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ALTERATIONS IN THE ULTRASTRUCTURE OF EPIPHYTIC LICHENS *HYPOGYMNIA PHYSODES* AND *ALECTORIA CAPILLARIS* CAUSED BY AIR POLLUTION

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The ultrastructure of *Hypogymnia physodes* and *Alectoria capillaris* grown or transplanted near a fertilizer plant and a pulp mill was compared to normal ultrastructure of these lichen species. The ultrastructural changes observed were highly similar in the symbionts of both species and near both the factories although the emissions are different. In the lichens grown near the factories the number of algae had clearly increased. The appearance of the chloroplasts was roundish compared to controls. The pyrenoglobuli and cytoplasmic storage bodies were smaller than normally and the number of polyphosphate bodies had increased. Also in mycobionts storage droplets were very small or absent and many vacuoles and dark inclusions appeared to hyphae in contrast to controls. In transplanted lichens there existed mainly the same ultrastructural changes as in the lichens grown near the factories. Near the fertilizer plant the damage was, however, more severe because all the lichens died during 6–7 months after transplantation. Near the pulp mill part of the lichens survived and seemed to adapt to air pollution.

INTRODUCTION

Epiphytic lichens are considered as good bioindicators of air pollution according to many physiological and mapping studies. The ultrastructural changes in lichen symbionts under pollution stress are still unknown, however. The aim of the present study has thus been 1) to acquire basic information on air pollution injuries at cellular level in epiphytic lichens 2) to find out if different emissions cause different changes in lichen ultrastructure and 3) if morphologically different lichen species have different symptoms at ultrastructural level.

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MATERIALS AND METHODS

Samples of visibly damaged *Hypogymnia physodes* and *Alectoria capillaris* growing on spruce branches were collected from the vicinity of a fertilizer plant (about 2500 m NW) and a pulp mill (about 3000 m NW). The fertilizer plant (Kemira Oy, Siilinjärvi) releases SO^2 (2500–3000 t/y), NO_2 (400 t/y), NH_3 (250 t/y), fluorides (1 t/y) and some fertilizer dust. The main emission of the pulp mill (Savon Sellu Oy, Kuopio) is SO_2 (about 4000 t/y) but also some NO_2 and ammonia is emitted. The colour of damaged lichens was dark greyish green instead of the normal pale greenish grey colour and the thalli were often stunted near both factories. In January 1979 spruce branches bearing these two lichen species were also transplanted from the control area (Itä-Karttula) to both industrial sites in the region of the lichen desert about 500 m NW from the factories.

The specimens for electron and light microscopy were taken in March, May, July, August and September 1979 both from transplanted lichens and those grown in industrial environments. Small pieces from the middle parts of the thalli were fixed in 3 % glutaraldehyde and 1 % OsO_4 in 0.05 M or 0.075 M phosphate buffer. Thicker sections for light microscopy were stained with toluidine blue and thin sections for electron microscopy with uranylacetate and lead citrate.

RESULTS

Light microscopy

The algal layer of normal *Hypogymnia physodes* and *Alectoria capillaris* consisted of about 2 cell layers (Table 1 and Figs. 1 and 3) including usually some collapsed appearing dead algae. Near both the factories the number of phycobionts had clearly increased (Table 1 and Figs. 2 and 4). In some sections there were more dead algae than in controls, but usually the number of dead cells was quite small. In *Hypogymnia physodes* the cortex layer was also thinner than in controls (Fig. 4) and in *Alectoria capillaris* the thickness of the cortex was uneven near both the factories.

In transplanted lichens there were many dead algae after 2–4 months from transplantation near both the factories. After 6–7 months all the algae were dead near the fertilizer plant, but near the pulp mill the algae had increased by this time and many aplanospores could be found in these specimens.

Table 1. The thickness of algal layer in *Alectoria capillaris* and *Hypogymnia physodes* measured as phycobiont cells

	controls	fertilizer plant	pulp mill
Alectoria capillaris	1.8	4.2[1]	3.8[1]
Hypogymnia physodes	2.0	3.9	3.1

[1]) The longitudinal section was often quite full of algae.

Electron microscopy

Normal ultrastructure

The normal ultrastructure of the *Trebouxia* phycobionts of *Hypogymnia physodes* and *Alectoria capillaris* was very similar (Figs. 5 and 6). One large lobate chloroplast occupied most of the cell. In cholorplast there was a great number of thylakoids and a central pyrenoid with dark staining pyreneglobuli which were dispersed throughout the pyrenoid. The thylakoids were usually swollen to some extent especially near the pyrenoid (Figs. 5 and 6). In *Hypogymnia physodes* small starch grains could occasionally be seen near the pyrenoid or there were small granulous deposits in the same region (Fig. 5). Outside the chloroplast in cytoplasm there were a nucleus, some mitochondria, storage bodies, dictyosomes, ribosomes, myelinlike figures and vesicular complexes sometimes with dark or grayish staining inclusions (polyphosphate bodies).

The study of the mycobionts was concentrated on the algal layer and upper medulla in both lichen species. The ultrastructure of the mycobionts of these two lichen species was also very similar. In dense cytoplasm only light staining storage droplets (Fig. 5), mitochondria and concentric bodies (Fig. 9) were clearly discernible. The cell membrane had also small infoldings which is typical to lichen fungi in algal layer. The cell wall had many layers staining with different intensities. In both lichen species the mycobiont and phycobiont had many close contacts but haustoria were seen only occasionally.

Ultrastructural changes in industrial environments

The ultrastructural changes observed in lichens collected from industrial environments were also very similar in both species and in both areas. In phycobionts the lobes of the chloroplast were not as long and sharp as in controls. So the appearance of the chloroplast was roundish (Fig. 7). No changes in the number and structure of thylakoids were, however, observed. Specially in *Alectoria capillaris* the size of the pyrenoglobuli was smaller than in controls and frequently their position was peripheral (Fig. 7). The number and size of polyphosphate bodies was bigger than in controls (Figs. 7 and 8). The cytoplasmic storage bodies were very small compared to controls or they were completely absent. The number of myelinlike figures in cytoplasm, in vesicular complex and between cell wall and cell membrane had also slightly increased.

In mycobionts of algal layer and upper medulla the storage droplets were also very small or absent. These fungi had many vacuoles in their cytoplasm in contrast to controls (Fig. 9). These vacuoles were the biggest and most abundant in *Alectoria capillaris* near the fertilizer plant. The mycobionts had also often small very dark dots or bigger dark bodies in cytoplasm or in small vacuoles (Fig. 9). The bigger dark bodies appeared especially in those hyphae which were situated near dead algal cells. There were no detectable changes in the number and structure of haustoria.

In transplanted lichens the living phycobionts had many large polyphosphate bodies after 2–6 months from transplantation near both factories. The size of the storage bodies decreased during the transplantation time both in mycobionts and phycobionts. Also the vacuoles and dark bodies appeared to mycobionts. These were most abundant near the fertilizer factory where the damage was more severe than near the pulp mill. However, no changes in the chloroplast shape and in the size of the pyrenoglobuli were observed during 7 months. In dead algae the ultrastructure was granulous without any details.

DISCUSSION

The increase in the number of phycobionts caused by air pollution proved a surprising result which, however, easily explains the dark green colour of the damaged lichens. Also the thinner cortex may change the thallus colour. The increase of algae is previously reported in *Cladonia stellaris* tranplanted near a fertilizer factory in Oulu (KAUPPI 1976). This increase was explained by the effects of fertilizer dust released by the factory. In Siilinjärvi fertilizer dust is propably one effecting agent but there must be also other reasons for the increase of algae because the same phenomenon could be seen also near the pulp mill. Propably the gaseous nitrogen compounds and SO_2 in lower concentrations have fertilizing effects on lichen algae. Some other possible explanations could also be mentioned 1) compensation of decreased photosyntethesis 2) better illumination through thinner cortex layer 3) declined fungal control on algae in symbiosis 4) high reproduction capacity of the genetically most pollution resistant algae. The first possibility is supported by the small size of the fungal and algal storage bodies and pyrenoglobuli which are also considered as storage bodies of later assimilation products (JACOBS and AHMADJIAN 1969, HESSLER and PEVELING 1978). The decrease of the different storage compounds in algae and fungi could also be a sign of icreased consumption in fungi. The fungal growth in cortex layer seems also limited possibly because of starvation.

The exact function of vesicular complexes and polyphosphate bodies is not known. Polyphosphate bodies may also be storage material but some authors consider the vesicular complexes as lysosomelike structures (PEVELING 1976). They may dissolve the sevage materials of the algal cell or convert the stored substances suitable for transport. The number of polyphosphate bodies have veen observed to increase also in ageing phycobionts and under dryness (GALUN et al. 1971, PEVELING and GALUN 1976). Thus the increase in the number and size of these bodies could indicate increased transport between symbionts or encouraged decaying process caused by air pollution.

The vacuoles in the lichen mycobionts may also have a lysosomatic function (GALUN et al. 1971). So they propably indicate cell degeneration caused by air pollution in this material. The dark granules or inclusions have not generally been reported in lichen fungi. The most probable explanation is that they are sevage material indicating cell degeneration because they were seen especially when the lichens were severely damaged. The dark bodies could also be some alternative storage material under pollution stress.

The specimens were collected from the distance of 2.5 to 3 km from the factories because the lichen desert extends more than 2 km from both factories (TAKALA et al. 1978). Fluorides are very reactive compunds which probably do not disperse very long from the source. Thus the symptoms in the lichens near the fertilizer plant could be caused mainly by SO_2 and nitrogen compounds in the distance of 2.5 km. The round shape of the chloroplasts may also be an indication of SO_2 pollution as it has proved to be in conifer needles (SOIKKELI and TUOVINEN 1979). However, the rapid death of transplanted lichens at the distance of 500 m from the fertilizer factory was propably caused by flucrides, because the SO_2-level is generally lower near the fertilizer factory than near the pulp mill where part of the lichens survived.

The ultrastructural damage symptoms were mainly similar in *Hypogymnia physodes* and *Alectoria capillaris* although these lichens are morphologically different. This can be explained by the similar fine structure of the symbionts. *Alectoria* species are generally considered as more sensitive to air pollutants than *Hypogymnia* physodes. The sensitivity seems to be more dependant on thallus morphology and permeability of cortex than the ultrastructure of symbionts.

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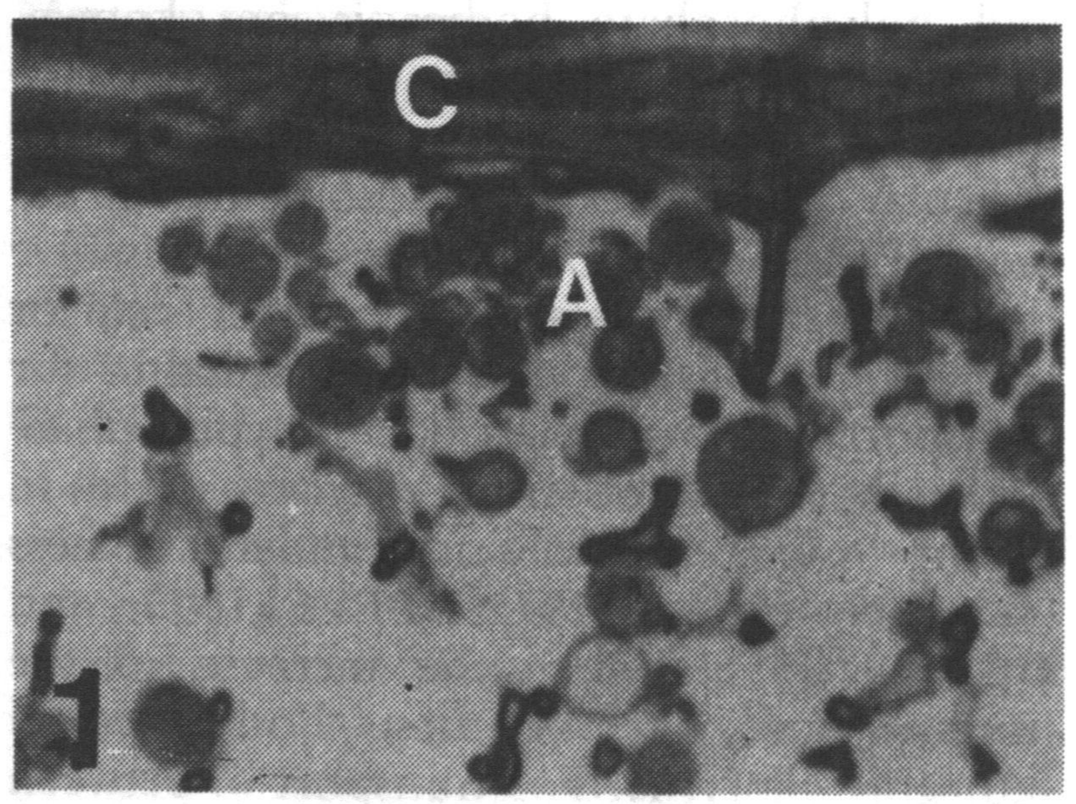

Fig. 1. A light micrograph from a normal *Alectoria capillaris*. A = algae, C = cortex layer. × 400

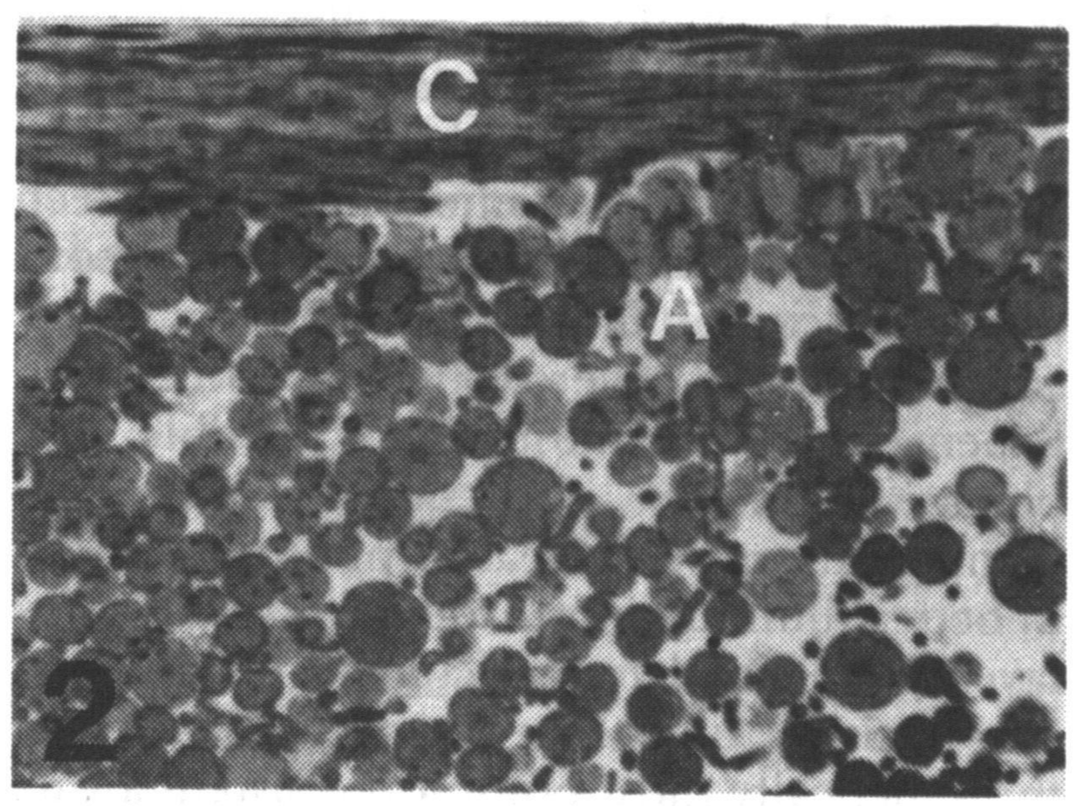

Fig. 2. Shows the increased number of algae in *Alectoria capillaris* near the fertilizer plant. × 400

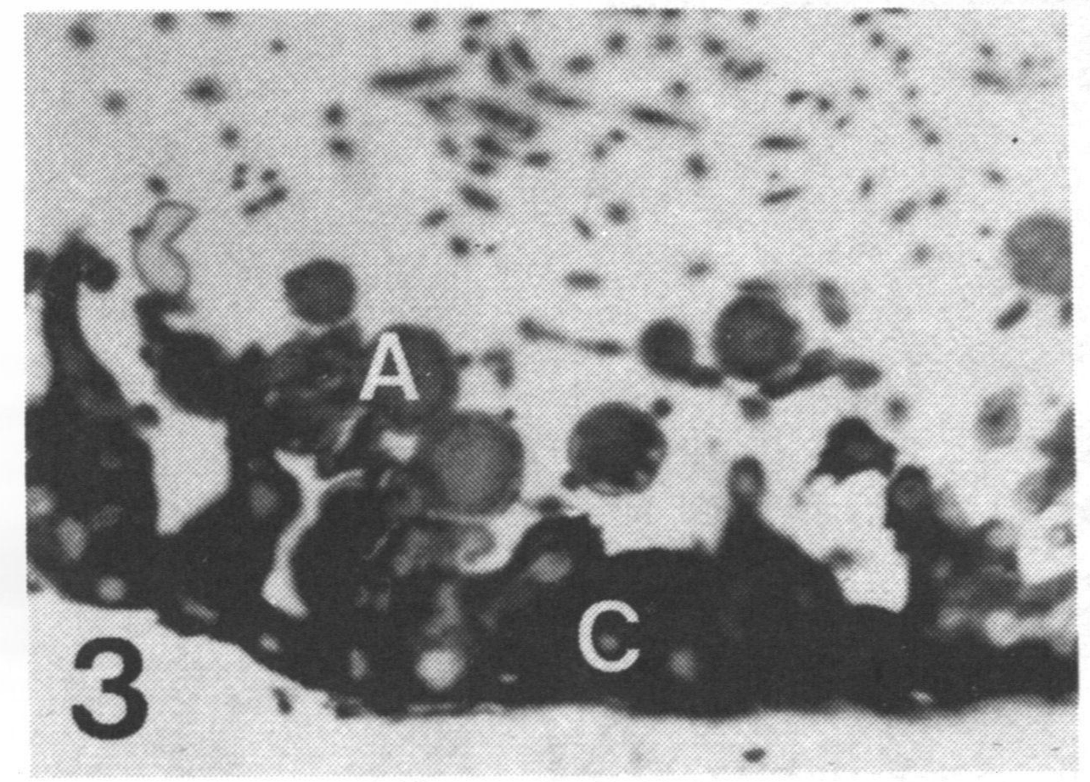

Fig 3. A light micrograph from a normal *Hypogymnia physodes*. A = algae, C = cortex layer. × 400

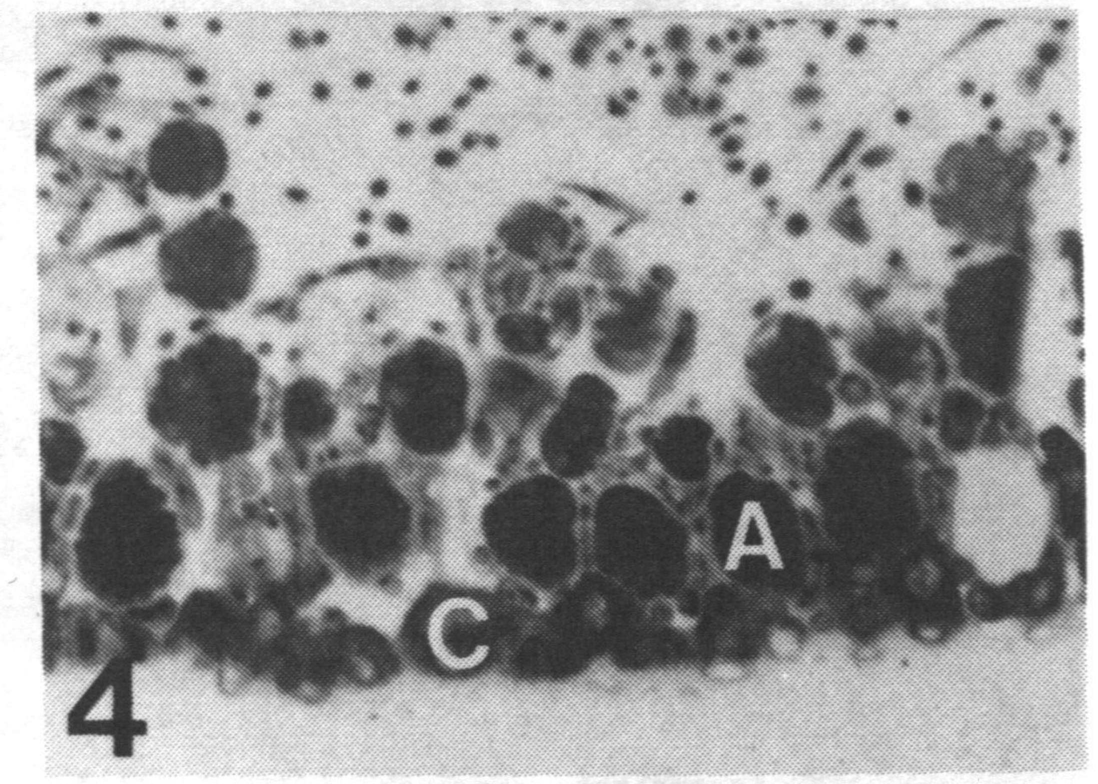

Fig. 4. shows the increase of algae and the decrease of cortex thickness in *Hypogymnia physodes* near the pulp mill. × 400

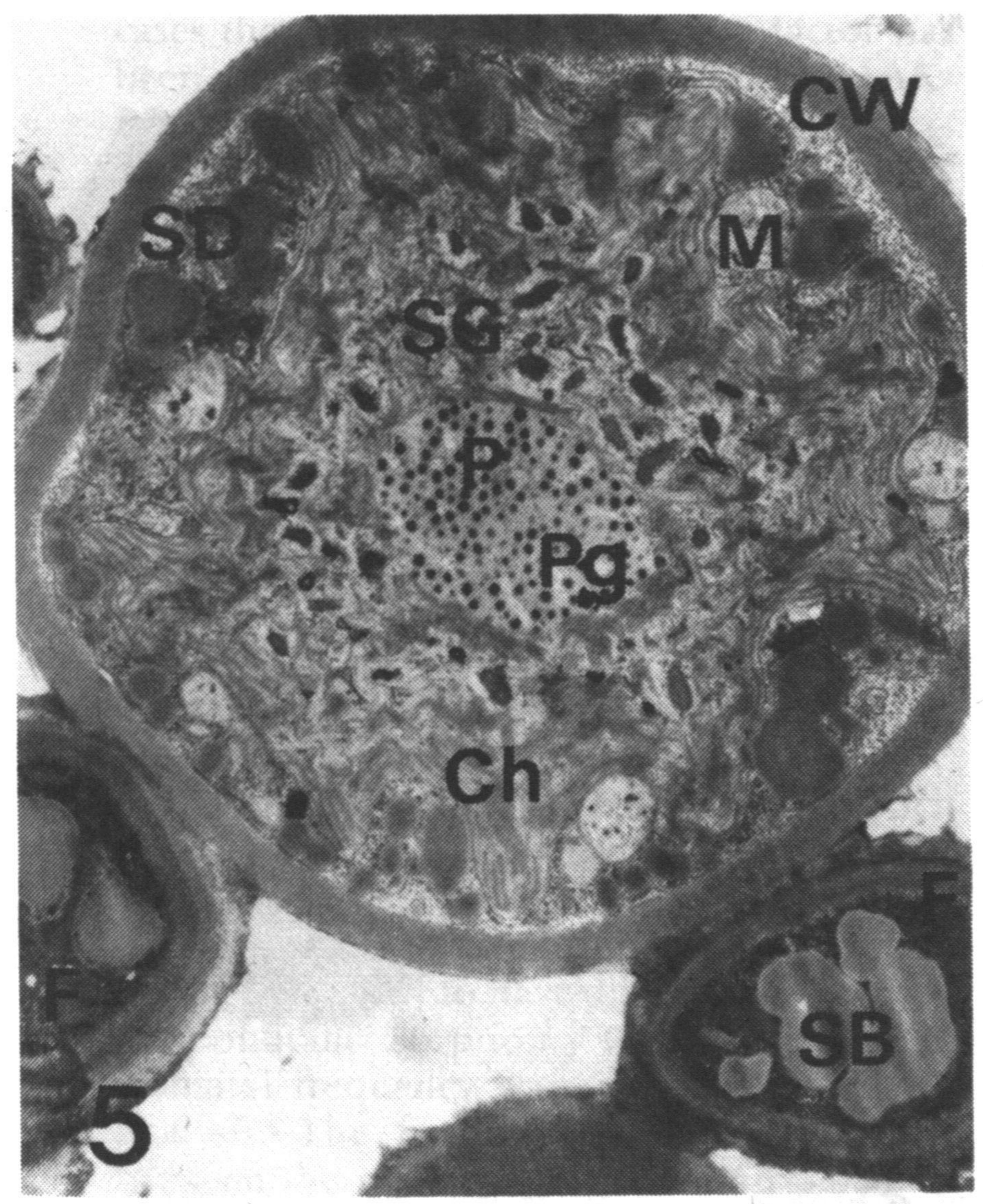

Fig. 5. An electron micrograph from normal *Hypogymnia physodes*. A *Trebouxia* cell with cell wall (CW), a chloroplast (Ch), pyrenoid (P), pyrenoglobuli (Pg), starch grains (SG), mitochondria (M) and storage droplets (SD) is surrounded by hyphae (F) in which large storage bodies (SB) can be seen. × 8400

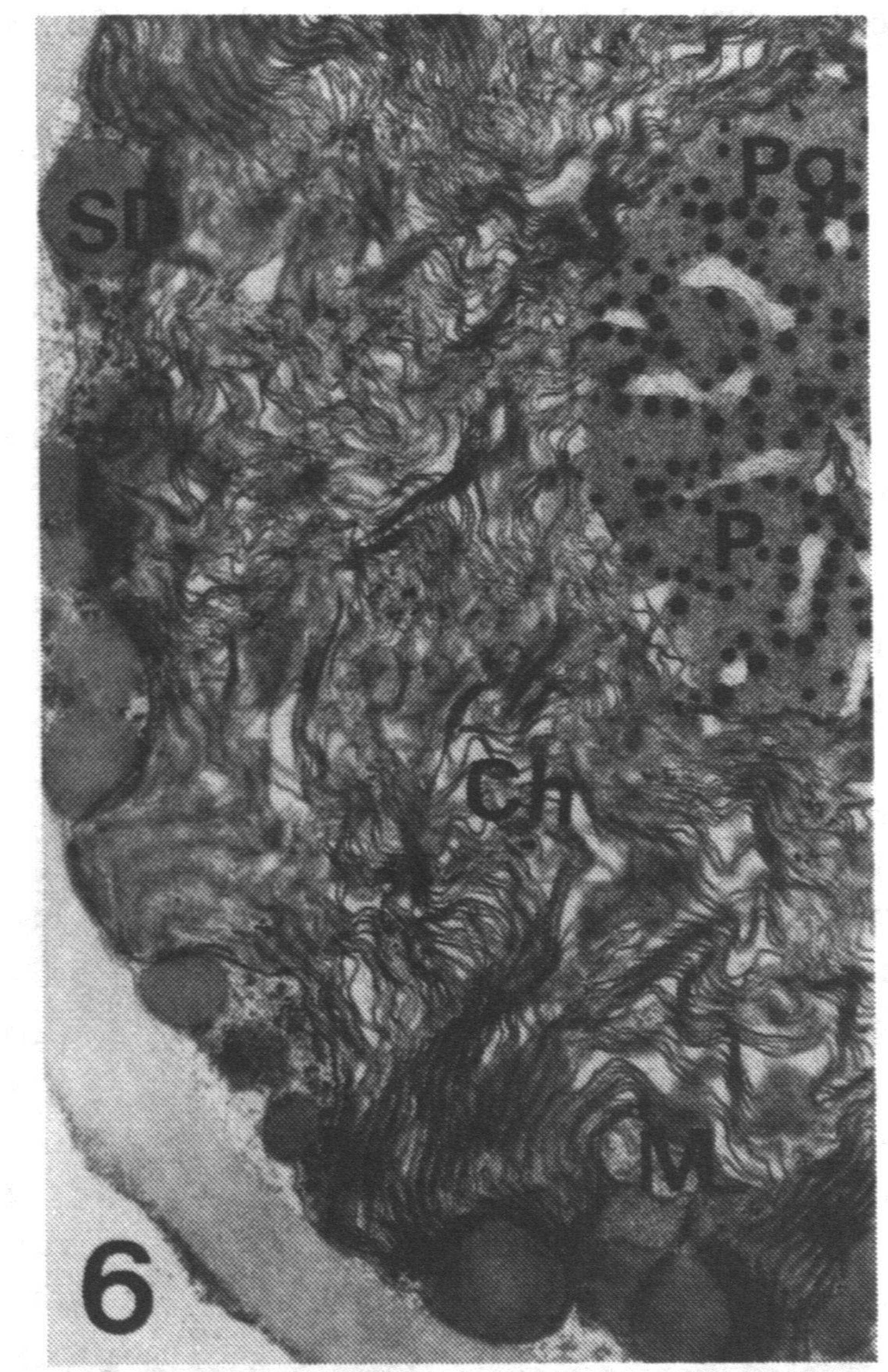

Fig. 6. Part of a *Trebouxia* cell from normal *Alectoria capillaris* with a chloroplast (Ch), pyrenoid (P), pyrenoglobuli (Pg), mitochondria (M) and storage droplets (SD). × 12000

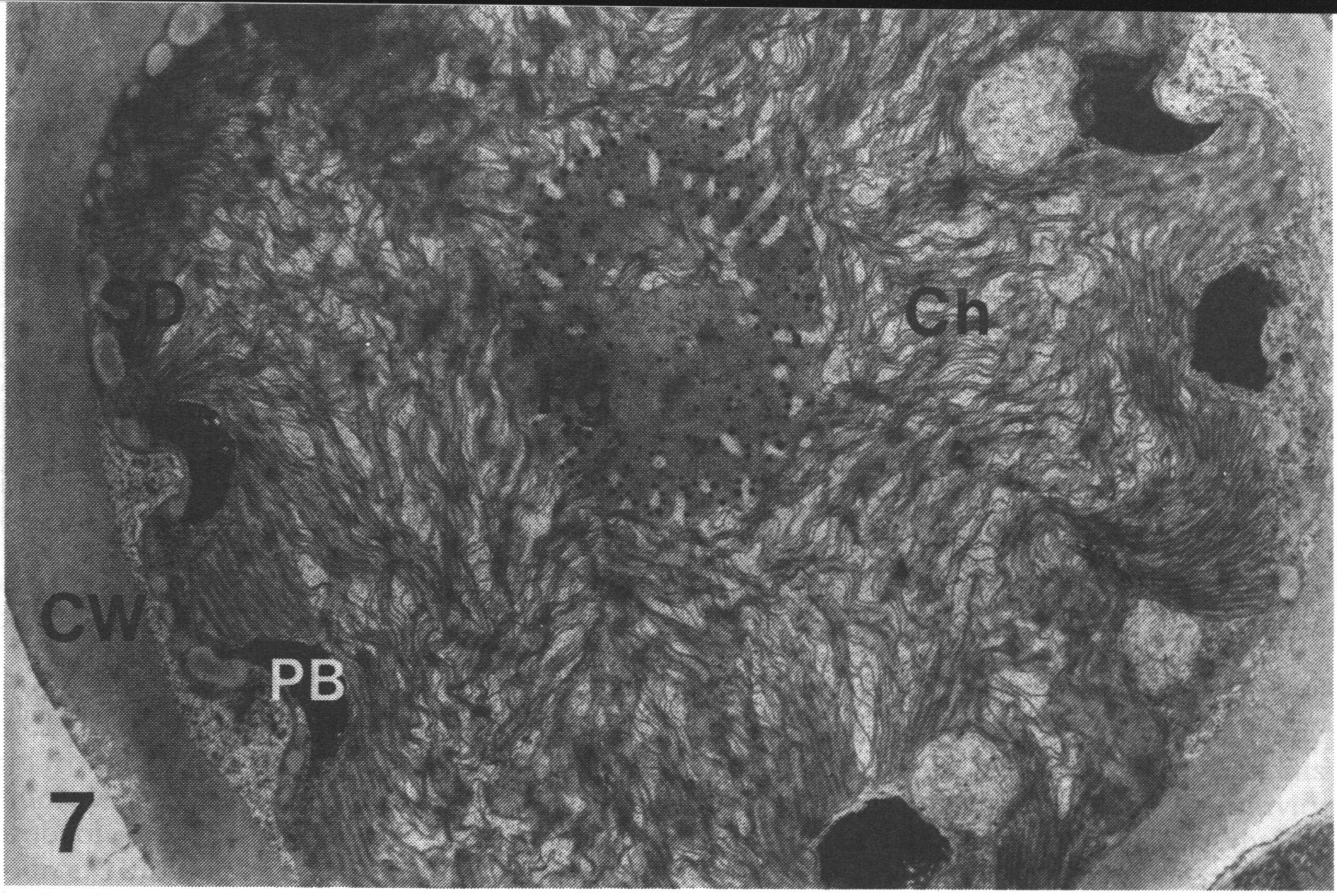

Fig. 7. A *Trebouxia* cell from *Alectoria capillaris* grown near the fertilizer plant. The appearance of the choloroplast (Ch) is roundish and the pyrenoglobuli (Pg) are very small and peripheral. Near the cell wall (CW) there are small storage droplets (SD) and dark polyphosphate bodies (PB). × 10500

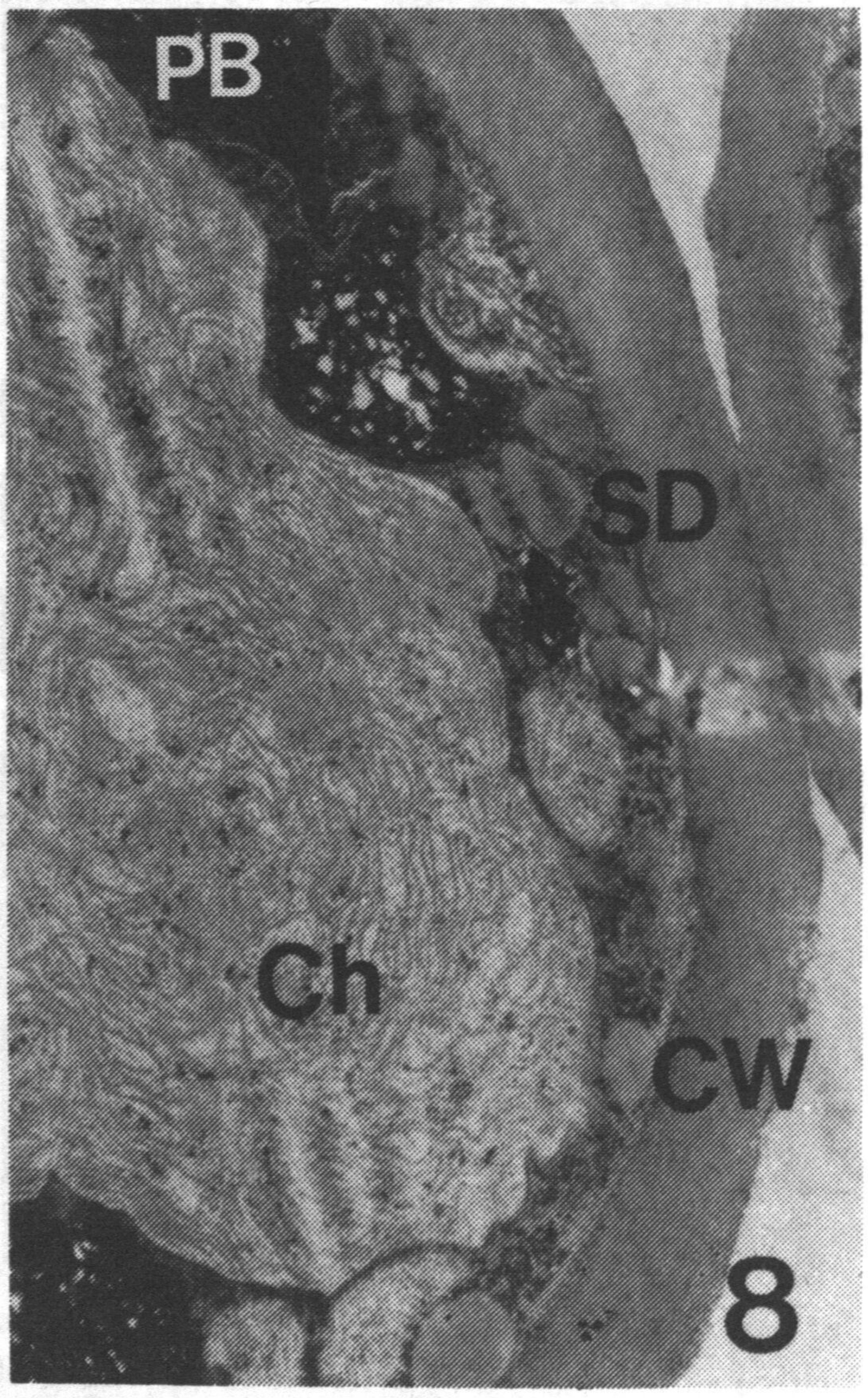

Fig. 8. Polyphosphate bodies (PB) with mottled appearance in *Hypogymnia physodes* transplanted near the fertilizer plant. Ch = chloroplast, SD = storage droplets, CW = cell wall. × 11000

Fig. 9. A hypha from *Alectoria capillaris* transplanted near the fertilizer plant showing typical pollution symptoms; vacuoles (V) and dark inclusions of different sizes. Cb = concentric bodies. × 40700