STUDIES ON PHLOEM EXUDATION FROM YUCCA FLACCIDA HAW. IX. PASSAGE OF CARBON BLACK PARTICLES THROUGH SIEVE PLATE PORES OF YUCCA FLACCIDA HAW.

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SUMMARY

A suspension of carbon black particles was sucked in by sieve-tubes. Particles of 200–700 Å were found on both sides of plates with plugged pores. This indicates that open connections of sufficient diameter must have been present during passage.

1. INTRODUCTION

Whether pores in sieve plates are normally open but plugged after injury, or whether they are always plugged is still a controversial question.

In electron-microscopy plugged pores are found, but with special fixation methods also open pores can be found (ESAU & CHEADLE 1961, ESCHRICH 1965, IE *et al.*, 1966, ANDERSON & CRONSHAW 1970).

The translocation of viruses through the phloem indicates a passage through the pores, although it is possible that the virus can be translocated as naked nucleic acid. Even more convincing are newly discovered micro-organisms, phloem pathogens, called *Mycoplasma* (DOI *et al.* 1967). These mycoplasmas are now known to occur in many species of higher plants. Their smallest particles, "elementary particles", are at least 500 Å in diameter and spherical. GIANNOTTI *et al.* (1970) have published an electron-micrograph showing a plugged pore with a mycoplasma-like body trapped in a closed pore and protruding at both sides. He supposed that this was the way they worked themselves through the pores. Another explanation, however, could be that the mycoplasma was strangled by the plugging mechanism.

Only the passage of lifeless particles can prove whether open connections are present in the pores during translocation.

Yucca, where exudation indicates that pores are closed less perfectly than in other plants, seemed a good object to suck in a carbon black suspension and to observe whether any particles could pass through the pores. In *Yucca*, IE *et al.* (1966) observed open pores with a few strands running through but also plugged ones were observed though all pores of one plate are always in the same condition.

2. MATERIAL AND METHODS

A carbon black suspension (India ink trade mark Talens) was diluted with 10 parts of water. For cryotome sections it was centrifuged for 4 hours at 3000 rpm to get rid of the larger particles. For electron-microscopy it was centrifuged for 17 minutes at 17000 rpm. India ink contains protein as a protective colloid and a trace of phenol as preservative. Also a suspension of pure carbon black was used with N Tamol as a disperser. This suspension was unsuitable because the particles adhered to every surface due to their electric charge. Stalks of inflorescences were stored for one night at 2 °C and treated and fixed at the same temperature. The low temperature prevents secretion of sugars into the sieve tubes. Otherwise an exudation flow out of the tubes could be expected (TAMMES *et al.* 1969).

Cross-sections of 3 mm were placed in porcelain dishes on a drop of the suspension and placed under an air current. The evaporation on the surface causes a suction force. After 5 hours the sections were fixed in cold glutaraldehyde 3% in 0.025 M phosphate buffer pH 7.4 and left overnight in the cold room.

For light-microscopy cryotome sections 40 μm thick were cut and embedded in glycerol.

For electron-microscopy after one night in the glutaraldehyde fixative, the sections were rinsed in the same buffer for 2 hours (4 changes) and chilled in ice. Meanwhile the disks were cut longitudinally in thin slices about 1 mm thick.

Afterwards the thin slices were postfixed in cold 1% OsO_4 in 0.025 M phosphate buffer pH 7.4 for 1 hour and chilled in ice. The fixed tissues were rinsed in the phosphate buffer, dehydrated in a graded series of ethanol concentrations and cut into convenient parts for embedding.

The tissues were embedded in pre-polymerized methacrylate mixture of butyl and methyl methacrylate (4:1) + 1.5% benzoyl peroxide, which was polymerized in gelatin capsules at 60 °C for 24 hours and then at 45 °C for 24 hours (PEASE 1960).

Selected specimens were sectioned with glass knives on an LKB Ultrotome-III ultramicrotome. The ultrathin sections were mounted on Formvar-coated copper grids, were stained first with uranyl acetate and then with lead citrate (REYNOLDS 1963) and were examined with a Siemens Elmiskop 1 electron-microscope operating at 80 kV.

3. RESULTS

The carbon black suspension is sucked short distances into xylem vessels, sieve tubes and intercellular cavities. In the sieve tubes agglomeration occurs and light-microscopy shows that the carbon particles are sieved out by the sieve plates (*fig. 1*). In one preparation in several phloem bundles faint grey tails appeared above the black dots at the sieve plates, indicating that some carbon black particles might have passed the pores. Smearing by the knive could be

Fig. 1. Suction of a carbon black suspension into sieve tubes. Particles agglomerate and are sieved out by the plates. The faint grey tails above the black dots (arrows) show that particles have passed the pores. Longitudinal cryotome section, 40 μ thickness.

excluded because the tails did not appear above xylem and intercellular cavities in the same preparation (*fig. 1*).

Electron-microscopy revealed that carbon black particles could be found in the sieve tubes (*fig. 2*) and on both sides of sieve plates (*fig. 3, 4, 5*). In *fig. 6* showing a plate with plugged pores carbon black particles are also found on both sides.

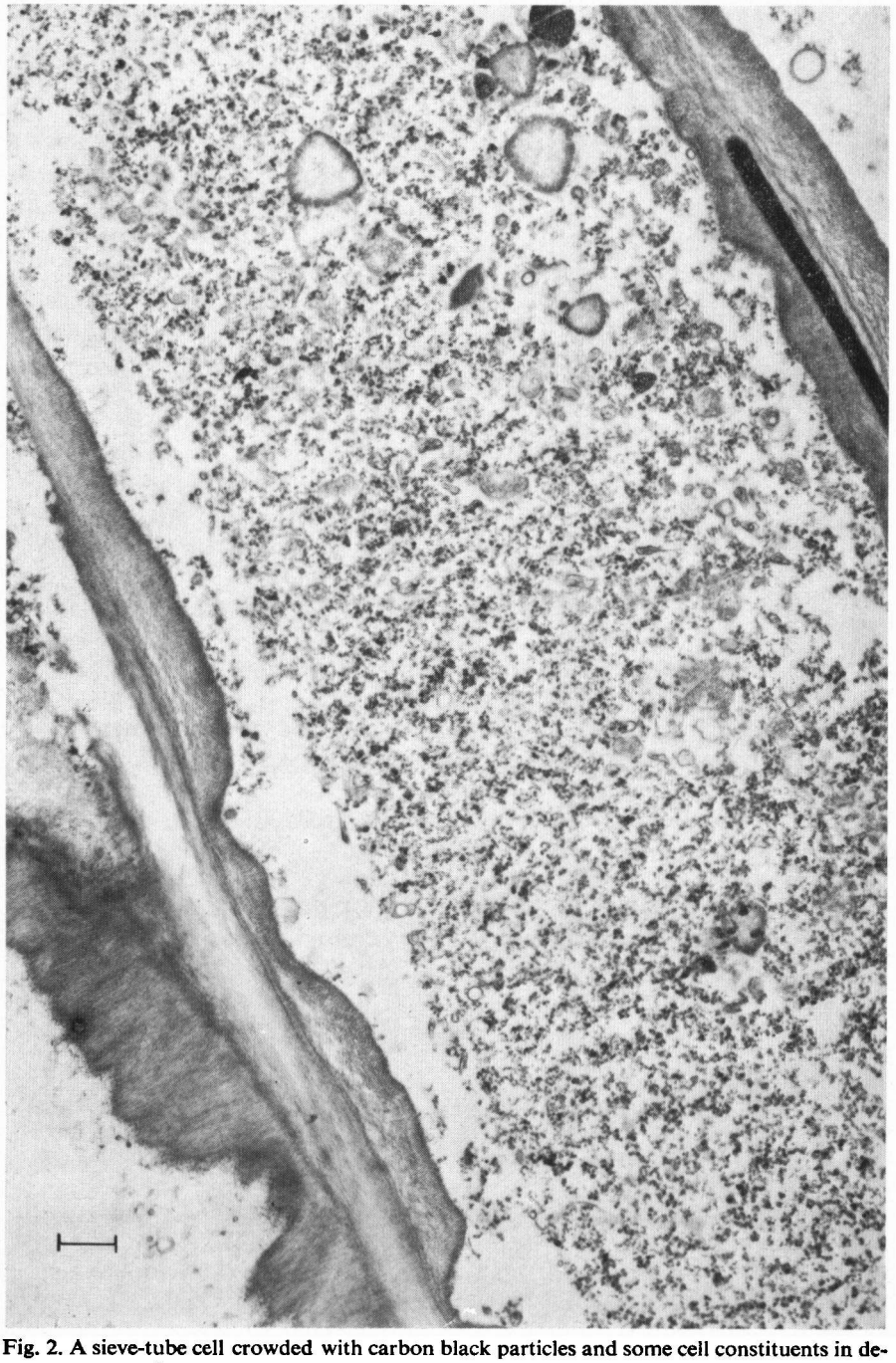

Fig. 2. A sieve-tube cell crowded with carbon black particles and some cell constituents in degenerated stage.

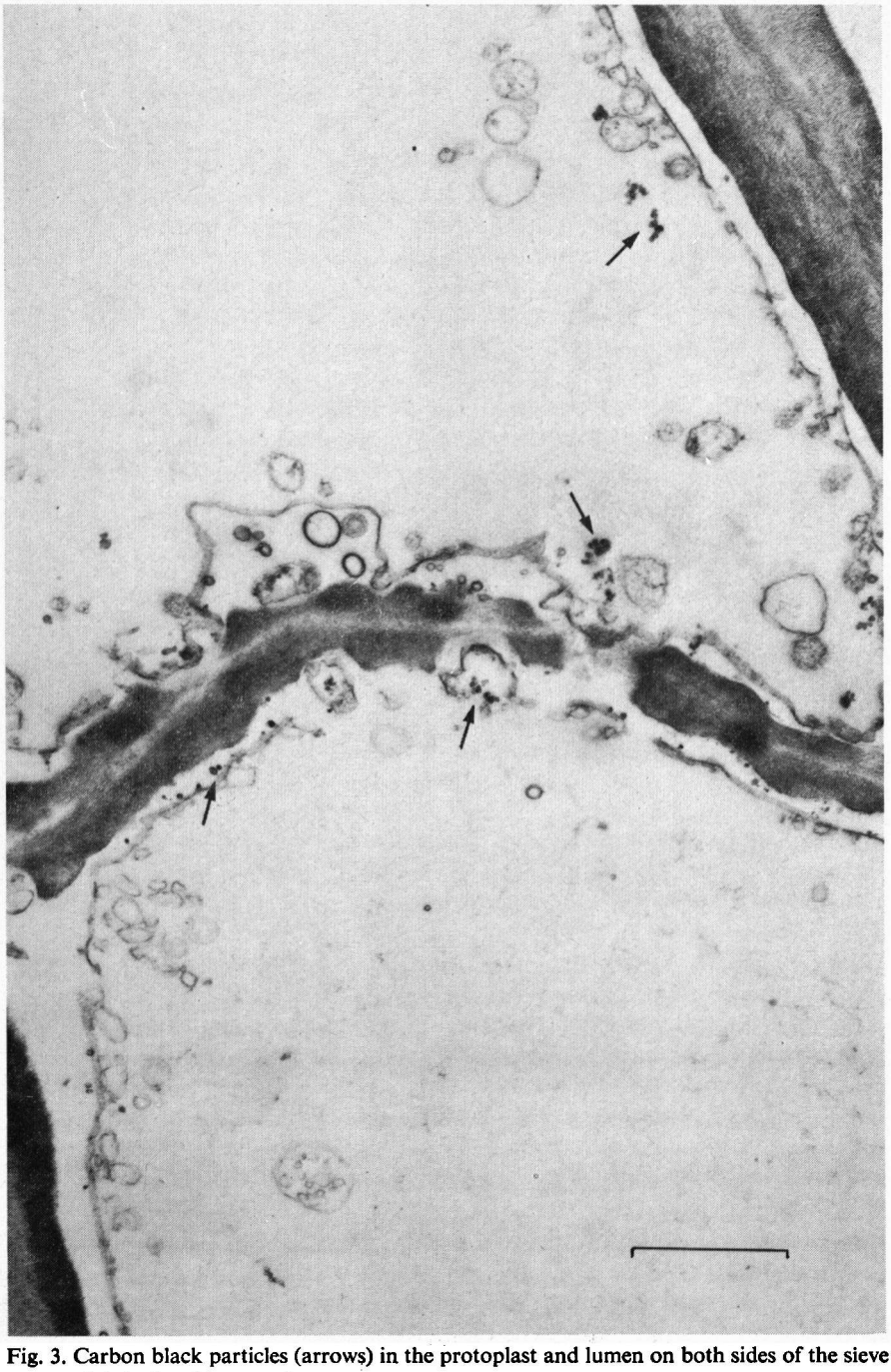

Fig. 3. Carbon black particles (arrows) in the protoplast and lumen on both sides of the sieve plate in the phloem system.

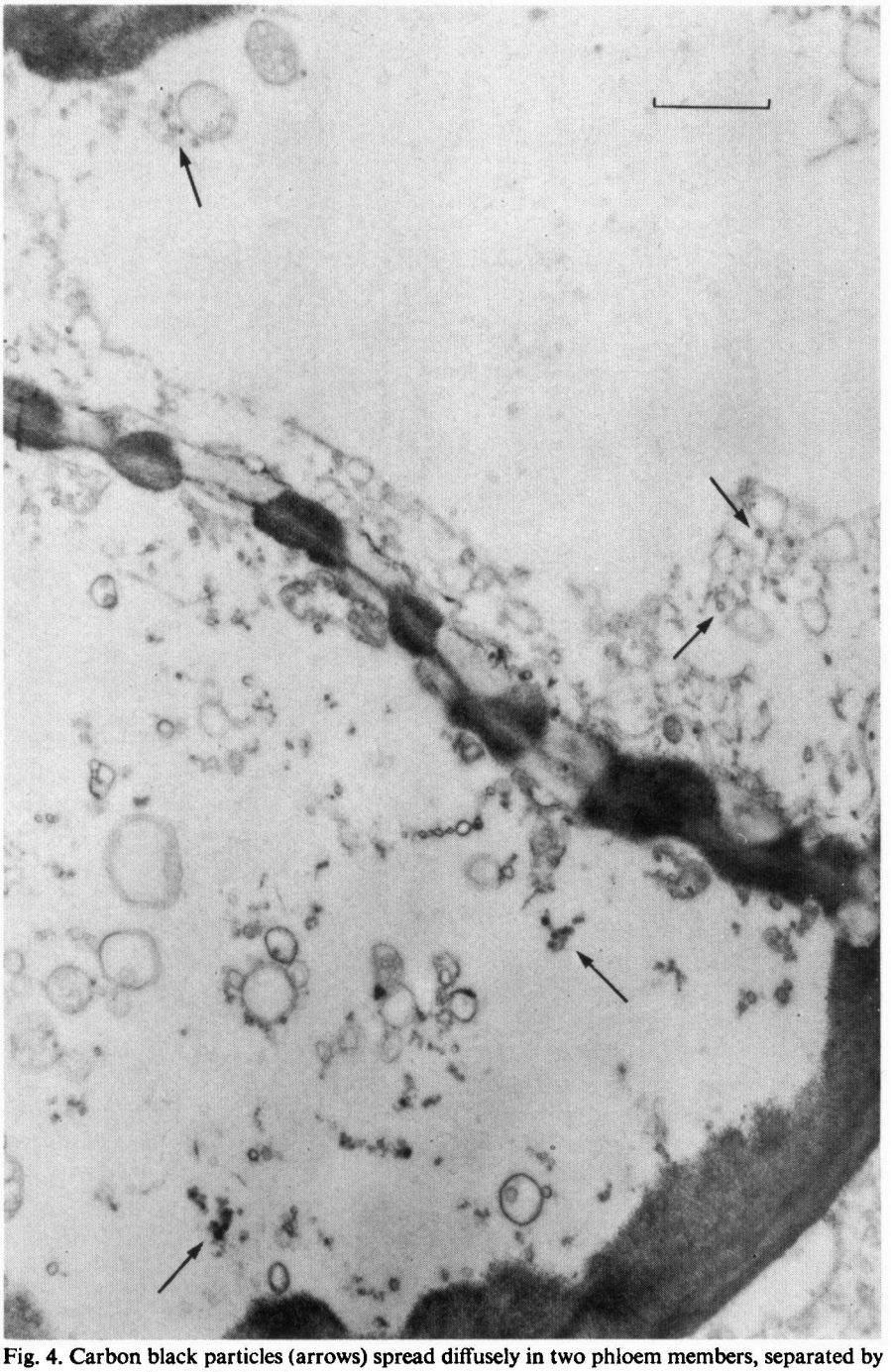

Fig. 4. Carbon black particles (arrows) spread diffusely in two phloem members, separated by a sieve plate with closed pores.

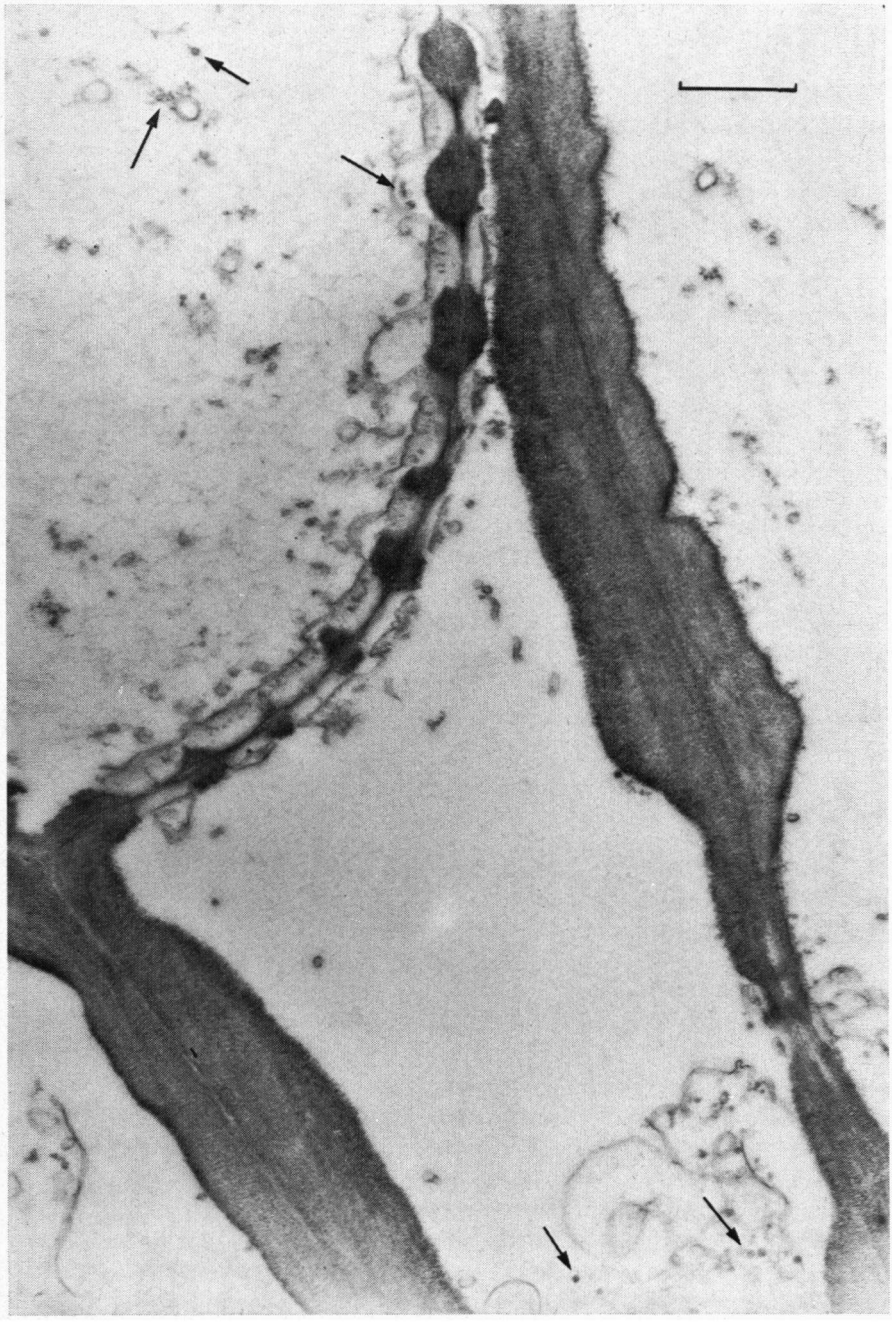

Fig. 5. Carbon black particles (arrows) located rarely in the lumen of the phloem cells, further away from the sieve plate area.

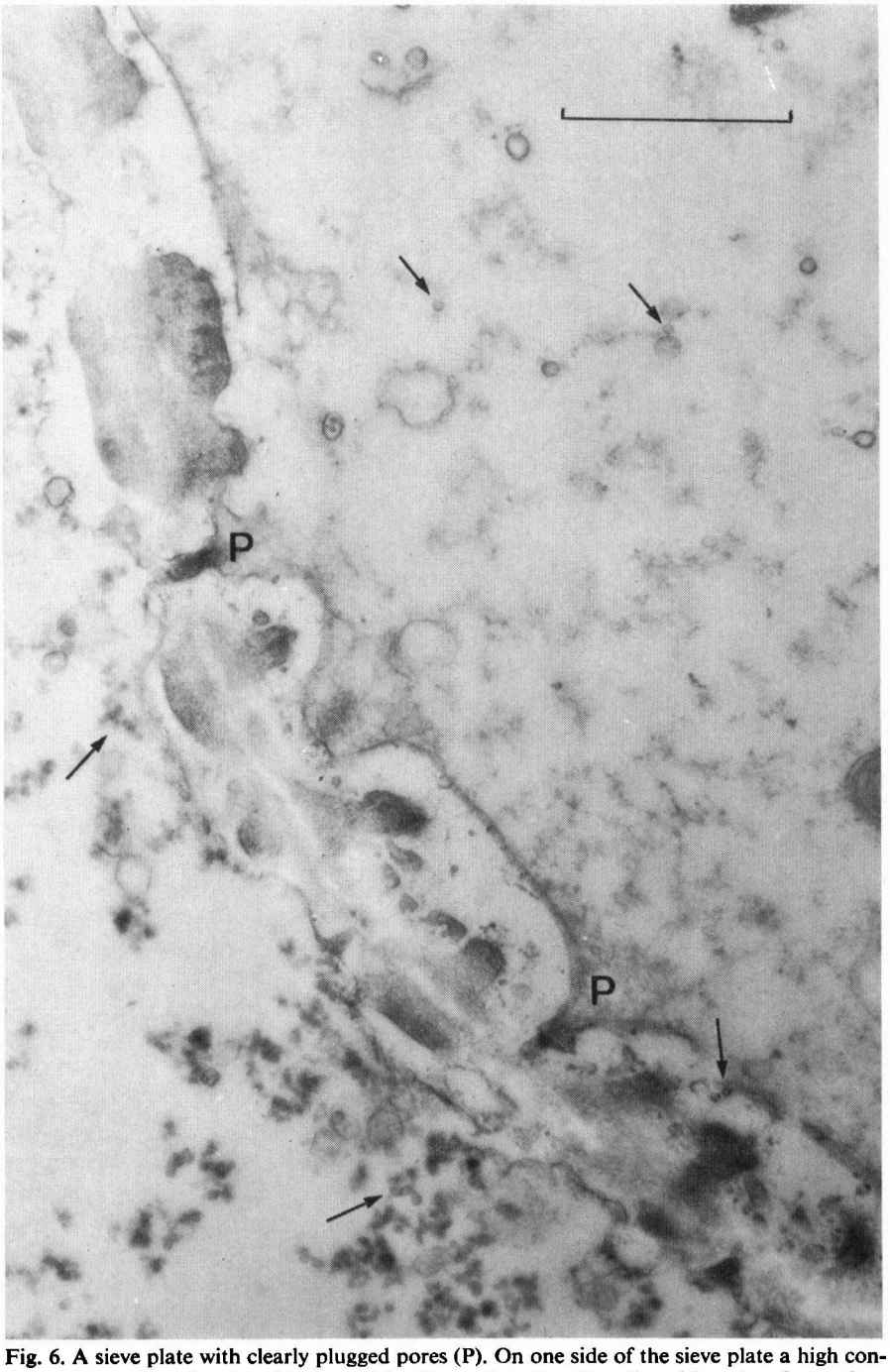

Fig. 6. A sieve plate with clearly plugged pores (P). On one side of the sieve plate a high concentration of carbon black particles (arrows) and on the other side only a few carbon black particles.

The carbon black particles were found in the cytoplasm and in the lumen but not in the callose, which is formed between plasmalemma and plate walls. The diameter of the particles was 200–700 Å with an average (n= 50) of 400 Å.

4. DISCUSSION

As carbon black particles were found on both sides of plugged pores, open connections of sufficient size to let the particles through must have been present. The pore must have been plugged after passage.

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