

# Behaviour of cotyledonal plastids during seed germination in some chloroembryophytes

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## Abstract

Chloroembryophyte seeds with both epigeal and hypogeal germination patterns and with different plastid situations were tested in order to establish the greening ability of the cotyledon tissues during germination. No relation exists between either epigeal or hypogeal germination and cotyledon greening ability, nor between the presence of chloroplasts in the quiescent cotyledons and the restarting of chlorophyll synthesis during germination. Thus the chloroembryophytes, which are all characterized by chlorophyll synthesis during embryogenesis, do not always express such ability in the germinating seeds. If this ability is evident it appears to be species or genus specific.

## Introduction

Chloroembryophytes are defined as those angiosperms able to differentiate green tissues during embryogenesis (Pinfield *et al.*, 1973; Ryczkowsky and Szewczyk, 1973, 1975; Szewczyk, 1974; Zhukova, 1975, 1978; Periasamy and Vivekanandan, 1981; Palanisamy and Vivekanandan, 1985). This characteristic is distinctive of none or all of the species belonging to a given genus (Dahlgren 1980). A plant is a chloroembryophyte if it synthesizes chlorophyll during embryogenesis, independently of the fact that the chlorophyll still persists in the mature seed or is present only during some steps of seed development (Yakovlev and Zhukova, 1980).

In numerous chloroembryophytes, in fact, the chloroplasts of embryo and, in particular, of cotyledonal leaves, undergo a dedifferentiation process in the seeds about to reach complete maturity, becoming amyloplasts or proplastid-like organelles devoid of thylakoids and pigments (Zhukova, 1981; Fischer *et al.*, 1988). Only in some species do the plastids maintain the inner membrane system and the cotyledons remain green in the mature quiescent seed (Zhukova, 1981; Whatley and Price, 1983; Casadoro and Rascio, 1987; Rascio and Casadoro, 1989).

Recent observations on germinating seeds of different chloroembryophytes belonging to the genera *Citrus* and *Fortunella* (Casadoro and Rascio, 1987; Rascio and Casadoro, 1989) showed that in all the species examined, in spite of the hypogeal model of germination, the cotyledons turned green in light, reaching levels of photosynthetic activity comparable with those of the primary leaves.

The present research has been carried out, as a consequence of such findings, to search for answers to the following questions. (1) Are the cotyledons of the chloroembryophytes all able to differentiate chloroplasts during embryogenesis, and is the ability to restart synthesizing chlorophyll during seed germination a common feature in the cotyledonal leaves? (2) Is the greening ability of cotyledons quite independent of the hypogeal or epigeal model of seed germination? (3) In the event that not all the chloroembryophyte cotyledons synthesize chloro-



phyll during germination in light, is this ability also shown by those species whose cotyledons remain green in the quiescent seeds? These questions must also be considered in relation to the hypothesis of Marin and Dengler (1972) and the work of other investigators (e.g. Zhukova, 1975), who indicated that the persistence of chloroplasts in quiescent cotyledons might be regarded as storage of photosynthetic membranes useful during the early stages of germination. Thus, in order to elucidate such problems four different chloroembryophytes were chosen and studied.

### Materials and methods

The first two species used in this work had white quiescent seeds and belonged to the same genus *Phaseolus*, but were characterized by a different model of seed germination, which was hypogeal in *Phaseolus multiflorus* Willd. and epigeal in *Phaseolus vulgaris* L. The other two, pea (*Pisum sativum* L.) and pistachio (*Pistacia vera* L.) were known for having hypogeal germination and green cotyledons in the quiescent seeds.

Seeds of the four species were soaked overnight in water in the dark and, after removal of coats, were germinated at 25°C on moistened filter paper in Petri dishes either in darkness or under continuous illumination, at a photofluence rate of 120  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

#### Electron microscopy

Cotyledon samples, collected at different times after germination, were fixed and prepared for transmission electron microscopy as described by Rascio *et al.* (1979).

#### Chlorophyll determination

The chlorophyll was determined spectrophotometrically according to Moran and Porath (1980) using the extinction coefficients proposed by Inskeep and Bloom (1985).

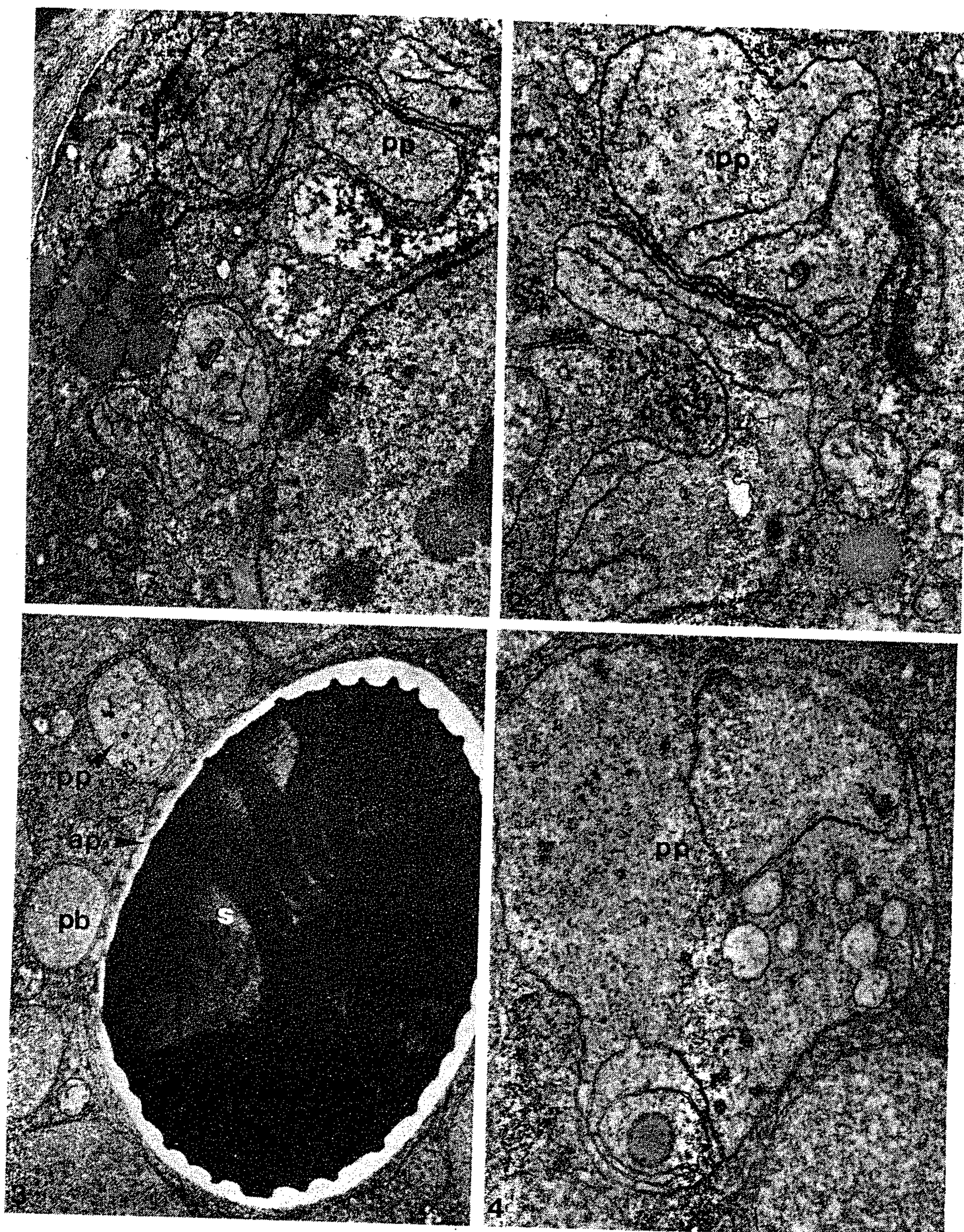
### Results and discussion

#### *Phaseolus* species

Comparing the two species of *Phaseolus*, the cellular structure in the white quiescent cotyledons (here shown in *P. multiflorus*) was similar. In both cases there were a few outer cell layers, almost lacking in reserves (Figure 1), with several proplastids devoid of starch (Figure 2). In the inner tissues, the reserves were abundant (Figure 3) and essentially were represented by proteins in numerous protein bodies and starch granules filling voluminous amyloplasts. In these cells, two different plastid populations could be seen. In fact, besides the very large amyloplasts, small proplastids (Figure 3) like those present in the outer cotyledonal region (Figure 4) were recognizable.

During germination in light, the cotyledons of both the epigeal and the hypogeal bean turned green, reaching comparable levels of chlorophyll (Figure 7), and showing similar ultrastructural features of plastids (here shown in the hypogeal





Figures 1 to 4 Quiescent hypogeal cotyledon of *Phaseolus multiflorus*; ap, amyloplast; pb, protein body; pp, proplastid; s, starch.

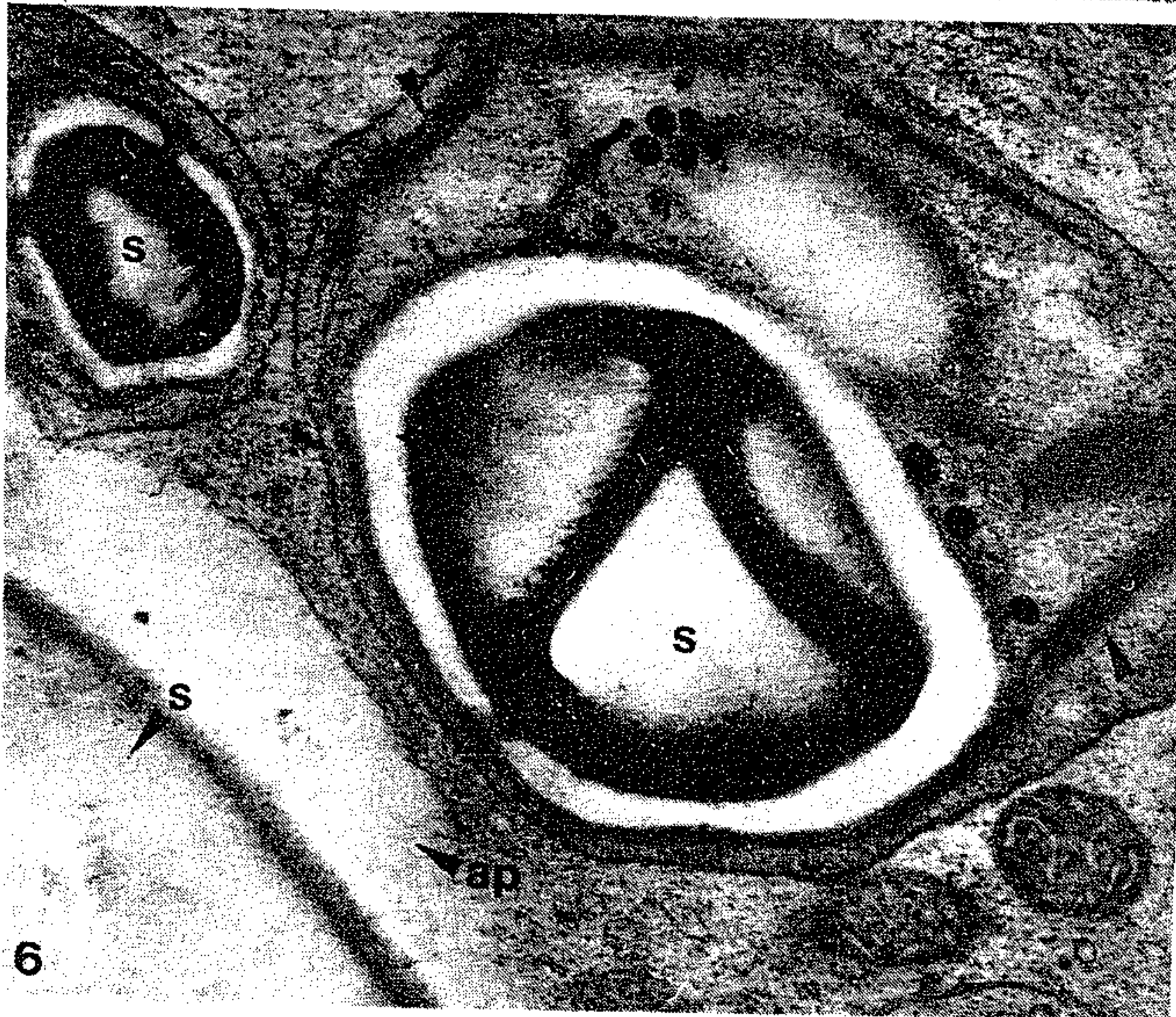
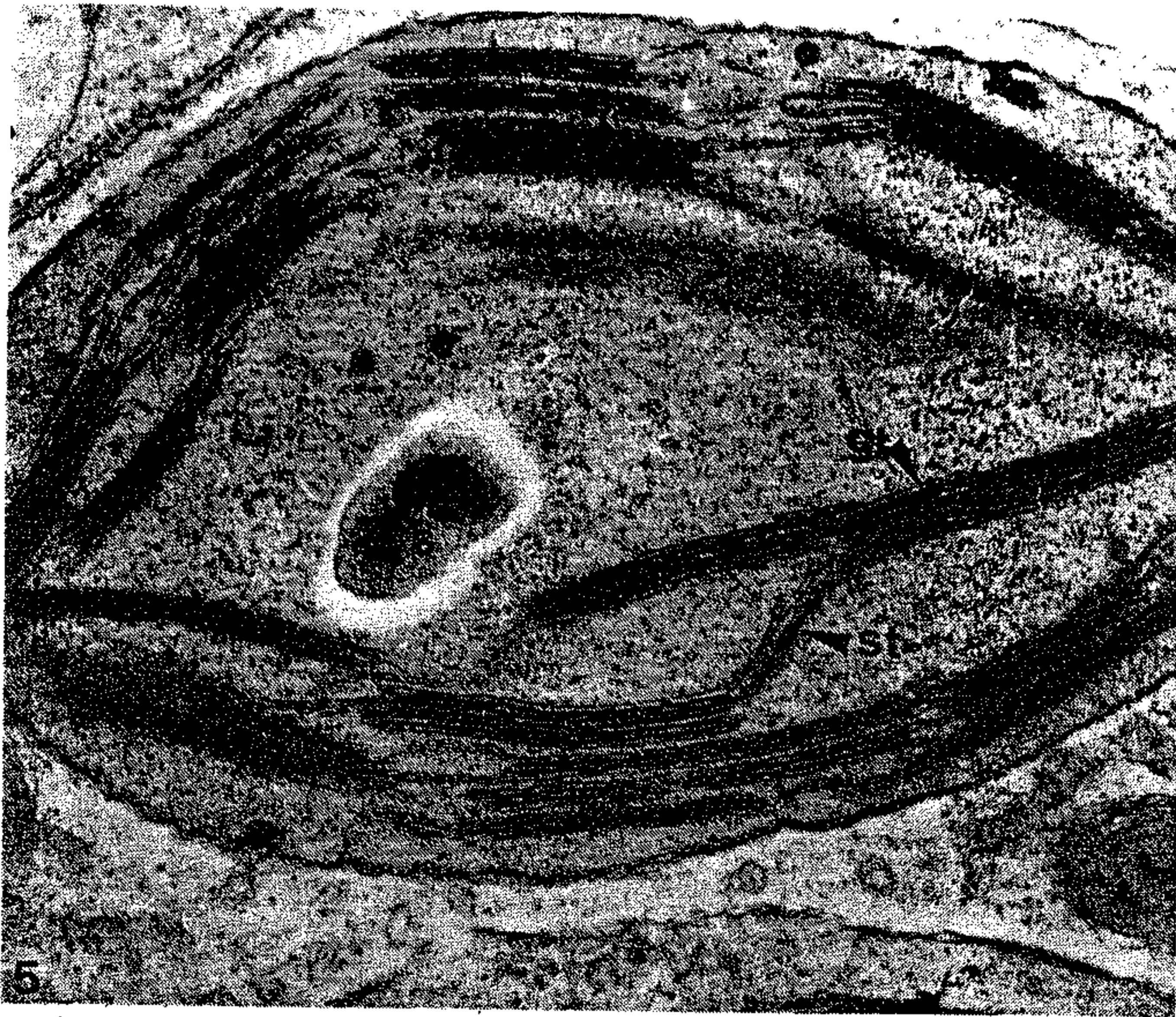
Figure 1 Proplastids in an outer cell almost devoid of reserves. x10,000.

Figure 2 Note the very reduced membrane system of the organelles. x18,000.

Figure 3 In the inner cell a voluminous amyloplast and a small proplastid are visible. x8,000.

Figure 4 The proplastid of an inner cell shows very few membranes. x28,000.



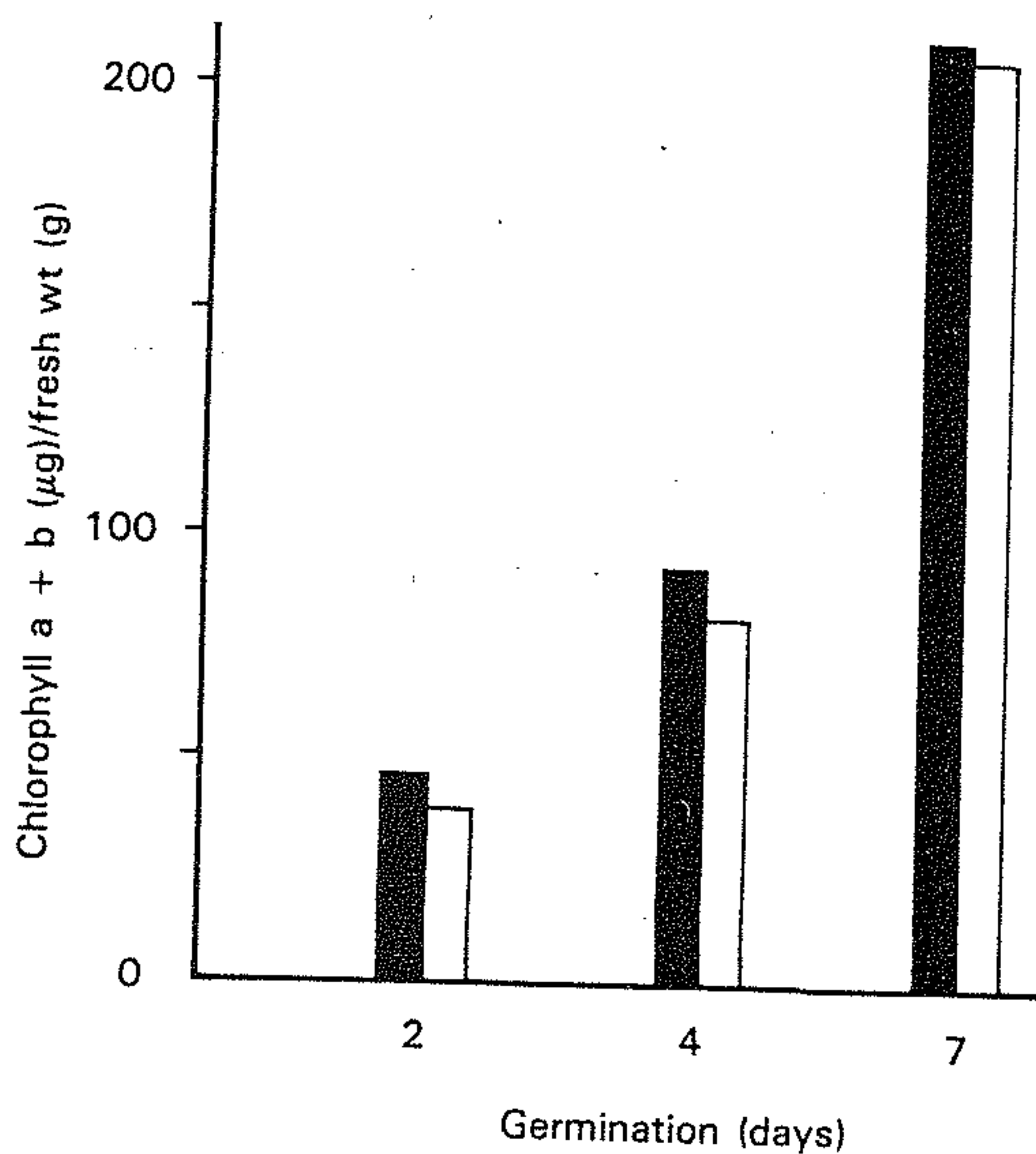


**Figures 5 and 6** Hypogeal cotyledon of *Phaseolus multiflorus* 7 days after germination in light. gt, Granal thylakoids; st, stroma thylakoids.

**Figure 5** The chloroplast from the outer region shows a well organized membrane system. x28,000.

**Figure 6** In the inner cell a chloroplast is evident with a less abundant thylakoid system (arrows) and a starch grain. In the bottom left corner a region of a still present amyloplast is visible. x28,000.





**Figure 7** Histogram of chlorophyll contents in bean cotyledons at different periods from germination. Solid columns, *Phaseolus multiflorus*; open columns, *Phaseolus vulgaris*.

species). Only the proplastid population demonstrated the ability to build up thylakoids. Thus, 7 days after germination, chloroplasts with a well organized membrane system were present in the outer cell layers (Figure 5), while in the deeper tissues, chloroplasts having a less abundant thylakoid system and some starch granules were visible in the cells near the still present huge amyloplasts (Figure 6).

The behaviour of the bean cotyledon tissues was typical of epigeal organs. Interestingly, such behaviour was exhibited also by the seeds of *P. multiflorus*, whose cotyledons are hypogeal and hence destined to remain underground in natural conditions.

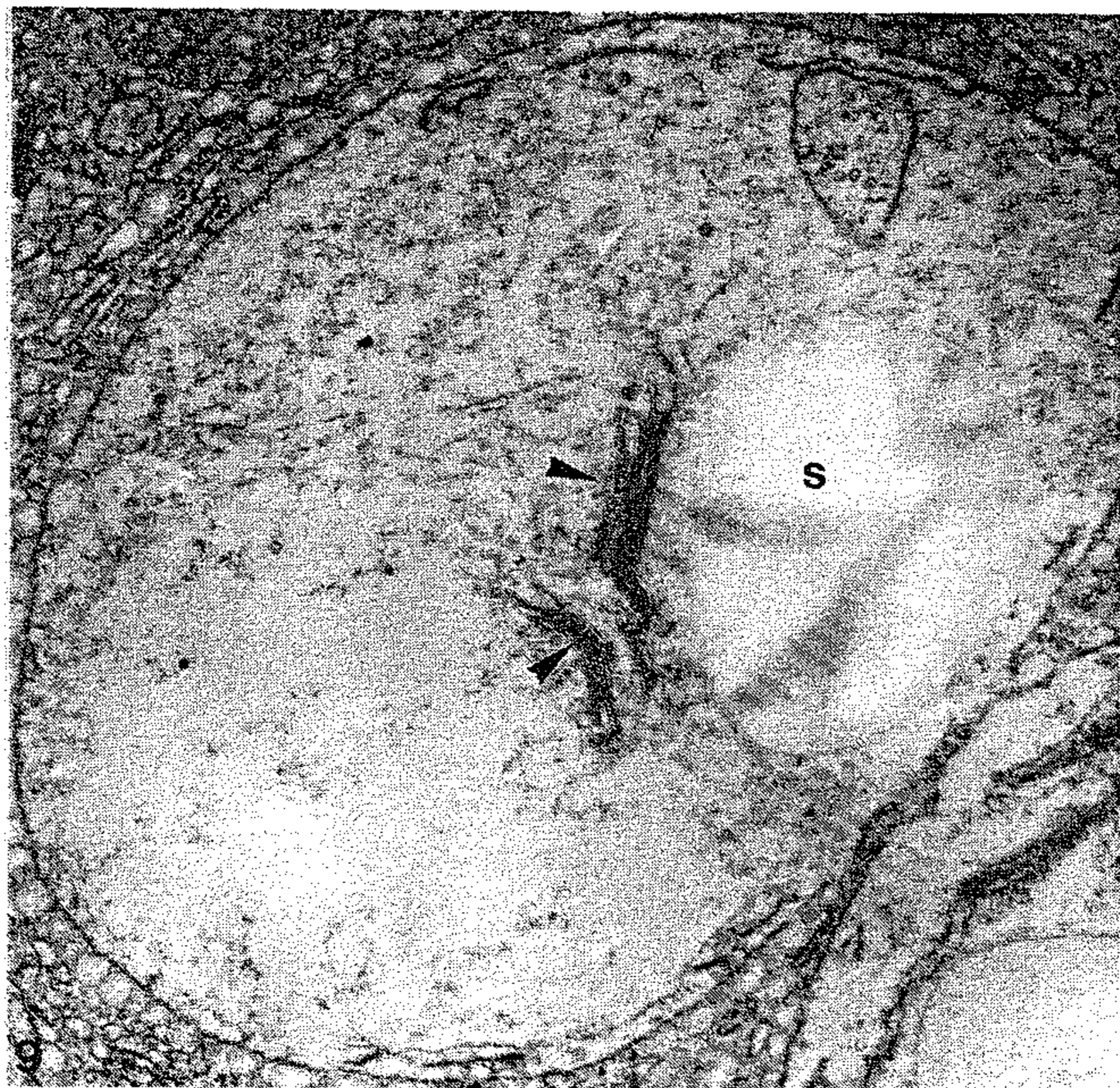
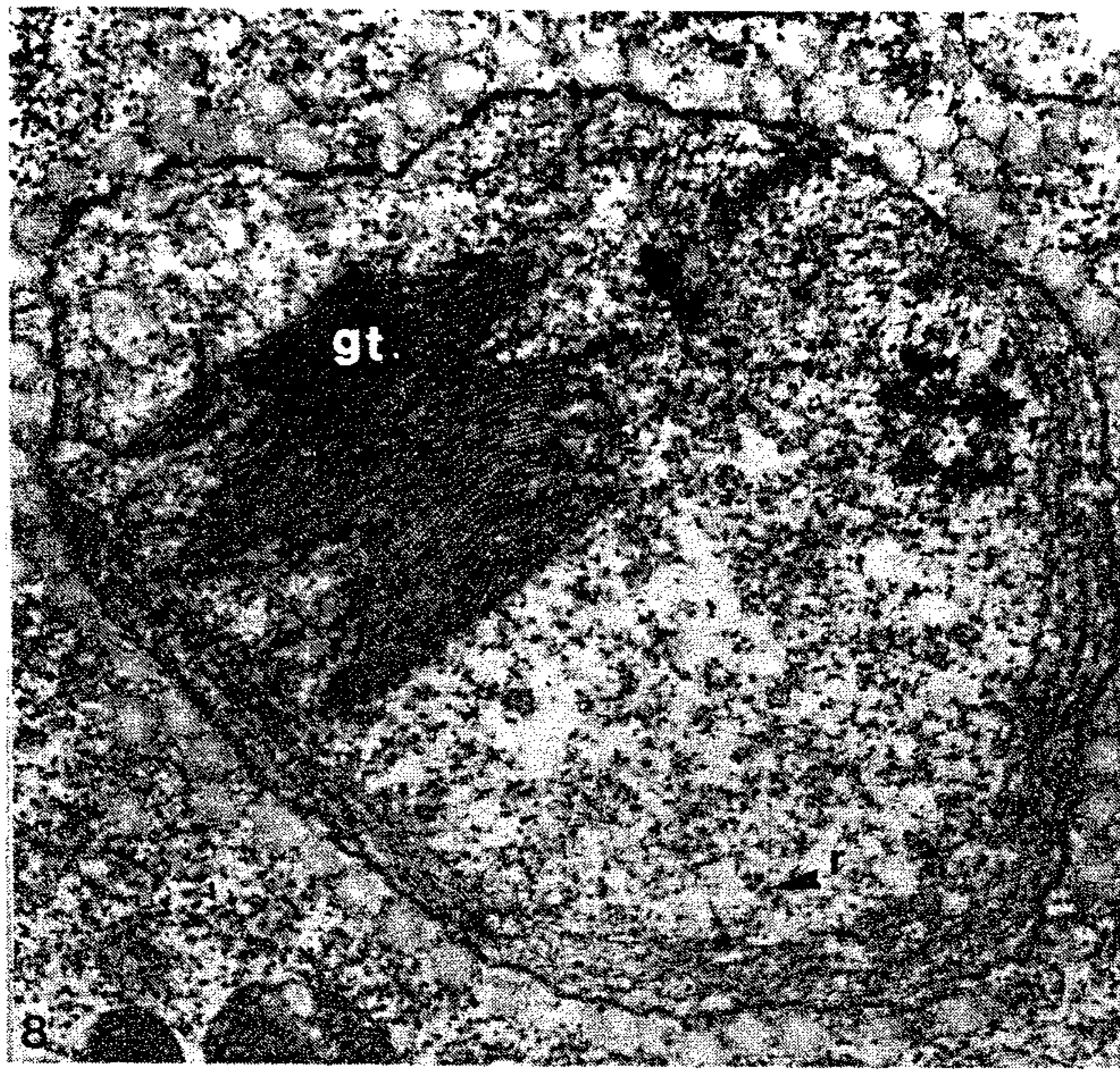
The two different species belonging to the genus *Phaseolus* showed the same response of cotyledon tissues to light. Thus, in these chloroembryophytes the plastid population which originated from the final dedifferentiation of chloroplasts formed during embryogenesis, namely the proplastids (Öpik, 1968), seems to have only temporarily interrupted chlorophyll and thylakoid synthesis.

The potential capability of cotyledon plastids to synthesize photosynthetic membranes during seed germination was also shown by the differentiation in the dark of aetioplasts with paracrystalline prolamellar bodies (Figure 15). This meant that the 'greening' programme was not triggered by the light, but was always expressed in the cotyledonal leaves, leading to the synthesis of pigment and membranes according to a model determined by the environmental conditions, with the differentiation of chloroplasts in light and of aetioplasts in the dark.

#### Pea

In the quiescent seed of pea the cotyledons were green, as shown by the chlorophyll content (Figure 10). In pea, as well as in bean, two different populations of plastids were visible in cotyledonal tissues. In this case they





**Figure 8** Chloroplast from a quiescent cotyledon of pea. Note the very large thylakoid stack and the ribosomes (r) in the stroma. x17,500.

**Figure 9** Plastid from a pea cotyledon after 6 h of germination in light. The thylakoids (arrows) are reduced and damaged. Ribosomes are not visible in the vacant stroma. x15,400.

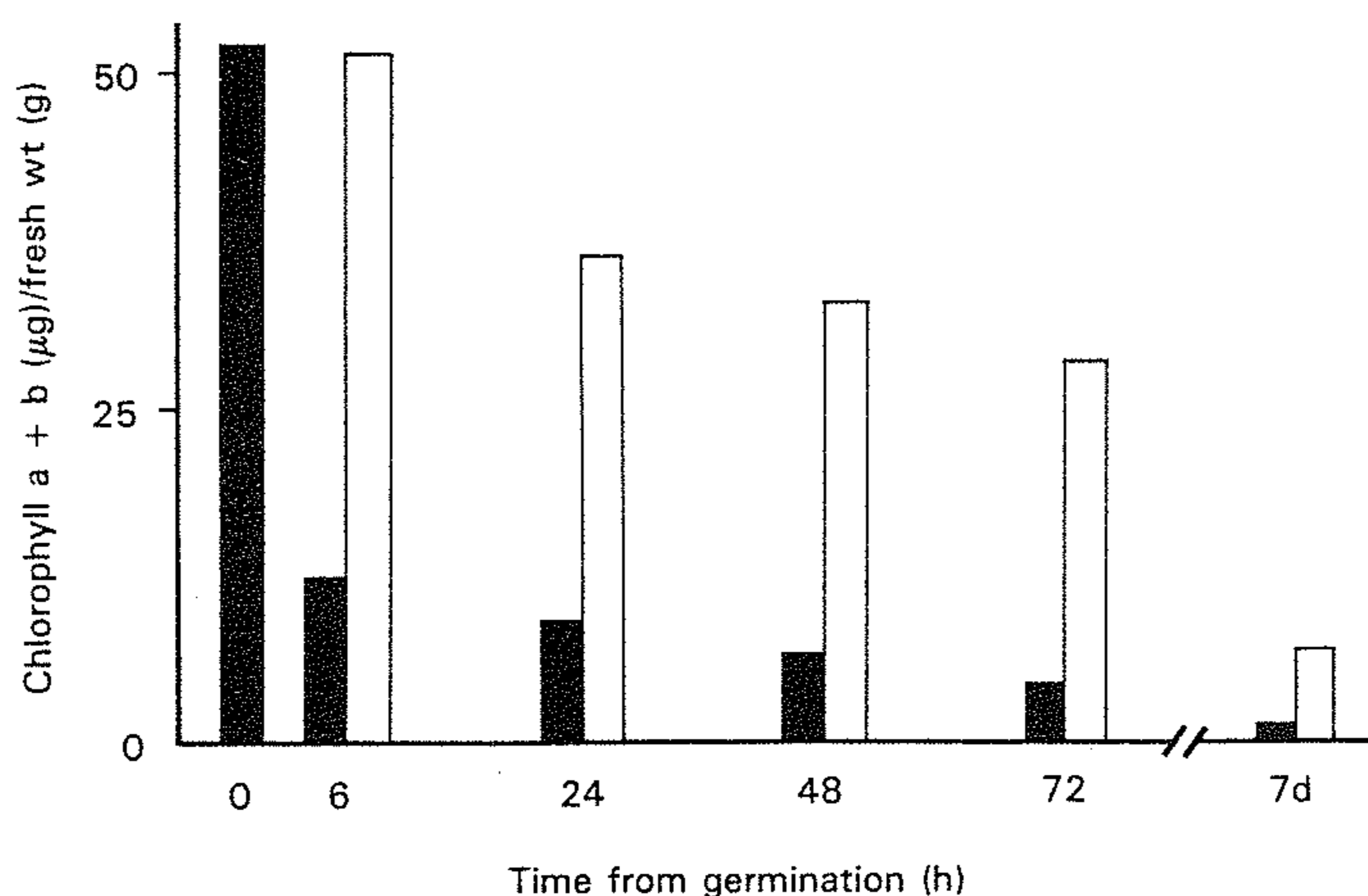


consisted of voluminous amyloplasts filled with starch granules and of chloroplasts which, less frequent in the inner tissues, became the only kind of plastid in the outer cell layers. These chloroplasts (Figure 8) were devoid of starch and contained numerous thylakoids forming very large stacks isolated in the stroma. Such a peculiar organization of the thylakoid system is frequent in chloroplasts from quiescent cotyledons of chloroembryophytes and has been noticed in several other species (Orsenigo, 1964; Marin and Dengler, 1972; Pinfield *et al.*, 1973; Casadoro *et al.*, 1980; Whatley and Price, 1983; Casadoro and Rascio, 1987; Rascio and Casadoro, 1989).

When the pea seeds were germinated in light, the cotyledons showed an immediate decrease in colour. In fact, after 6 h in light they looked yellowish and contained only a very small quantity of chlorophyll (Figure 10). Indeed, dramatically altered chloroplasts could be seen in the cells (Figure 9), with a few irregular stacks of thylakoids and vacant stroma lacking ribosomes, which were clearly visible in the organelles from the quiescent cotyledon (Figure 8).

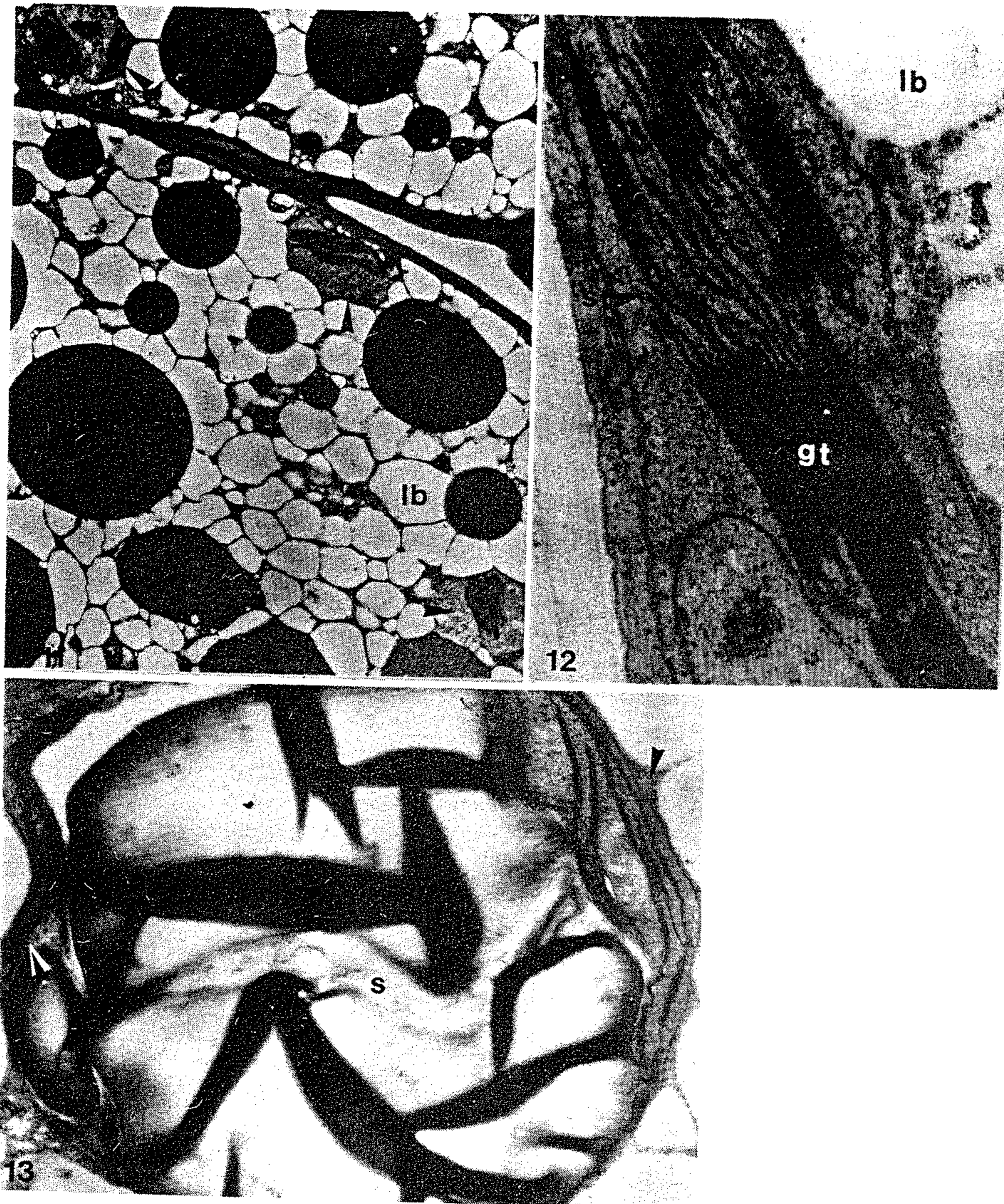
The reduction in chlorophyll was slower in cotyledons of seeds germinated in darkness (Figure 10), thus stressing the striking effect of light on the cotyledonal plastid bleaching. In the pea, the restarting of metabolic activity during germination was not associated with a reacquisition of the capability to synthesize chlorophyll by the green cotyledonal plastids. Moreover, the membranes built up during embryogenesis and retained in the quiescent seed showed an evident instability and a particularly high photolability.

The inability of plastids to synthesize *de novo* thylakoid membranes was also shown in seeds kept in darkness. After 7 days from germination, no prolamellar bodies were present in the altered organelles (Figure 17). Thus, the cotyledons of pea seeds, even if originally green, are programmed to carry out the exclusive function of storage organs, typical of the hypogeal model of germination (Kirk and Tilney-Bassett, 1978).



**Figure 10** Histogram of chlorophyll contents in quiescent pea cotyledons (0h) and in cotyledons at different periods from germination in light (solid columns) and in darkness (open columns).





**Figures 11 and 12** Quiescent cotyledons of pistachio.

**Figure 11** Chloroplasts (arrows) can be seen in the cells, among the reserves. lb, Lipid body. x3,420.

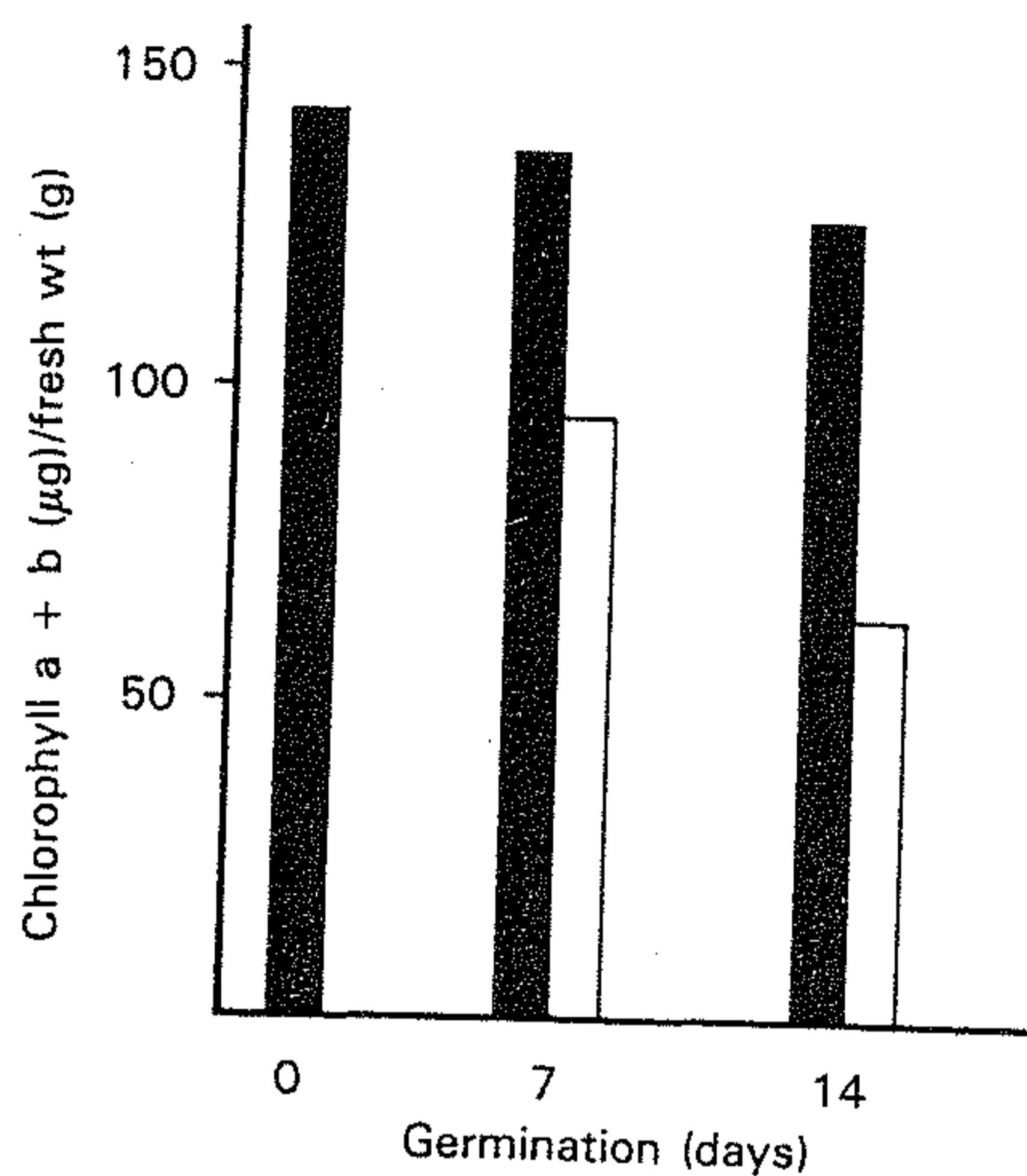
**Figure 12** The membrane system is organized in grana and stroma thylakoids (st). x38,475.

**Figure 13** Plastid from a pistachio cotyledon 14 days after germination in the light. Thylakoids (arrows) are still visible in the stroma, together with a large starch grain. x13,000.

### Pistachio

The quiescent seeds of pistachio had green cotyledons (Figure 14) in whose tissues chloroplasts were present among the reserves contained in numerous protein and lipid bodies (Figure 11). The chloroplasts, usually devoid of starch, showed an abundant membrane system, with large grana and several intergrana thylakoids (Figure 12).





**Figure 14** Histogram of chlorophyll contents in quiescent pistachio cotyledons (Oh) and in cotyledons at different periods from germination in light (solid columns) and in the dark (open columns).

During germination in light, the chlorophyll content in cotyledonal tissues decreased very slowly, so that after 14 days the cotyledons still looked green and contained plastids with a still distinguishable thylakoid system among the large starch grains (Figure 13). Unlike the observations in pea, the chlorophyll reduction in pistachio cotyledons in light was slower than that occurring in cotyledons of seeds germinated in darkness (Figure 14). This suggests that these cotyledonal chloroplasts are able, even if to a very limited extent, to synthesize chlorophyll during germination, so managing to partially sustain the pigment turnover in light.

Their ability to synthesize pigments and membranes, on the other hand, was shown also by the presence of paracrystalline prolamellar bodies (Figure 16) in cotyledon organelles after 2 weeks from germination in darkness. However, in spite of some greening ability, which only delayed the tissue bleaching, it is plausible to assert that pistachio cotyledons, as well as those of pea, have the main function of storage organs.

### Conclusions

In reaching conclusions it is evident that, although they are all chloroembryophytes, and hence able to synthesize chlorophyll during embryogenesis, the plants studied show very different behaviour during seed germination, as far as chlorophyll synthesis is concerned. In pistachio, whose seeds exhibit hypogeal germination, the cotyledon chloroplasts seem to have the ability, even if limited, to produce chlorophyll. This, however, only allows them to partially produce the pigment, without bringing about a rise in the chlorophyll content of the cotyledon tissues. The cotyledons of hypogeal seeds of pea, on the other hand, are not able to synthesize chlorophyll during germination. Moreover, the chloroplasts maintained in the quiescent cotyledons show a striking photolability, with light which dramatically damages pigment and membranes, as well as the stroma and the ribosomal component.





Figures 15 to 17 Plastids from cotyledons germinated in darkness. plb, Prolamellar body.  
**Figure 15** A large paracrystalline prolamellar body is present in the organelle of hypogeal bean cotyledon 7 days after germination. x21,375.  
**Figure 16** A prolamellar body is also visible among thylakoids (arrows) in the plastid of pistachio cotyledon 14 days after germination. x22,800.  
**Figure 17** No prolamellar body is seen in the organelle of pea cotyledon at 7 days from germination. Note the swollen thylakoids (arrows). x22,000.



The behaviour in light of germinating seeds of the hypogeal and epigeal species of bean appears quite different. In both cases, in fact, the white cotyledons turn green, and chloroplasts with a well organized inner membrane system differentiate from the initial proplastids. In these cotyledons, therefore, as well as in the epigeal ones of soybean (Harris *et al.*, 1986a,b; Brown and Huber, 1987), the reserve function is possibly associated with the photosynthetic process.

It can now be asserted that the common ability to synthesize chlorophyll during embryogenesis is not related in chloroembryophytes to an equally common ability to restart synthesizing these pigments during seed germination. In fact as far as this characteristic is concerned different species may show very different abilities, as can be seen from the behaviour of the plants examined.

A correlation does not even exist between the persistence of green plastids in quiescent cotyledons and the ability to synthesize chlorophyll during germination. This is clearly shown by the fact that the cotyledonal chloroplasts of pea are not able to produce chlorophyll, whose synthesis, on the contrary, is carried out by the initially white plastids of bean. This disproves the hypothesis that the persistence of chloroplasts in quiescent cotyledons is significant in photosynthetic membrane storage, which is useful during germination (Marin and Dengler 1972; Zhukova 1975). In chloroembryophytes, the benefit of synthesizing chloroplasts is limited to embryogenesis, when the production of green and photosynthesizing cotyledons (Pinfield *et al.*, 1973; Ryczkowsky and Szewczyk, 1973, 1975; Szewczyk 1974; Periasamy and Vivekanandan 1981) can, undoubtedly, contribute to the nutrition of the growing embryo. The persistence or not of chloroplasts in the mature seed is, then, a characteristic which depends on the species, but that does not lead to any real advantage during seed germination and seedling growth, as shown in other chloroembryophytes by Rascio and Casadoro (1989).

The cotyledon ability to synthesize chlorophyll is not even related to the germination model. In bean this ability is shown by both the species considered, one of them with epigeal, the other one with hypogeal germination. Thus, the ability to synthesize chlorophyll, which characterizes embryogenetic steps of the chloroembryophyte species is not always expressed in the germinating seeds of the same plants. Indeed, if such a capability is manifest, it seems to be species or genus specific, rather than depend on the model of seed germination, *i.e.* on the epigeal or hypogeal programming of cotyledons.

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# Review

## Bacterial Genetics in Natural Environments

Edited by J. C. Fry and M. J. Day

This is an overview of current thinking on bacterial genetics. The release of genetically engineered micro-organisms into environments may have great potential for both agriculture and industry, but much basic research is still required.

The book is the outcome of a meeting on Bacterial Genetics and Ecology held at the University of Wales in 1989. Some 60 participants, producing about 20 papers, discussed modern developments in the genetics of bacteria in aquatic and terrestrial habitats. Some of the introductory topics included genetic approaches to the study of gene transfer; factors influencing the dissemination of DNA by bacterial conjugation; gene transfer in bacteria; and phage genetics and ecology.

In the aquatic habitat papers included plasmid transfer in the epilithon; modern methodology; gene transfer in marine environments and inactivated sludge. In the terrestrial habitat plasmid transfer between soil bacteria and gene transfer in polluted soils, the use of wide host range promoters to monitor the fate of recombinant DNA in soil, the role of soil bacteria in risk assessment analysis, and gene transfer between streptomycetes in soil, are discussed. There is also a paper on plasmid transfer and the release of genetically engineered bacteria in nature.

The present interest in the use of genetically engineered micro-organisms and of the potential danger in their use makes this book an important contribution to current knowledge. Little is known about the transfer, spread, establishment, expression or survival of genes in such circumstances, and all those in the disciplines of physiology, biochemistry, molecular biology and microbiology should find this small volume well worth reading.

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