

Effect of kinetin and chloramphenicol on chlorophyll synthesis and chloroplast development in detached lupin cotyledons under low light intensity

FORTUNAT MŁODZIANOWSKI and EWA GEZELA

Department of General Botany, Institute of Biology, A. Mickiewicz University,
Poznań,

(Received: June 13, 1973)

Summary

Chlorophyll synthesis in detached lupin cotyledons under low light intensity was stimulated by kinetin at 20 mg/l and inhibited by chloramphenicol at 50 mg/l. Kinetin not only counteracted the inhibitory effect of chloramphenicol, but stimulated the chlorophyll synthesis to a greater level than in the control material.

Kinetin accelerated the starch degradation and the development of chloroplast; its prolonged action, however, produced some abnormalities, such as an excessive growth of plastids resulting in some cases in bursting of their envelopes, the formation and release from plastids of numerous membrane-bound bodies and the accumulation in released and swollen thylakoids of electron-dense substance.

In the presence of chloramphenicol, some disturbances in structure of the stroma thylakoids and the appearance of vesicular structures in the stroma and the enlargement of grana and swelling of their thylakoids were observed. Kinetin prevented some of these abnormalities.

INTRODUCTION

The synthesis of chlorophyll and the development of individual cellular organelles during the germination of seeds were the subject of many studies (Horner and Arnott, 1966; Öpik, 1966 and 1968; Engelbrecht and Weier, 1967; Lovell and Moore, 1970; Moore et al., 1972, and others). Detached cotyledons represent an especially convenient material for studying these process and the effects exerted on them by different experimental conditions and by stimulators and inhibitors of chlorophyll synthesis (Knypl, 1970;

Knypl and Mazurczyk, 1972; Fletcher and McCullagh, 1971).

It is known that cytokinins not only inhibit the decrease in chlorophyll level and the decay of plastids in ageing tissues (Shaw and Manocha 1965; Sveshnikova et al., 1966; Dennis et al., 1967; Młodzianowski and Ponitka, 1973; Młodzianowski and Kwintkiewicz 1973), but also stimulate chlorophyll synthesis in cotyledons (Fletcher and McCullagh, 1971; Khokhlova et al., 1971) or even determine the chloroplast development from proplastids and the chlorophyll synthesis, as e. g. in tobacco tissue cultures (Stetler and Laetsch, 1965; Kaul and Sabharwal, 1971).

Chloramphenicol blocks the light-induced synthesis of chlorophyll and simultaneously produces structural changes in differentiating plastids in algae (Drawert and Mix, 1961), mosses (Młodzianowski and Szweykowska, 1971) ferns (Bergfeld, 1968), and flowering plants such as wheat (Sarkissian and Huffaker, 1962), tomato (Döbel, 1963), bean (Margulies, 1966; Wrischer, 1967) and pea (Srivastava et al., 1971).

One of the effect of kinetin on chloroplast structure is an increase in the chloroplast volume and the enlargement of grana (Dennis et al., 1967; Khokhlova et al., 1971; Farinneau and Rousseaux, 1970). The formation of massive grana has been also observed under the influence of chloramphenicol (Döbel, 1963; Margulies, 1966). Kinetin applied together with chloramphenicol acted synergistically in the formation of abnormally developed grana in the protonema of *Funaria hygrometrica* (Młodzianowski and Szweykowska, 1971).

The aim of the present investigation was to study the chlorophyll level and chloroplast development in detached lupin cotyledons and the effect of kinetin and chloramphenicol on these processes.

MATERIAL AND METHODS

Incubation of the cotyledons. Lupin seeds (*Lupinus luteus* L. cv. 'Express') were swollen for 24 h in water and then germinated for 24 h in darkness. Subsequently the cotyledons were excised and placed with their inner surface (the palissade mesophyll side) into Petri dishes with filter paper. The Petri dishes contained each 10 ml of the following incubation media: distilled water (control), kinetin at 20 mg/l, chloramphenicol at 50 mg/l, and kinetin + chloramphenicol at 20 mg/l and 50 mg/l, respectively. The dishes with the cotyledons were exposed to white fluorescent light of 14 lux. In a preliminary

experiment it was shown that under light of such a low intensity the stimulating effect of kinetin on chlorophyll synthesis is most distinct. The incubation solutions were changed every other day. For chlorophyll analyses and electron microscopic studies the material was taken after 2, 3, 6, 9 and 12 days of incubation.

Chlorophyll determination. Chlorophyll was extracted with ethyl alcohol from 5 cotyledons for each experiment. The extracts were made up to an equal volume and their light absorption was determined at the wavelength of 665 nm. Each experiment was repeated 3 times.

Electron microscopy. The cotyledons were cut into 1 mm² pieces consisting of epidermis, palissade mesophyll and several layers of spongy mesophyll, fixed in 5% glutaraldehyde in 0,1 M phosphate buffers, pH 7, for 2 h at room temperature and overnight at 4° C. After the fixation the material was washed 3 times with 0,1 M phosphate buffers, pH 7, postfixed in 2% OsO₄ in the same buffer for 2 h at room temperature, washed with buffer as before, dehydrated in alcohol-propylene oxide and embedded in Epone 812. Sections were cut with a Tesla ultramicrotome, contrasted with uranyl acetate and lead citrate according to Reynolds (1963) and examined in a JEM 7A electron microscope.

RESULTS

1. Chlorophyll synthesis and fresh weight increase

Kinetin at 20 mg/l considerably stimulated chlorophyll synthesis and growth of the cotyledons (Fig. 1 and Table 1).

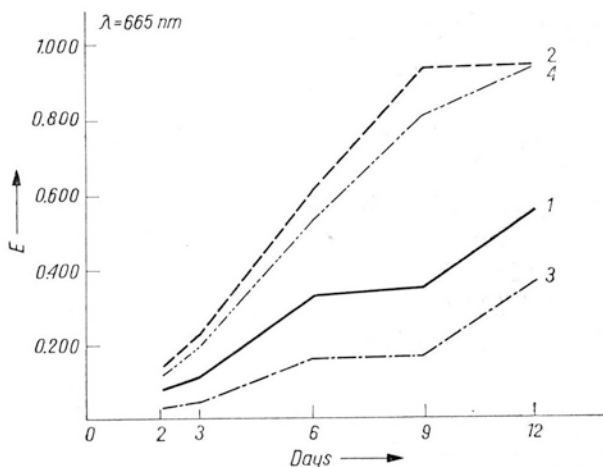


Fig. 1. The effect of kinetin and chloramphenicol on the chlorophyll content in the detached cotyledons of lupin.

1 — water (control); 2 — kinetin (20 mg/l); 3 — chloramphenicol (50 mg/l); 4 — kinetin + chloramphenicol at 20 mg/l and 50 mg/l respectively

Table 1

Changes in the fresh weight of the lupin detached cotyledons after exposing them to light

Days	Growth, mg/cotyledon			
	Water	KIN	CAP	KIN+CAP
2	93	96	96	91
3	109	111	99	95
6	134	173	140	165
9	174	203	165	191
12	198	246	216	229

KIN — kinetin at 20 mg/l; CAP — chloramphenicol at 50 mg/l;

KIN + CAP — kinetin and chloramphenicol at 20 mg/l and 50 mg/l, respectively

Preliminary experiments showed that chloramphenicol inhibited chlorophyll synthesis in cotyledons, without showing any toxic effects, at concentrations up to 100 mg/l. At higher concentrations (beginning with 500 mg/l) there was no increase in weight and no greening, and necrotic spots were observed. When a concentration of 50 mg/l was applied, there was an inhibition of the chlorophyll synthesis without a noticeable inhibition of growth of the cotyledons.

Kinetin overcame the inhibitory effect of chloramphenicol on the chlorophyll synthesis (Fig. 1).

2. Plastid development in control material

In the cotyledons of dry seeds plastids are in the proplastid stage. They are enclosed by a double membrane often forming a characteristic creased line on the cross section. Nucleoid-like regions containing the DNA fibrils, aggregations of phytoferritin and numerous osmophilic bodies — plastoglobuli occur in the matrix. It is impossible to identify that certain granulations in the matrix correspond to ribosomes. No traces of the inner membrane system or starch were observed (Fig. 2). On cross sections of plastids from cotyledons of swollen (24 h) and germinated in darkness (24 h) seeds, distinct starch grains were present (Fig. 3). They were formed most likely as a result of transformation of some other materials, such as hemicelluloses, lipids or proteins. The starch grains still enlarged during 2 days after the exposition of the cotyledons to light (Fig. 4). Prolamellar bodies and the thylakoid system in which they developed became visible only after a partial disappearing of starch, which was observed on the 6th day of light exposition (Fig. 5). From that moment, two types of plastids could be distinguished in the cotyledons. The first type, which on the cross-section had most often

a spindle-shaped outline, was characterized by a dense matrix, while the second one, with circular contours, by a transparent matrix and less numerous thylakoids. These two types of plastids are further termed as "A" and "B" according to De Vecchi (1971) who observed similar plastid differentiation in oat leaves. Plastids of the "B" type occurred sporadically in the control material and their development lagged behind that of the "A" type of plastids (Figs. 6 and 7). Prolamellar bodies were observed in both types of plastids even after 12-days of experiment.

3. Kinetin effect on chloroplast development.

Under the influence of kinetin an accelerated differentiation of chloroplasts was observed. After 6 days of light exposure, grana and thylakoids developed completely in the "A" type of plastids. No starch grains were observed and the prolamellar bodies occurred only sporadically (Fig. 8). The number of the "B" type of plastids distinctly increased. For some time, the thylakoid system inside these plastids developed normally from large prolamellar bodies (Figs. 9 and 11). On the boundary of the plastids their derivatives were often observed in form of spherical bodies containing homogeneous matrix and surrounded by one (Fig. 9) or two membranes (Figs. 10 — 13). Similar chloroplast protuberances in leaves of some *Oenothera* species were demonstrated by comparative light and electron microscopic investigation (Schötz et al., 1971). After 12 days of experiment the maximum growth of the "B" type of plastids or even their overgrowth accompanied by disruption on the plastidal envelope were observed. The margins of thylakoids grew larger forming vesicular protrusions of considerable size. It was interesting that their inside was electron dense, which might indicate accumulation of protein substances. Some pictures seemed to indicate that spherical bodies were separated from individual thylakoids (Fig. 15).

4. Chloramphenicol effect on chloroplast development.

Prolamellar bodies appeared in the presence of chloramphenicol (Fig. 18). The thylakoids looked swollen in cross-section (Figs. 17 — 19) and the areas inside the thylakoids were electron-transparent. After 12 days of exposure to light, in the "A" type of plastids the grana were larger than in the control material, but the development of the stroma thylakoids was markedly inhibited. The inner membrane of the

plastidal envelope often formed small invaginations into the matrix indicating separation of vesicles (Fig. 16). In some "A" type plastids dense arranged concentric thylakoids on the periphery of matrix and scattered grana in their centre were observed (Fig. 17). Among the matrix granulations some might be recognized as ribosomes. In the "B" type of plastids of the 12 day old material, the matrix was transparent, the thylakoids occurred on the plastid periphery and were swollen, and in addition some starch was found as a residue of transformations taking place in the first stage of the plastid development (Fig. 18). Kinetin prevented changes caused by chloramphenicol, which was first of all manifested in a normal development of thylakoids but the prolamellar bodies in both type of plastids were present (Fig. 19 and 20).

DISCUSSION

In experiments described in this paper, the chlorophyll synthesis and chloroplast development from proplastids in detached cotyledons of lupin incubated in water took place in conditions of low light intensity. Under light of higher intensity the chlorophyll level would certainly increase more rapidly and the chloroplast development would be more complete. The use of a dim light allowed, however, to reveal better the stimulating effect of kinetin on the chlorophyll synthesis. It is in good agreement with the opinion, that there appears to be close involvement of cytokinins in light-sensitive processes; in some cases cytokinins appear to exert an action similar to that of phytochrome-dependent reactions such as chlorophyll synthesis and formation and maintenance of chloroplasts (see review by Hall, 1973). Results similar to those of the present paper showing the stimulating action of kinetin on chlorophyll synthesis were also obtained by Stetler and Laetsch (1965) in tobacco tissue cultures, by Banerji and Laloraya (1967) and by Mikulovich et al., (1971) in pumpkin cotyledons and by Fletcher and McCullagh (1971) in cotyledons of cucumber. The report of Narain and Laloraya (1970) on the inhibiting influence of kinetin on the chlorophyll synthesis in cucumber cotyledons seems to be isolated and contradictory to the studies of Fletcher and McCullagh (1971), who propose the use of cucumber cotyledons (in conditions of the proper plant age, light and temperature) as a sensitive and specific material for a cytokinin test.

It seems very likely, that in lupin cotyledons greening under low light intensity kinetin stimulated chlorophyll synthesis and chloroplast differentiation as well as prevented the reaccumulation of protochlorophyllide and the reformation of prolamellar bodies. Such reformation

was observed by Henningsen and Boynton (1970) in etioplasts of barley and by Ikeda (1971) in cucumber seeds greening in low light. Indeed, since in the "A" type of kinetin treated lupin chloroplasts prolamellar bodies were observed only sporadically, in the control material these bodies were always present. But we are not sure, if in our long lasting culture and under low light intensity, the presence of prolamellar bodies is a result of incomplete differentiation of thylakoids from these bodies or an effect of their reformation.

Kinetin accelerates the starch degradation in lupin cotyledons and which is in agreement with some evidence suggested that cytokinins affect carbohydrate metabolism in plants (Boothby and Wright, 1952; Sugiura et al., 1962; Berridge and Ralph, 1971).

The stimulation by cytokinins of the differentiation of plastids was found in tobacco callus tissue Stetler and Laetsch, 1965) and in the cotyledons of pea (Farinneau and Roussaux, 1970) and pumpkin (Khokhlova et al., 1971). In the present study, too, the thylakoids developed more rapidly in the kinetin-treated material than in the control. However, an excessive growth of grana as e. g. observed under the influence of kinetin in the protonema of *Funaria* (Młodzianowski and Szweykowska, 1971) or in the pea cotyledons (Farinneau and Roussaux, 1970) was not observed. In lupin cotyledons kinetin caused an increase in plastid dimensions, similarly as in young leaves of brussels sprouts (Dennis et al., 1967) or parsley (Młodzianowski and Ponitka, 1973). These tendencies to a strong growth of plastids in lupin, as well as the formation of derivative spherical bodies, the swelling of thylakoidal margins in the last stage of the plastid existence and the accumulation of some substances in the distensions formed indicate a stimulation by kinetin of the synthesis of material building these structures. From the electron micrographs presented in this study it follows that in the presence of kinetin even after the disruption of the envelope and the release of thylakoids, active syntheses probably continued in their insides.

Although in the darknes chloramphenicol inhibits the disintegration of chlorophyll (Knypl, 1970, Knypl and Mazurczyk, 1972), in light it inhibits its synthesis in many plants such as broad bean and barley (Osipova et al., 1967, Nikolayeva et al., 1970), kidney bean (Margulies, 1966) tomato (Döbel, 1963) and pumpkin (Banerji and Laloraya, 1967). The inhibiting action of chloramphenicol on the chlorophyll synthesis has been also shown in this study. The effect of chloramphenicol on the differentiation of plastids in lupin cotyledons results in an incomplete development of the stroma thylakoids, in the irregular arrangement of enlarged grana and the appearance of vesicular structures.

These ultrastructural changes were similar to those observed by Döbel (1963), Margulies (1966), Nikolayeva et al., (1970), Srivastava et al., (1971) and it is possible that they result from an inhibition of the synthesis of a protein precursor which is normally incorporated into the lamellae (Margulies, 1966; Ellis and Forester, 1972). If so, this protein must be synthesized on the plastid ribosomes, as chloramphenicol is a well known inhibitor of this synthesis (Eisenstadt and Brawermann, 1964; Spencer and Wildman, 1964; Margulies, 1970). This kind of protein must be responsible among other more for the arrangement of grana into the plastid matrix than for the synthesis of some protein particles (Goodenough and Staechelin, 1971) involved in the stacking process of grana thylakoids. However, Wellburn and Wellburn (1973) investigated the developmental changes in isolated etioplasts with the use of some antibiotics, and observed that chloramphenicol prevented the bi- and poly-thylakoid state of lamellae formation. Similar effect in chloramphenicol treated chloroplast of *Euglena* was observed by Bishop and al. (1973). These results are in opposition to our observations and support the suggestion that chloramphenicol inhibits the synthesis of proteins responsible for the fusion of individual lamellae in the chloroplast. Yet it may be, that the arrangement of thylakoids in chloramphenicol stimulated giant grana undergoes in different pattern than the stacking process of thylakoids in normally chloroplast differentiation but this should be confirmed first by other techniques.

The synergistic action of kinetin and chloramphenicol on the formation of giant grana as found in the protonema of *Funaria hygrometrica* (Młodzianowski and Szweykowska, 1971) has not been observed in lupin cotyledons. On the contrary, kinetin prevented some destructive changes induced by chloramphenicol in the thylakoidal arrangement.

In lupin cotyledons kinetin not only overcame the inhibitory effect of chloramphenicol on the chlorophyll synthesis but caused the chlorophyll level to increase much above that of the control material.

Banerji and Laloraya (1967) investigated in detail the influence of kinetin and chloramphenicol on chlorophyll synthesis in pumpkin cotyledons. These authors concluded that kinetin markedly promotes the synthesis of protochlorophyll and chloramphenicol strongly inhibits the development of both chlorophyll, a and b. Kinetin can overcome chloramphenicol inhibition of chlorophyll b synthesis to a greater extent than of chlorophyll a synthesis. During the induction of greening in tobacco tissue cultures by kinetin, the production of chlorophyll b was favoured over that of chlorophyll a (Kaul and Sabharwal, 1971). These results indicated that synthesis of chloro-

phyll a and chlorophyll b occurs by different pathways and kinetin promotes synthesis of chlorophyll b more than that of chlorophyll a.

Some experiments are in progress to investigate in more details this problem in the lupin cotyledons with the use of inhibitors complementary to chloramphenicol.

We wish to thank Prof. A. Szweykowska for the facilities and critical reading of this report.

REFERENCES

- Banerji D., and M. M. Laloraya, 1967. Chlorophyll formation in isolated pumpkin cotyledons in the presence of kinetin and chloramphenicol. *Plant Cell Physiol.* 8: 263—268.
- Bergfeld L., 1968. Chloroplasten ausbildung und Morphogenese der Gametophyten von *Dryopteris filix-mas* (L.) Schott nach Applikation von Chloramphenicol und Actidion (Cycloheximid). *Planta (Berl.)* 81: 274—279.
- Berridge M. V. and R. K. Ralph, 1971. Kinetin and carbohydrate metabolism in chinese cabbage. *Plant Physiol.* 47: 562—567.
- Bishop D. G., J. M. Bain and R. M. Smillie, 1973. The effect of antibiotics on the ultrastructure and photochemical activity of developing chloroplasts. *J. Exp. Bot.* 24: 361—376.
- Boothby D. and S. T. C. Wright, 1962. Effects of kinetin and other plant growth regulators on starch degradation. *Nature* 196: 389—390.
- Dennis D., M. Stubbs and T. P. Coultate, 1967. The inhibition of brussel sprout leaf senescence by kinins. *Can. J. Bot.* 45: 1019—1024.
- De Vecchi L., 1971. Fine structure of detached oak leaves senescing under different experimental conditions. *Isr. J. Bot.* 20: 169—183.
- Döbel P., 1963. Untersuchung der Wirkung von Streptomycin, Chloramphenicol und 2-Thiouracyl Behandlung auf die Plastidenentwicklung von *Lycopodium obscurum*. *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 (Berl.)* 57: 51—70.
- Eisenstadt J. M. and G. Brawermann, 1964. The protein — synthesizing systems from the cytoplasm and the chloroplast of *Euglena gracilis*. *J. Molec. Biol.* 10: 392—402.
- Ellis R. J. and E. E. Forrester, 1972. The function of chloroplast ribosomes. *Proceedings of the Biochemical Society, Biochem. J.* 130: 28.
- Engelbrecht A. H. P. and T. E. Weier, 1967. Chloroplast development in the germinating sunflower (*Carthamus tinctorius*) cotyledons. *Amer. J. Bot.* 54: 44—54.
- Farinneau N. and M. J. Roussaux, 1970. Influence de la 6-benzylamino-purine sur l'évolution des amyloplastes de cotyledons de pois cultivés *in vitro*. *C. R. Acad. Sc. Paris.* 273: 181—191.
- Fletcher R. A. and D. McCullagh, 1971. Cytokinin — induced chlorophyll formation in cucumber cotyledons. *Planta (Berl.)* 101: 88—90.
- Goodenough U. W. and L. A. Staehelin, 1971. Structural differentiation of stacked and unstacked chloroplast membrane. Freeze etch electron microscopy of wild type and mutant strains of *Chlamydomonas*. *J. Cell Biol.* 48: 596—619.

- Hall R. H., 1973. Cytokinins as a probe of developmental processes. *Ann. Rev. Plant Physiol.* 24: 415—444.
- Henningsen K. W. and Boynton J. E., 1970. Macromolecular physiology of plastids. VIII. Pigment and membrane formation in plastids of barley greening under low light intensity. *The Journal of Cell Biology.* 44:
- Horner H. T. and H. J. Arnott, 1966. A histochemical and ultrastructural study of pre- and post-germinated *Yucca* seeds. *Bot. Gaz.* 127: 48—64.
- Ikeda T., 1971. Prolamellar body formation under different light and temperature conditions. *Bot. Mag. Tokyo.* 84: 363—375.
- Kaul K. and P. S. Sabharwal, 1971. Effects of sucrose and kinetin on growth and chlorophyll synthesis in tobacco tissue cultures. *Plant Physiol.* 47: 691—695.
- Khokhlova V. A., I. N. Sveshnikova and O. N. Kulaeva, 1971. The influence of phytohormones on the formation of chloroplast structure in isolated pumpkin cotyledons. *Cytology.* 13: 1074—1079.
- Knypl J. S., 1970. Wpływ retardantów wzrostu, kumaryny, gibereliny i cytokinin na kiełkowanie nasion oraz syntezę i rozpad chlorofilu, białek i RNA w liściach i liścieniach. Uniwersytet Łódzki.
- Knypl J. S. and W. Mazurczyk, 1972. Retarding effect of inhibitors of Protein and RNA synthesis on chlorophyll and protein breakdown. *Biologia Plantarum* 14: 146—154.
- Lovell P. H., and K. G. Moore, 1970. The effects of benzylaminopurine on growth and ¹⁴carbon translocation in excised mustard cotyledons. *Ibid.* 23: 179—186.
- 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.
- Margulies M. M., 1970. In vitro protein synthesis by plastids of *Phaseolus vulgaris*. V. Incorporation of ¹⁴C leucine into a protein fraction containing ribulose — 1,5-diphosphate carboxylase. *Plant Physiol.* 46: 136—141.
- Mikulovich, T. P., V. A. Khokhlova, O. N. Kulaeva, I. N. Sveshnikova, 1971. Effect of 6-benzyl aminopurin on isolated gourd cotyledons. *Soviet Plant Physiol.* 18: 98—106.
- Młodzianowski F. and M. Kwintkiewicz, 1973. The inhibition of kohlrabi chloroplast degeneration by kinetin. *Protoplasma*, 76: 211—226.
- Młodzianowski F. and A. Ponitka, 1973. Ultrastructural changes of chloroplasts in detached parsley leaves yellowing in darkness and the influence of kinetin on that process. *Z. Pflanzenphysiol.* 69: 13—25.
- Młodzianowski F. and A. Szweykowska, 1971. Fine structure of kinetin-treated protonema and kinetin-induced gametophore buds in *Funaria hygrometrica*. *Acta Soc. Bot. Pol.* 40: 549—555.
- Moore K. G., A. Cobb and P. H. Lovell, 1972. Effects of sucrose on rooting and senescence in detached *Raphanus sativus* L. cotyledons. *J. Exp. Bot.* 23: 65—74.
- Narain A. and M. M. Laloraya, 1970. Inhibition of chlorophyll synthesis by kinetin in *Cucumis* cotyledons. *Plant Cell Physiol.* 11: 173—175.
- Nikolayeva, M. K. M. P. Vlasova, and O. P. Osipowa, 1970. Variation of the pigment composition and structure of chloroplasts induced by chloramphenicol. *Soviet Plant Physiol.* 17: 5—13.
- Öpik H., 1968. Development of cotyledon cell structure in repening *Phaseolus vulgaris* L. during germination. *J. Exp. Bot.* 17: 427—439.

- Osipova O. P., M. K. Nikolayeva and C. J. Hein, 1967. On the action of chloramphenicol on the photosynthetic apparatus of plants. *Soviet Plant Physiol.* 14: 210—218.
- Reynolds E. S., 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* 17: 208—213.
- Sarkissian I. V. and R. C. Huffaker, 1962. Depression and stimulation by chloramphenicol of development of carboxylating enzyme activity in inbred and hybrid barley. *Proc. Nat. Acad. Sci. USA* 48: 735—743.
- Schötz F., L. Diers, P. Ruffer, 1971. Abgabe von Plastidanteilen in das Cytoplasma. Eine vergleichende lichtmikroskopische und elektronenmikroskopische Untersuchung. *Ber. Dtsch. Bot. Ges.* 84: 41—51.
- Shaw M. and M. S. Manocha, 1965. Fine structure in detached senescing wheat leaves. *Canad. J. Bot.* 43: 747—755.
- Spencer D. and S. G. Widmann, 1964. The incorporation of amino acids into protein by cell free extracts from tobacco leaves. *Biochemistry* 3: 954—959.
- Srivastava L. M., M. Vesik and A. P. Singh, 1971. Effect of chloramphenicol on membrane transformations in plastids. *Can. J. Bot.* 49: 587—593.
- Stetler D. A. and W. M. Laetsch, 1965. Chloroplast structure and function in cultured tobacco tissue. *Amer. J. Bot.* 52: 798—804.
- Sugiura M., K. Umemura and Y. Oota, 1962. The effect of kinetin on protein level of tobacco leaf discs. *Physiol. Plant.* 15: 457—464.
- Sveshnikova J. N., O. N. Kulaeva and Y. P. Bolyakina, 1966. Formation of lamellae and granae in the chloroplasts of yellow leaves induced by 6-benzilaminopurine. *Soviet. Pl. Physiol.* 13: 681—686.
- Wellburn A. R. and F. A. M. Wellburn, 1973. Developmental changes of etioplasts in isolated suspensions and in situ. *Ann. Bot.* 37: 11—19.
- Wrósch M., 1967. The effects of inhibitors of protein synthesis on the differentiation of plastids in etiolated bean seedlings. *Planta (Berl.)* 173: 324—327.

Authors' addresses:

Dr. Fortunat Młodzianowski
mgr. Ewa Gezela
Institute of Biology,
Adam Mickiewicz University,
ul. Stalingradzka 14,
61-713 Poznań, Poland

Wpływ kinetyny i chloramfenikolu na syntezę chlorofilu i rozwój chloroplastów w odciętych liściach łubinu w warunkach słabego oświetlenia

Streszczenie

Przy słabym oświetleniu kinetyna w stężeniu 20 mg/l stymulowała, a chloramfenikol w stężeniu 50 mg/l hamował syntezę chlorofilu w izolowanych liściach łubinu. Kinetyna nie tylko znosiła hamujący efekt chloramfenikolu, lecz stymulowała w jego obecności syntezę chlorofilu do poziomu wyższego niż w materiale kontrolnym.

Kinetyna przyspieszała degradację skrobi i rozwój chloroplastów, jednak jej dłuższe działanie powodowało szereg anormalności, jak pękanie otoczek u pewnych form, tworzenie licznych obłonionych, kulistych ciałek na ich obwodzie oraz akumulowanie w uwolnionych tylakoidach elektronowo-gęstej substancji.

W obecności chloramfenikolu obserwowano pewne zakłócenia w budowie tylakoidów stromy, występowanie struktur pęcherzykowatych w stromie oraz powiększanie gran i pęcznienie ich tylakoidów. Kinetyna zapobiegała pewnym anormalnościom.

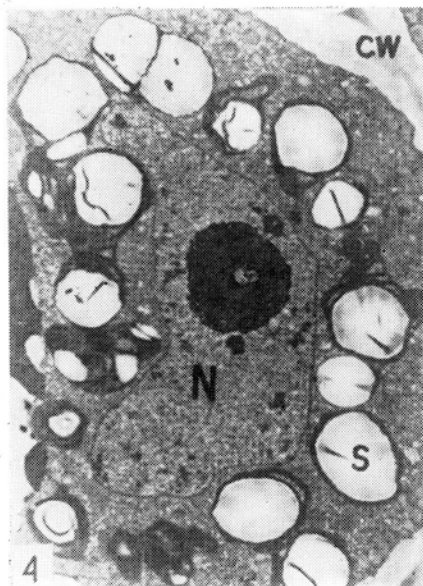
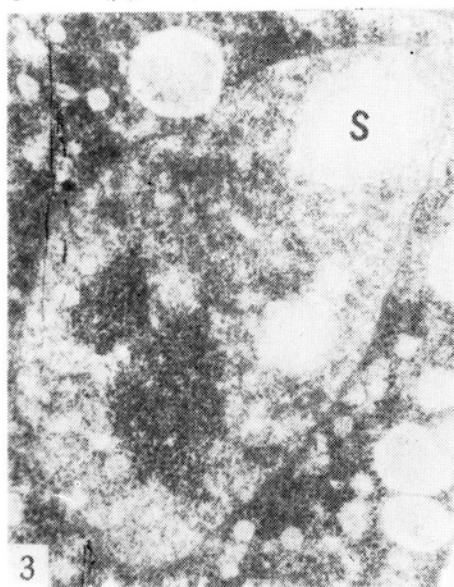
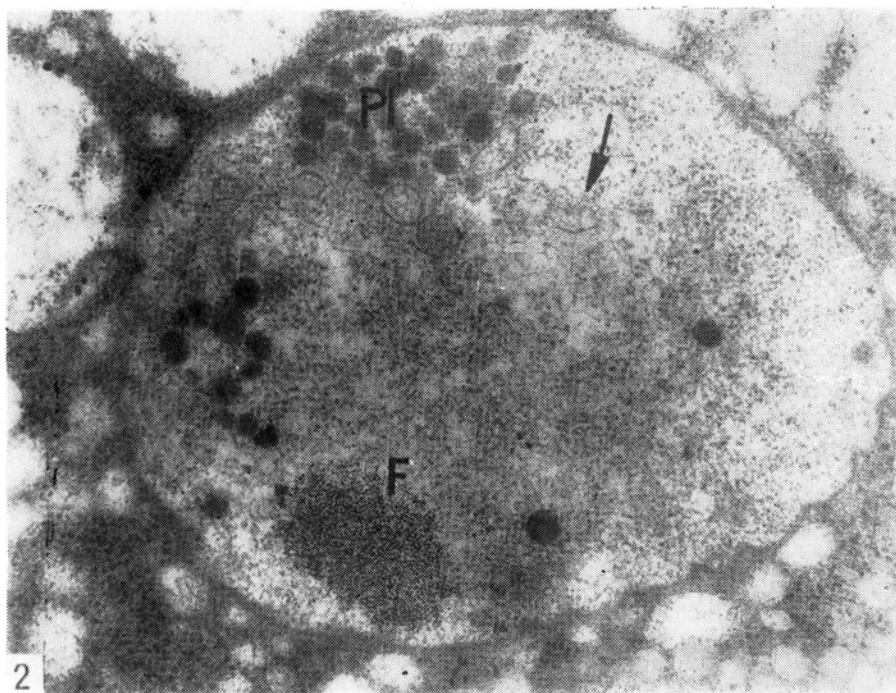


Fig. 2. Proplastid of a cotyledon from dry seed.

F — phytoferritin, Pl — plastoglobuli, Nucleoid — like area (arrow) with visible DNA fibrils. $\times 36\,000$.

Fig. 3. Proplastid with starch (S) of a cotyledon from a swollen seed. $\times 24\,000$.

Fig. 4. Cell fragment of the cotyledon exposed to light for 2 days.

N — nucleus, CW — cell wall, S — starch. $\times 3\,000$.

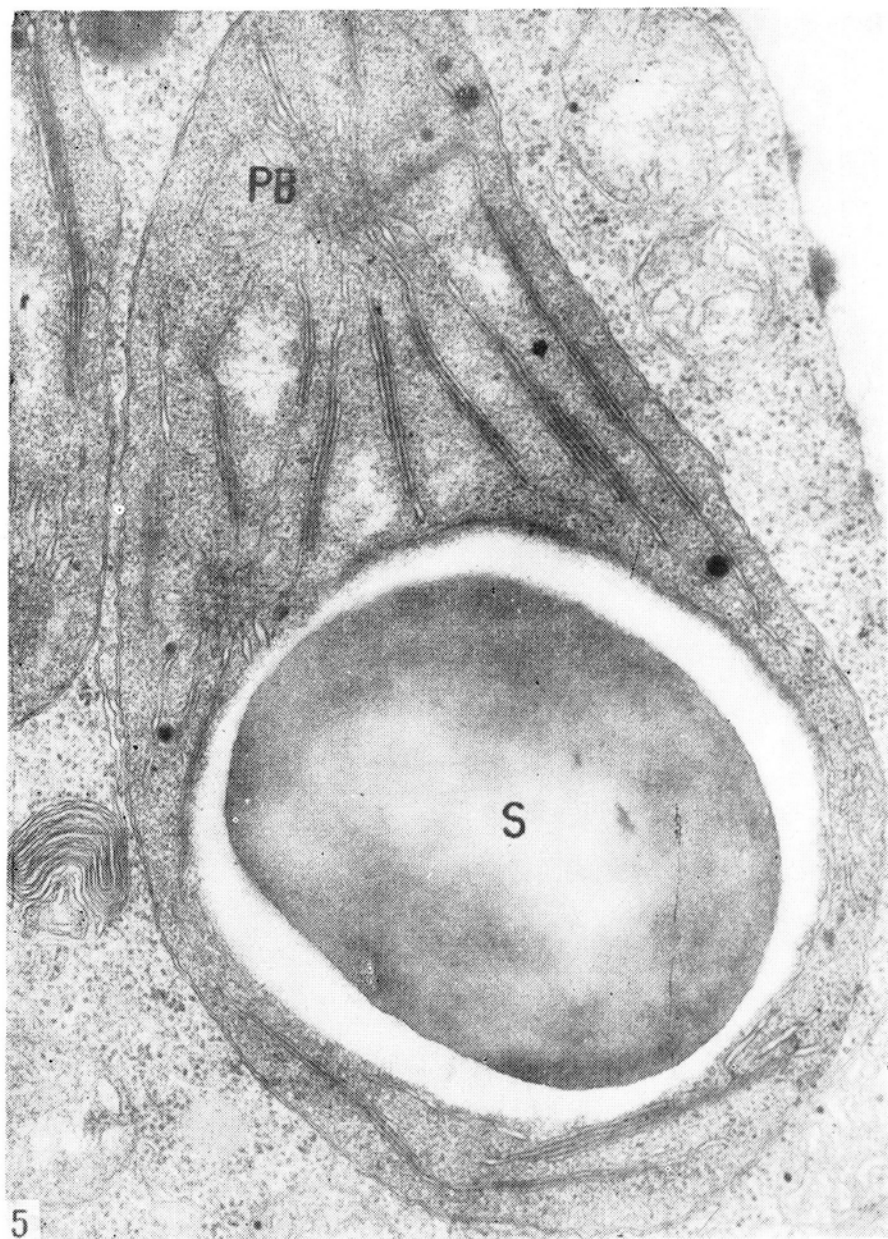


Fig. 5. The development of thylakoids from prolamellar bodies (PB) and the disintegration of starch (S) after 6 days of exposing the cotyledons to light. $\times 30\,000$.

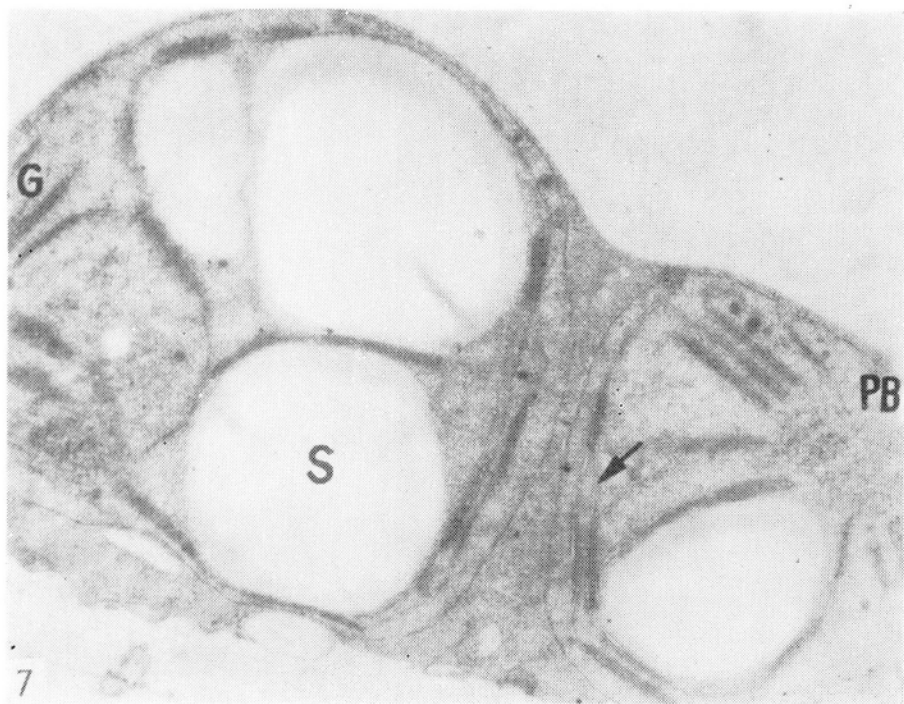
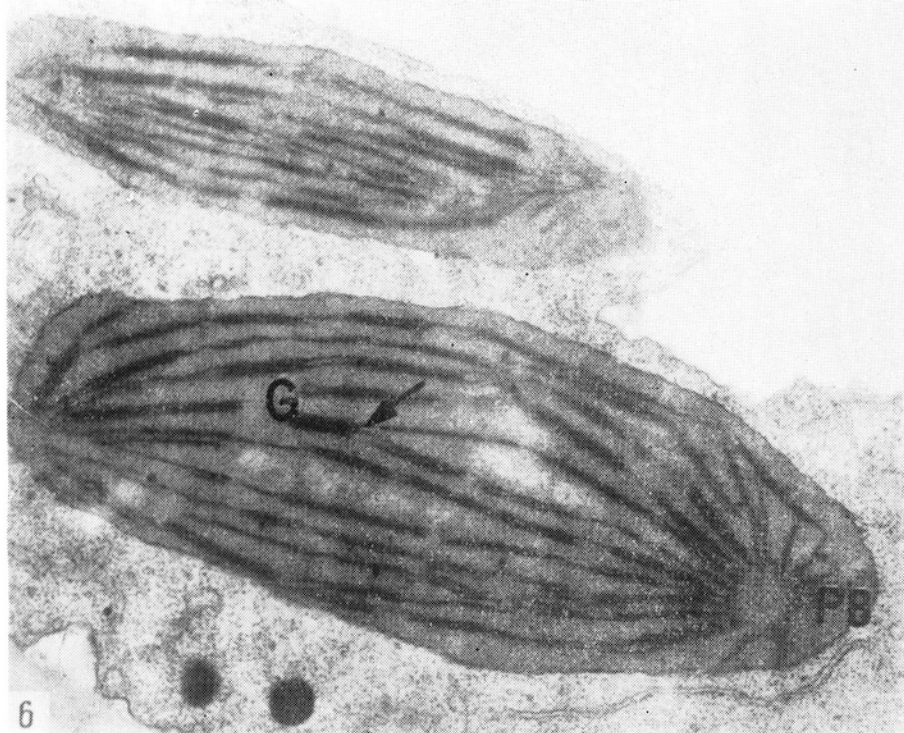


Fig. 6. Fully developed grana thylakoids (G) and stroma thylakoids (arrow) within the "A" type plastids of the control material after 12 days exposing the cotyledons to light. PB — prolamellar body. $\times 17\,000$.

Fig. 7. "B" type of plastid of the control material after 12 days of exposing the cotyledons to light. Grana — G, stroma thylakoids (arrow), prolamellar body (PB), starch (S), $\times 16\,000$.

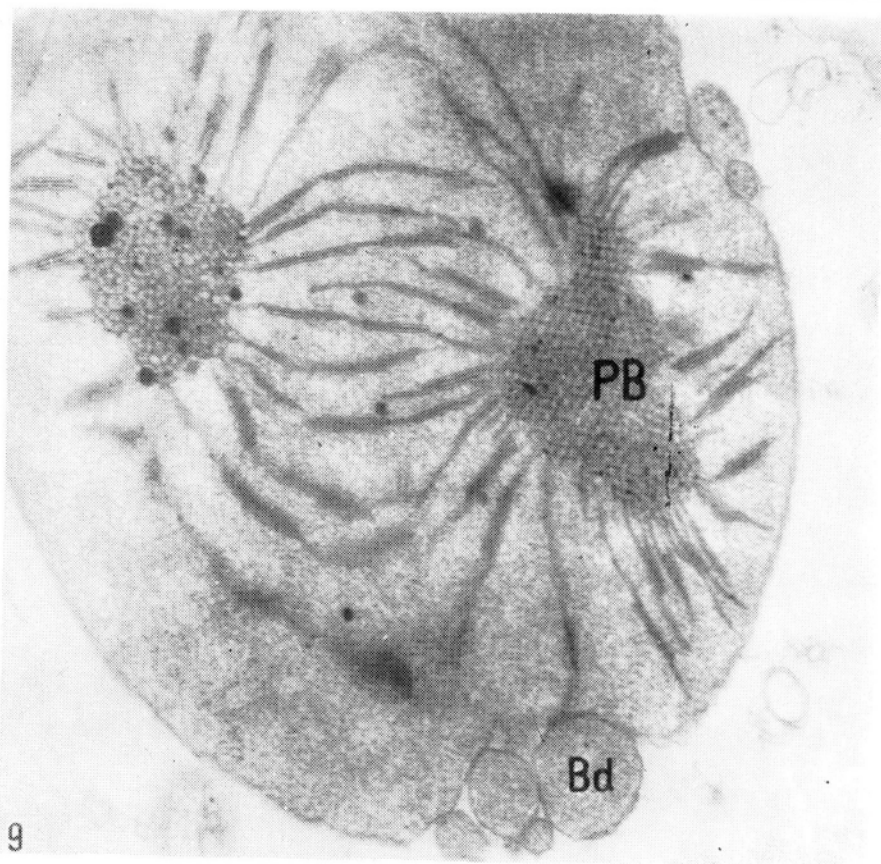
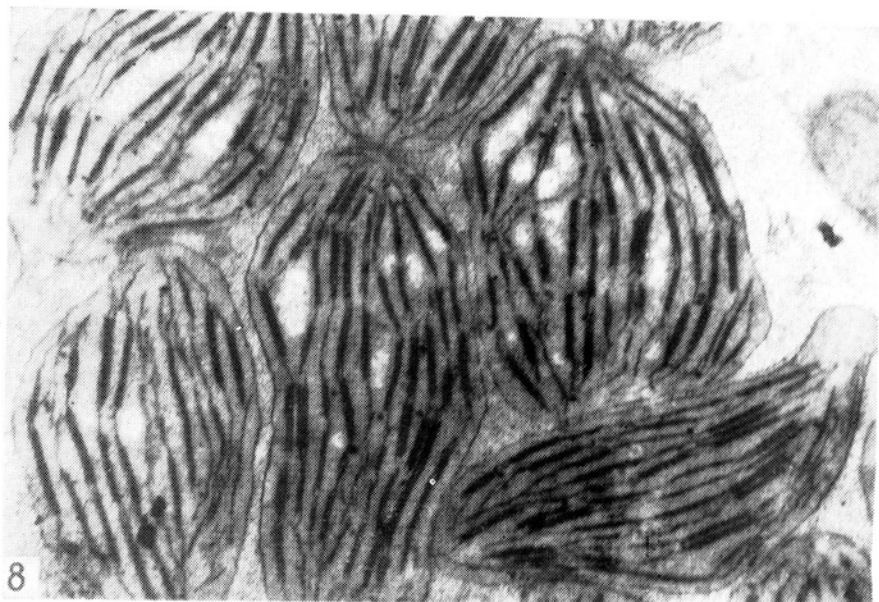


Fig. 8. Chloroplast of the "A" type with a normally developed system of grana and stroma thylakoids in the kinetin treated material after 6 days of its exposure to light. $\times 13\,000$.

Fig. 9. Chloroplasts of a "B" type with a large prolamellar body (PB) in kinetin-treated material after 6 days of exposure to light. Spherical bodies (Bd) present at the boundary, $\times 26\,000$.

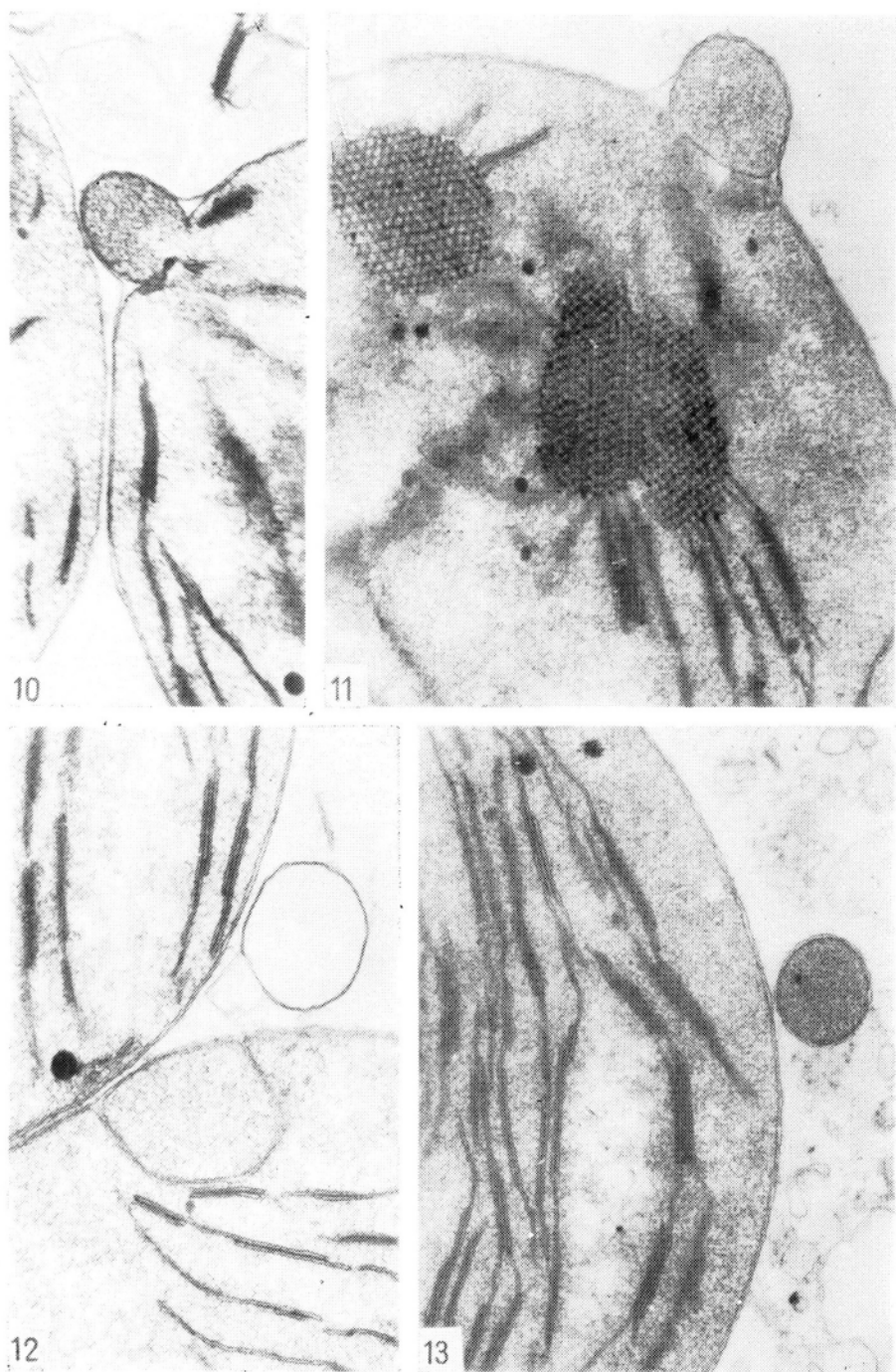


Fig. 10—13. Various forms of spherical bodies developing from swollen plastids ("B" type) in kinetin treated material after 6 days of exposure to light.

Each of the presented stages may theoretically give rise to a single — membrane body by a disruption of the outer membrane of the envelope as well as to a double-membrane body by a closing of the outer membrane. Fig. 13 — a double — membraned form.

Figs. 10 and 11 — $\times 34\,000$, Fig. 12 — $\times 29\,000$, Fig. 13 — $\times 27\,000$.

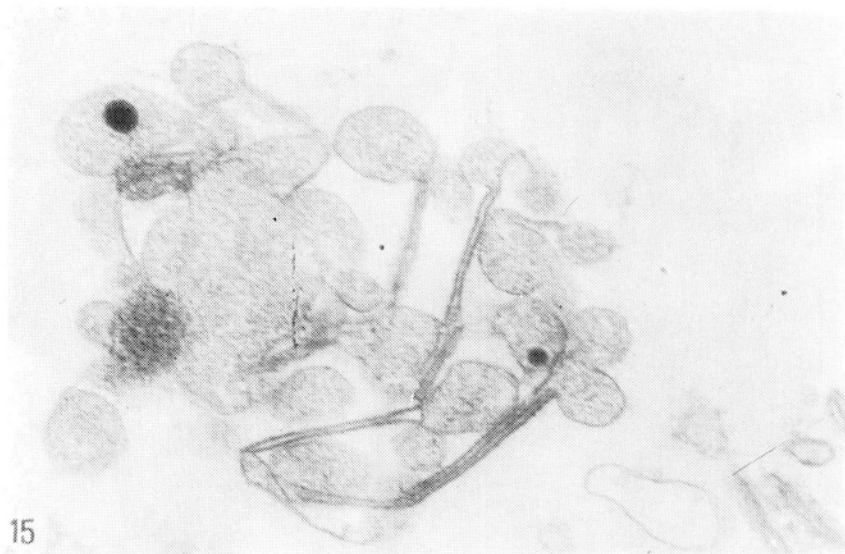
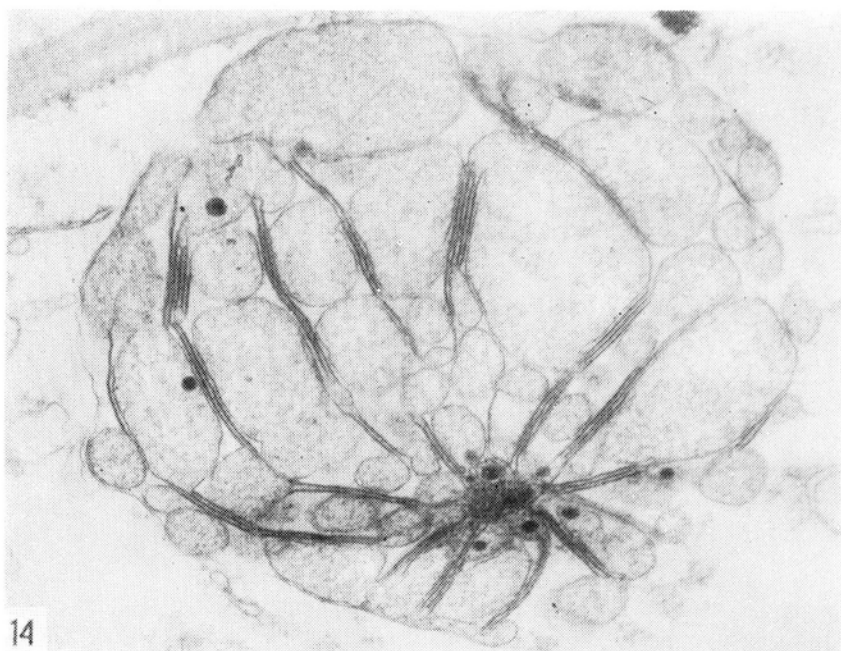
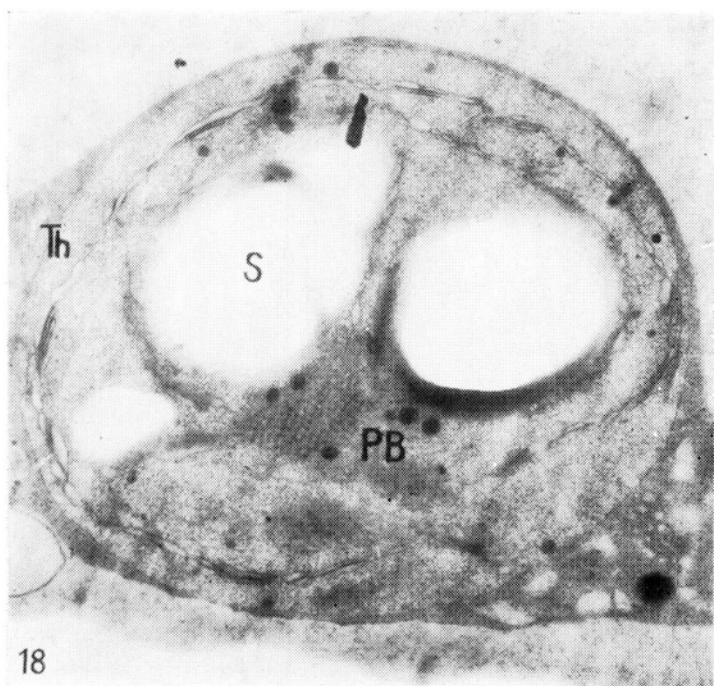
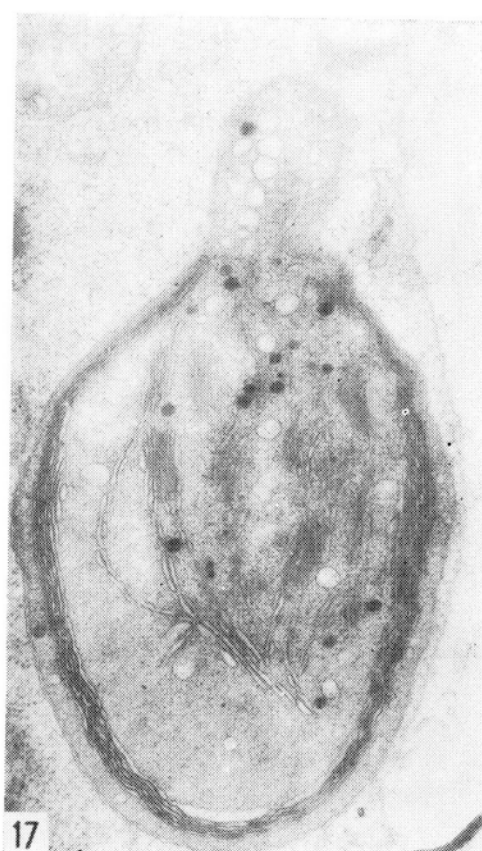


Fig. 14. Disintegration of a "B" type of plastid and a strong distension of thylakoids in the material treated with the kinetin and exposed to light for 12 days. $\times 27\,000$.

Fig. 15. Individual thylakoids with vesicular margins and electron — dense, fine granular content. $\times 17\,000$. $17\,000$.



Figs. 16—18. Chloroplasts of a cotyledon treated with chloramphenicol for 12 days.

Fig. 16 . Chloroplast of the "A" type. A noticeable lack of continuous stroma thylakoids; the large grana and vesicles separating from the inner membrane (arrow) are visible. $\times 23\,000$. On Fig. 17, long and concentric arranged thylakoids, scattered grana and vesicles are visible. $\times 20\,000$. Fig. 18. Chloroplast of the "B" type with swollen thylakoids (Th) on the periphery; starch grains (S) and prolamellar body (PB) are visible. $\times 13\,000$.

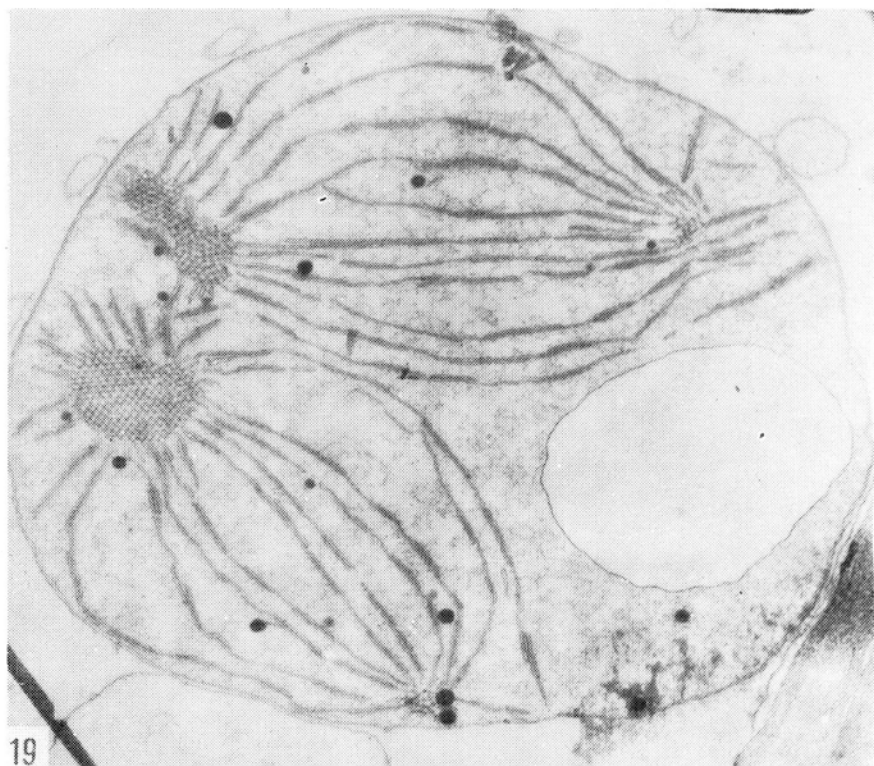


Fig. 19. Chloroplast of the "B" type after 12 days of the cotyledon development in the presence of kinetin and chloramphenicol. $\times 25\,000$.

Fig. 20. Chloroplast of the "A" type after 12 days of the cotyledon development with the presence of kinetin and chloramphenicol. Well developed grana and prolamellar bodies (reformation?) are visible. $\times 16\,000$.