastructure of the protonema chloroplasts in Funaria has been described in more detail in a previous paper (Młodzianowski 1970). Under the influence of chloramphenicol their structure underwent considerable changes. They consisted mainly in destruction of the stroma thylakoids, which at first disintegrated into linearly arranged bead-like structures (Fig. 6) and finally into vesicles irregularly distributed in the stroma (Figs. 5, 7, 8). The grana were free and irregularly arranged, and as a result of multiple divisions of their lamellae giant grana were formed composed of a large number of thylakoids amounting occasionally to 30 (Fig. 5). The thylakoids of giant grana were shorter and frequently the more distended the more they were distant from the center of the granum. Three to six grana of this kind were found in one plastid section. Similar giant grana, as well as the process of their formation in chloroplasts of leaves treated with chloramphenicol, have been described by Döbel (1963) in tomato and by Margulies (1966) in bean.

Some chloroplasts were almost completely filled with vesicle structures (Fig. 8). These structure in chloramphenicol treated chloroplasts seem to develop in two ways, depending mostly on the stage of plastid development at the moment of antibiotic action. In mature chloroplasts, they may origin as a result of disintegration of the lamellar system already existing. In developing chloroplasts, being in a post-division stage, vesicles are separated directly from invaginations of the inner plastid membrane. In normal conditions, thylakoids are formed by fusion of such vesicles (Sun 1966). This process is inhibited by chloramphenicol, probably as a result of inhibition of synthesis of proteins building the lamellar system of chloroplasts (Margulies 1966).

In chloramphenicol treated chloroplasts, even those with a high degree of structural degradation, starch grains were always found (Figs. 6 and 8). Between the membranes surrounding the plastid, oval bodies were frequently present with a homogeneous, granular structure, bounded by a distinct single membrane (Figs. 6 and 8). In the control material, such bodies were only occasionally observed. Similar bodies („Körperchen in der Plastidenmembran") were observed by Diers (1966) in chloroplasts of an *Oenothera* hybrid. It is not clear whether they pass into the cytoplasm, and nothing is known about their function.

A considerable swelling of the whole chloroplasts occurred under the influence of chloramphenicol. Chloroplasts with disrupted envelopes were also observed. Their contents in form of numerous vesicles were dischargeed to the cytoplasm (Fig. 9).

On fig. 9 a fragment of nucleus is visible, with numerous vesicles separating from evaginations of its outer membrane. Such pictures were also observed in the control material, it seemed, however, that this process was more intense in the chloramphenicol treated material.

A destruction of mitochondria in form of a considerable reduction of tubules was also observed in cells treated with chloramphenicol (Figs. 7 and 9).

DISCUSSION

The structural and functional changes induced in cells by antibiotica depend on various factors: the age of organ (Döbel 1963), the physiological condition of plant and the degree of development of the photosynthetic apparatus (Osipowa et al. 1967). Provasoli (1951) also indicates that the bleaching effect of antibiotica in *Euglena* appears only in dividing cells.

At the time of transferring the protonema to chloramphenicol containing medium, the material was relatively uniform, but some features as e.g. the division potencies of various cells were not identical. The cell divisions are much more frequent in apical cells of the protonema than they are in intercalary cells (Szweykowska, Guzowska and Gallas 1968). This may be the reason that various degrees of structure disintegration were observed in various cells, even in the same filament. Generally, following characteristic changes were observed in chloramphenicol treated cells:

1. The chloroplasts accumulated at the transverse walls forming compact complexes.
2. Increasing bleaching of chloroplasts was accompanied by following modifications in ultrastructure:
   - disintegration of stroma thylakoids,
   - free and irregular arrangement of grana,
   - an excess enlargement of some grana (giant grana),
   - appearence of numerous vesicle structures in the stroma,
   - formation of numerous bodies between membranes surrouding the chloroplasts,
   - swelling of chloroplasts and disruption of their boundary membranes.
3. A considerable reduction of tubules was observed in mitochondria.

The changes induced by chloramphenicol in chloroplast ultrastructure in *Funaria hygrometrica* are generally similar to those observed by Döbel (1963) in chloroplasts of chloramphenicol treated leaves in tomato and by Margulies (1966) in bean. Similar deformations of chloroplast structure were also observed with other antibiotica (Döbel 1963; Wriescher 1967), with various metabolic inhibitors (Ashton et al. 1963; Signol 1961), and in conditions of some mineral or organic deficiences (Thomson and Weier 1962; Sunderland and Wells 1968). Therefore, the structural changes induced by chloramphenicol cannot be taken as specific for this compound. They are probably induced as a result of protein synthesis inhibition which in case of chloramphenicol is not a complete one (Döbel 1963; Bergfeld 1968), but concerns first of all proteins involved in building the lamellar system of

chloroplasts (Margulies 1966). The presence of starch grains in chloroplasts showing various degrees of degeneration may indicate that starch is synthesized in the presence of chloramphenicol, or that starch deposited during the first days of experiments (in the presence of glucose and absence of chloramphenicol) has not been used then bacause of chloramphenicol induced inhibition of its enzymatic hydrolysis. Chloramphenicol is a specific inhibitor of protein synthesis in bacterial systems, where it combines with the subunit 50-S of the 70-S ribosomes. In eucariotic cells (e.g. yeasts, reticulocytes) possesing ribosomes 80-S instead of 70-S, the protein synthesis is not sensitive to this antibiotic (Hartmann et al. 1968). In plants, chloramphenicol has been found to affect preferentially synthesis of chlorophyll and of some insoluble protein fraction in chloroplasts probably involved in formation of lamellae (Margulies 1966; Osipowa et al. 1967). Among other organelles, mitochondria have been reported to show some sensitivity to chloramphenicol (Döbel 1963). The action of chloramphenicol on chloroplasts and mitochondria may be connected with structure of their ribosomes which are not identical with the ribosomes of the cytoplasm, but show some features of the bacterial ones, e.g. they have a sedimentation coefficient 60—70 S and are relatively sensitive to chloramphenicol (Eisenstadt and Brawerman 1964; Sissakian et al. 1965; Wilson et al. 1968). The sensitivity of some chloroplast and mitochondrial structures to chloramphenicol speakes for the autonomy of these organelles with respect to syntheses of some proteins, whereas others may be cytoplasm-dependent and less sensitive to this inhibitor. The proteins building the plastidal lamellar system and the mitochondrial tubules seem to be mainly sensitive to chloramphenicol and probably dependent on a specific system of ribosomes and protein synthesis.

The changes induced in the ultrastructure of the *Funaria* cells present a good example of a selective action of chloramphenicol on plant cells. This antibiotic causes also an inhibition of bud formation in the protonema of *Funaria* which indicates that bud induction may be at least partly dependent on protein syntheses localized in chloroplasts and/or in mitochondria.

SUMMARY

Changes induced by chloramphenicol in the fine structure of chloroplasts and other organelles in the protonema of *Funaria hygrometrica* have been described. The most characteristic changes were: disintegration of stroma thylakoids, free and irregular arrangement of grana, enlargement of some grana (giant grana), formation of numerous vesicle structures in the stroma, swelling of chloroplasts and disruption of their boundary membranes, and a considerable reduction of tubules in mitochondria. The selective effect of the inhibitor on some cell organelles indicates that the process of bud formation in the protonema of Funaria, on which chloramphenicol has an inhibitory effect, may be at least partly dependent on protein syntheses localized in chloroplasts and/or in mitochondria.

Department of General Botany
Adam Mickiewicz University
Poznań, Stalingradzka 14, Poland

(Entered: June 25, 1969.)

LITERATURE

Ashton F. M., E. M. Gifford., and T. Bisalputra., 1963, Structural changes in *Phaseolus vulgaris* induced by atrazine. II. Effects on fine structure of chloroplasts, Bot. Gaz. 134: 336—343.

Bergfeld R., 1968, Chloroplastenausbildung und Morphogenese der Gametophyten von *Dryopteris filix-mas* (L.) Schott nach Applikation von Chloramphenicol und Actidion (Cycloheximid), Planta 81: 274—279.

Diers L., 1966, Über die dreidimensionale Gestaltung des Thylakoid-systems in den Chloroplasten, Planta 70: 322—343.

Döbel P., 1963, Untersuchung der Wirkung von Streptomycin, Chloramphenicol-, und 2-Thiouracil-Behandlung auf die Plastidenentwicklung von *Lycopersicon esculentum* Miller Biol. Zbl. 82: 275—295.

Drawert H., and M. Mix, 1961, Licht-und elektronenmikroskopische Untersuchungen an Desmidiaceen. VI. Der Einfluss von Antibiotica auf die Chloroplastenstruktur bei Micrasterias rotata, Planta 57: 51—70.

Eisenstadt J. M., and G. Brawerman, 1964, The protein synthesizing systems from the cytoplasm and the chloroplasts of *Euglena gracilis*, Jour. Mol. Biol. 10: 392—402.

Hartmann G., W. Behr., K. A. Beissner., K. Honikel., and A. Sippel, 1968, Antibiotica als Hemmstoffe der Nucleinsäure- und Proteinsynthese, Angew. Chem. 80: 710—718.

Luft J. H., 1956, Permanganate- a new fixative for electron microscopy, J. Biophys. Biochem. Cytol. 2: 799—802.

Margulies M. M., 1966, Effect of chloramphenicol on formation of chloroplast structure and protein during greening of etiolated leaves of Phaseolus vulgaris, Amer. Jour. Bot. 41: 992—1003.

Młodzianowski F., 1970, Fine structure of protonema in *Funaria hygrometrica*, Acta Soc. Bot. Pol. 39: 45—50.

Osipowa O. P., M. K. Nikołajewa, and C. J. Chejn, 1967, K woprosu o diejstwii kłoramfenikoła na fotosinteticzeskij apparat rastienij, Fizjoł. Rast. 14: 210—218.

Provasoli L., and I. P. Pinter, 1951, Destruction of chloroplast by streptomycin, Cold. Spr. Harb. Symp. Quant. Biol. 16: 113:120.

Reynolds E. S., 1963, The use of lead citrate and high pH as an electron opaque stain in electron microscopy, J. Cell. Biol. 17: 208—212.

Signol M., 1961, Comparison de l'action de la dihydrostreptomycine à celle de l'acide 3-(α-iminoethyl)-5 methyltetronique sur l'infrastructure des chloroplastes de *Zea mays* L., Compt. Rend. Acad. Sci. Paris 252: 1993—1995.

Sissakian N. M., I. I. Filipowich, E. N. Svetailo and K. A. Aliyev, 1965, On the protein-synthesizing system of chloroplasts, Biochim. Biophys. Acta 95: 474—485.

Sun C. N., 1962, Submicroscopic structure and development of the chloroplasts of *Funaria hygrometrica* and *Bryum* sp., Cytologia (Tokyo) 27: 333—342.

Sunderland N., and B. Wells, 1968, Plastid structure and development in green callus tissues of *Oxalis dispar*, Ann. Bot. 32: 327—346.

Szweykowska A., and J. Schneider, 1967, Action of 8-azaguanine and of inhibitors of protein synthesis on bud inducing activity of kinetin in the moss *Ceratodon purpureus*, Acta Soc. Bot. Pol. 36: 735—738.

Szweykowska A., I. Guzowska, and J. Gallas, 1968, Studies on the activity of kinetin in cultures of *Funaria hygrometrica*. II. Effect of kinetin on isolated protonema cells, Acta Soc. Bot. Pol. 37: 201—206.

Szweykowska A., W. Ratajczak, and J. Schneider, 1968, Studies on the activity of kinetin in cultures of *Funaria hygrometrica*. I. Bud-inducing activity of kinetin and protein synthesis, Acta Soc. Bot. Pol. 37: 145—155.

Thomson W. W., and T. E. Weier, 1962, The fine structure of chloroplasts from mineral deficient leaves of *Phaseolus vulgaris*, Amer. Jour. Bot. 49: 1047—1055.

Wilson R. H., J. B. Hanson, and H. H. Mollenhauer, 1968, Ribosome particles in corn mitochondria, Plant Physiol. 43: 1874—1877.

Wrischer M., 1967, The effects of inhibitors of protein synthesis on the differentiation of plastids in etiolated bean seedlings, Planta 73: 324—327.

Wpływ chloramfenikolu na ultrastrukturę chloroplastów w splątku Funaria hygrometrica

Streszczenie

W pracy opisane zostały zmiany w ultrastrukturze chloroplastów i innych organelli splątka *Funaria hygrometrica* wywołane chloramfenikolem. Do najbardziej widocznych można zaliczyć: rozpad tylakoidów stromy, wolne i nieregularne ułożenie gran, powiększenie pewnych gran, tworzenie licznych pęcherzykowatych struktur w stromie, pęcznienie plastydów i pękanie ich osłonek oraz znaczną redukcję tubul w mitochondriach. Selektywny wpływ inhibitora na pewne organelle komórkowe świadczy, że hamowany przez chloramfenikol proces tworzenia pączków gametoforowych w splątku Funarii może przynajmniej częściowo zależeć od syntezy białka odbywającej się na terenie chloroplastów lub/oraz mitochondriów.