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Effect of Soybean Lecithin on Cucumber Powdery Mildew, *Sphaerotheca fuliginea*, at Various Growth Stages*

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Lecithin obtained from soybean plants at the time of producing soybean oil controls effectively several powdery mildews and rice blast disease. Since lecithin is a ubiquitous component in living organisms and its safety as a food additive is established, it is expected that this compound may be a safe agricultural fungicide and is applicable to vegetables even during harvest. Therefore, we examined the effect of lecithin on the growth of the cucumber powdery mildew fungus, *Sphaerotheca fuliginea* with a scanning electron microscope. After spraying 2000 ppm of the lecithin on the lower surfaces of cucumber cotyledons, conidiospores of the pathogen were inoculated on the same place. Spore germination, hyphal elongation, conidiophore formation and sporulation were observed at certain intervals. The lecithin did not inhibit remarkably spore germination, but gave some effects on spore germination, hyphal elongation and sporulation. That is, germ tubes on treated lower surfaces were stunted and became shorter than that of control. Hyphal elongation on treated surfaces was delayed, and conidiophore formation and sporulation were poorer than those on control lower surfaces. It was a striking feature that hyphal tips on the lower surfaces of cucumber cotyledon treated with lecithin became deformed, namely thin membrane-like substance appeared around hyphal tips and expanded with time. More delayed and fewer conidiophores and conidiospores were observed on the lower surface treated with the lecithin than those on the control lower surfaces. In addition, many conidiospores which were unable to release from conidiospore chain were observed on conidiophores, and such conidiospores dangled like osmund from the conidiophores on lower surfaces of cucumber cotyledon treated with the lecithin.

INTRODUCTION

It was found that soybean lecithin which was obtained at the process of producing oil from soybeans was effective on various vegetable powdery mildews and rice blast disease.[1] The results of field tests were excellent for the cucumber powdery mildew, caused by *Sphaerotheca fuliginea*. It is known that powdery mildews frequently occur under dried condition. The inhibitory effect of the lecithin on various vegetable powdery mildews seems to be based on inhibition against some stages of life cycle of the pathogen. It is supposed that the lipophilic group of the lecithin has an affinity to the lipophilic group of *Sphaerotheca fuliginea* or hygroscopic character[2] of the lecithin inhibits the fungal growth which is susceptible to moisture. From this point of view, we selected *S. fuliginea*, a pathogen of cucumber powdery mildew which is expected to be controlled by the lecithin and decided to examine the effect of the lecithin on the growth stages of this pathogen. It is supposed that the

*Studies on the Mode of Action of Soybean Lecithin (Part 1) lecithin may adsorb on the pathogen, and the fungal growth may be inhibited by the surfactant nature of the lecithin, since the pathogen has a habit of creeping on the surface of cucumber leaves, namely it is external parasitic.[3] The effect of the lecithin on the growth stages of the pathogen was observed by scanning electron microscopy. This paper reports the results of the morphological investigation.

MATERIALS AND METHODS

Drugs and application. Lecithin used was composed of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol (Fig. 1), and soybean oil.

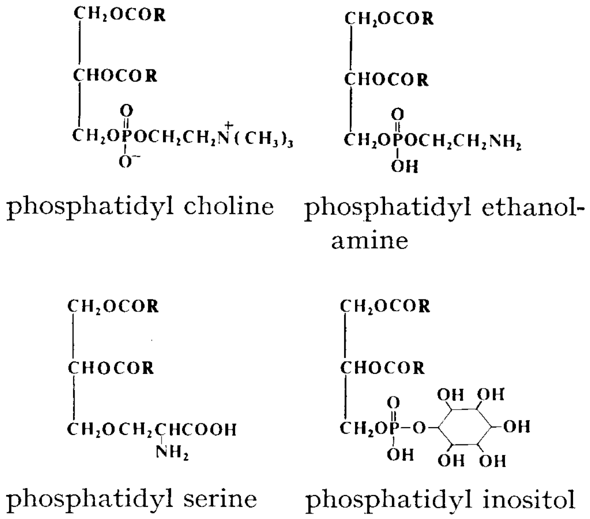

Fig. 1 The structures of main components of soybean lecithin.
R: palmitic acid, stearic acid, oleic acid or linoleic acid

The lecithin preparation was diluted with water at the concentration of 2000 ppm. This solution was sprayed on the lower surface of cucumber cotyledons (variety: Sagamihanjirō) with spray gun at 2 kg/cm^2 before inoculation.

Organism and inoculation. Fungus of cucumber powdery mildew (*Sphaerotheca fuliginea*) used in this experiment was isolated from naturally infected cucumber (variety: Sagamihanjirō) at the greenhouse, and maintained on cucumber in the greenhouse. Conidia formed freshly were used as the inoculum. Inoculation was done soon after drying of the sprayed lecithin solution, and then the inoculated plants were kept in the greenhouse.

Preparation of materials for the scanning electron microscopy. Effect of lecithin on *S. fuliginea* was observed by the scanning electron microscope, JEOL JSM U3, on spore germination after 2 days of inoculation, on hyphal elongation after 5, 8, 13 days of inoculation, on mycelial growth after 7, 13 days of inoculation and on conidial formation after 13 days of inoculation, respectively.

These inoculated cucumber leaves were cut into 10 mm×10 mm in size and fixed with formalin vapor at room temperature for 20 hr. After that, the materials were attached to specimen stubs with silver-conducting paint, and coated with very thin films of carbon and gold under high vacuum. The specimens were observed and photographed at accelarating voltage of 5 kV.

OBSERVATIONS

1. *Spore Germination*

Spore germination on the lower surface of cucumber cotyledons was observed 2 days after inoculation. The germ tube is normal (Plate I–1). While, spores which were inoculated on the lower surface of cucumber cotyledons treated with 2000 ppm of the lecithin germed 2 days after inoculation, but these germ tubes were stunted and shorter (Plate I-2) than control germ tubes. These results indicate that the lecithin did not inhibit spore germination, but retarded slightly the elongation of germ tubes.

2. *Hyphal Elongation and Mycerial Growth*

Five days after inoculation. Normal elongation of hypha on the lower surface of cucumber cotyledons was observed 5 days after inoculation (Plate I–3). Hyphal elongation was long as a whole, in addition to it thin membrane-like substance was observed at hyphal tips. Hyphal tips on the lecithin treated cucumber cotyledon were examined by higher magnification (Plate I–4). In this Plate, thick membrane-like substances appeared at hyphal tips. They seem to have thicker membrane-like substances than those of the control hyphal tips.

Eight days after inoculation. Some thin membrane-like substances around hyphal tips can not be seen on the control cucumber lower surface (Plate I–5). On the other hand, the tips of hyphae grown on the surface of cucumber cotyledon treated with lecithin had thicker membrane-like substances as shown in Plate I–6.

The thick membrane-like substances grew larger than those seen three days before.

Thirteen days after inoculation. Hyphal tips on the surface of control cucumber cotyledons had no distinct membrane-like substance (Plate II–7), while, hyphal tip 13 days after inoculation on the lecithin treated surface of cucumber cotyledons (Plate II–8) seems to have the largest and thickest membrane-like substance among those seen before. It is not distinct whether this eel tail-like substance around hyphal tip is induced from the pathogen or from the host plant. Otherwise, this substance may be a product derived from conjugation of the thin substance around hyphal tip as shown in Plate I–3 with the lecithin.

A large number of mycelia were observed on the surface of control cucumber cotyledon 13 days after inoculation (Plate II–9). While, so many mycelia were not found on the lecithin treated surface of cotyledons (Plate II–10). This result corresponds with the retarded hyphal elongation on the lecithin treated surface.

Mycelial growth on the lower surface of cucumber cotyledons 7 days after inoculation are shown in Plate III–11. A number of mycelia are distributed all over the surface of lower side of cucumber cotyledon and they are also very fresh in comparison with mycelia on the lecithin treated surface (Plate III–12). Besides, several conidiophores are formed in the similar proportion as shown in Plate III–11. On the other hand, in the case of the lecithin treated surface of lower side of cucumber cotyledon, mycelial growth was rough. However, the growth was not so vigorous as mycelia grown on the control surfaces. This phenomenon seems to be caused by the effect of the lecithin. In addition, several osmund-like conidial chains were found.

3. *Conidiophores and Conidia*

Conidiophores formed on the surface of control cucumber cotyledon are shown in Plate III–13. This micrograph was taken 13 days after inoculation. Conidiophores formed on the surface of lecithin treated cucumber cotyledon seem to be normal (Plate III–14). Therefore, the formation of conidiophores seemed to be unaffected by the lecithin. In order to clarify the formation of osmund-like conidiospore chains on the lecithin treated and control surfaces, scanning electron micrographs were taken at higher magnifications. Namely, conidiospores on the surface of the control lower side of cotyledon did not dangle on the conidiophore (Plate III–15), while conidiospores on the surface of the lecithin treated lower side of cotyledon dangled on the conidiophore without release, and such conidiospores seemed to be burst (Plate III–16). This might be a result of some effects of the lecithin on them, because osmund-like conidiospore chains were not found on the control lower side of cotyledons. It is not clear whether the effect of the lecithin on the conidiospores is due to its killing action to conidiospores or its spore-sticking action.

DISCUSSION

Soybean lecithin is mainly composed of four components; phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine and phosphatidyl inositol.[4] Lecithin is an essential element for all organisms and it is found in abundance in brain lung, liver, nervous tissue, egg yolk, leucocyte, and in various plant seeds, especially in soybeans. This compound is mixed with groceries, such as margarin, chocolate, ice cream, caramel etc..[2] On the other hand, not only this substance is utilized as a medicine for arteriosclerosis and cirrhotic liver, but also, saturated dipalmitoyl lecithin plays an important role as a surfactant of liver in gas exchange in human body.[5] However, it is known very little on physiological function. Lecithin is distributed in the natural world in such forms as previously mentioned, in addition to it, lecithin seems to play an important role with its physiological function.[6] Therefore we supposed that this substance might not cause environmental pollution or might induce little toxicological and residue problems, because lecithin has been added into groceries or it is used as a medicine.

During the course of a screening of naturally occurring substances effective against rice blast disease, soybean lecithin like amino acid derivatives[7] was found to protect rice plant from blast disease. Furthermore, we checked inhibitory activities of this compound against other plant diseases, and it was found that lecithin had an activity to control powdery mildews of several kinds of vegetables including cucumber powdery midew.[1] Powdery mildew is one of the most important among cucumber diseases, therefore, we selected it as a vegetable disease to examine and investigate the effect of lecithin on the various growth stages of the pathogen.

By microscopical observation it was found that the lecithin (2000 ppm) did not extremely inhibit spore germination of *S. fuliginea*. Nevertheless, germ tubes were stunted and became shorter on the lecithin treated surface than that on the control surface.

After then, elongation of the hyphae was slightly inhibited at the same concentration. We found some morphological abnormalities which have been shown by conventional agricultural chemicals.[8] It was characteristic that eel tail-like membrane came out at the hyphal tips three days after inoculation and it developed gradually with time. On the other hand, thin membrane-like substance at the hyphal tips of the pathogen was not always found on the surface of the control plant. Even if it was observed, it did not develop with time. Therefore, it can be supposed that the expansion of hyphal tip membrane observed on the surface of the lecithin treated cucumber seems to be due to the presence of the lecithin. But it is not obvious whether the lecithin and fungal secretion react each other or not.

These results indicate that the lecithin inhibits slightly germ tube elongation and hyphal elongation on the leaf surface of cucumber. Because of being slightly inhibited by the lecithin, the growth of mycelial colonies paralleled with both stages, germ tube elongation and hyphal elongation. The growth of mycelial colonies was not so dense on the lecithin treated leaf surfaces in comparison with the control leaf surface.

Though the lecithin has not fungicidal activity against *S. fuliginea*, the growth of mycelial colonies treated with the compound is generally rough and not rapid. This may be due to the fungistatic activity of the lecithin. Therefore, some conidiophores are found on the lecithin treated surface 7 days after inoculation, besides, osmund-like conidial chains are found on their tops. These phenomena were particularly observed on the lecithin treated leaf surfaces.

Conidiophores were formed 5 and 7 days after inoculation on the control and the lecithin treated leaf surfaces respectively. Conidiophores on the lecithin treated leaf surface were found at a growth stage and formed later than those on the control leaf surface. However, the morphology of conidiophores once formed did not differ from those formed on the control leaf surface. The lecithin has inhibitory effect on several growth stages of this fungus, but its direct effect on the morphology of the conidiophores is not considered.

Generally speaking, conidiospores formed on conidiophores are used to release in order as then mature.[3] As shown in Plate I–2, Plate II–10, and Plate III–12 each growth stage of *S. fuliginiea* after treated with the lecithin on the leaf surface was inhibited slightly. On the other hand, conidiospores formed and matured on the lecithin treated surfaces are not always released from conidiophores, but hang on them. This phenomenon was revealed by several experiments. This means that the lecithin has some activity to inhibit the release of the conidia from conidial chains. Such conidiospores often became withered and more folded than normal ones, and they appear to have lost their vital power. However, from these results, it is not clear why conidiospores are not released from conidiophores and why they become more withered and more folded.

On the other hand, it can be considered that the hydrophilic groups which the lecithin has in its structure (Fig. 1) contain moisture. It is said that this fungus is weak to water. Therefore, it may be assumed that wetness inhibits the release of conidiospores and takes off their vital power.

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要 約

キュウリうどんこ病菌 *Sphaerotheca fuliginea* を供試し，本菌の生育過程に及ぼす大豆レシチンの影響を検討した．キュウリ子葉裏面に大豆レシチン2000ppmを処理し，うどんこ病菌分生胞子を接種し，分生胞子の発芽，菌糸の伸長，分生子梗，分生胞子の形成を経時的に走査電顕により観察した．分生胞子の発芽には著しい抑制はみられなかったが，無処理区に比し，発芽管がやや太く，短くなり，菌糸の伸長が遅くなる傾向がみられた．もっとも顕著な影響は，処理区の接種3日目以降の菌糸先端部に現われた．すなわち，伸長した菌糸の先端部周縁に薄膜が見られ，うなぎ尾状を呈し，日数の経過とともに膜の部分が拡大することであった．また無処理区に比し，分生子梗，分生胞子の形成が遅れ，数も少なく，とくに分生胞子が分生子鎖から離脱せずに，そのまたれ下がり，全体的にゼンマイ状を呈するものがみられた．

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Explanation of Plates

Plate I

1 Conidiospore germination on the control surface 2 days after inoculation. G: Germ tube. Bar: 10 μm.

2 Conidiospore germination 2 days after inoculation on the lecithin (2000 ppm) treated surface. G: Germ tube. Bar: 10 μm.

3 Hyphal elongation on the control surface 5 days after inoculation. MS: Membrane-like substance. Bar: 5 μm.

4 Deformity of hyphal tip on the lecithin (2000ppm) treated surface 5 days after inoculation. MS: Membrane-like substance. Bar: 1 μm.

5 Hyphal tip on the control surface 8 days after inoculation. Bar: 2.5 μm.

6 Deformity of hyphal tip on the lecithin (2000 ppm) treated surface 8 days after inoculation. MS: Membrane-like substance. Bar: 2.5 μm.

Plate II

7 Hyphal tip on the control surface 13 days after inoculation. Bar: 1 μm.

8 Deformity of hyphal tip expanded with time on the lecithin (2000 ppm) treated surface 13 days after inoculation. MS: Membrane-like substance. Bar: 2.5 μm.

9 Mycelial growth on the control surface 7 days after inoculation. Bar: 25 μm.

10 Mycelial growth on the lecithin (2000 ppm) treated surface 7 days after inoculation. Bar: 50 μm.

Plate III

11 Mycelial growth and conidiophores on the control surface 13 days after inoculation. Cp: Conidiophore. Bar: 50 μm.

12 Mycelial growth and conidiophores on the lecithin (2000 ppm) treated surface 13 days after inoculation. Cp: Conidiophore. OCp: Osmund-like conidiophore and conidial chain. Bar: 100 μm.

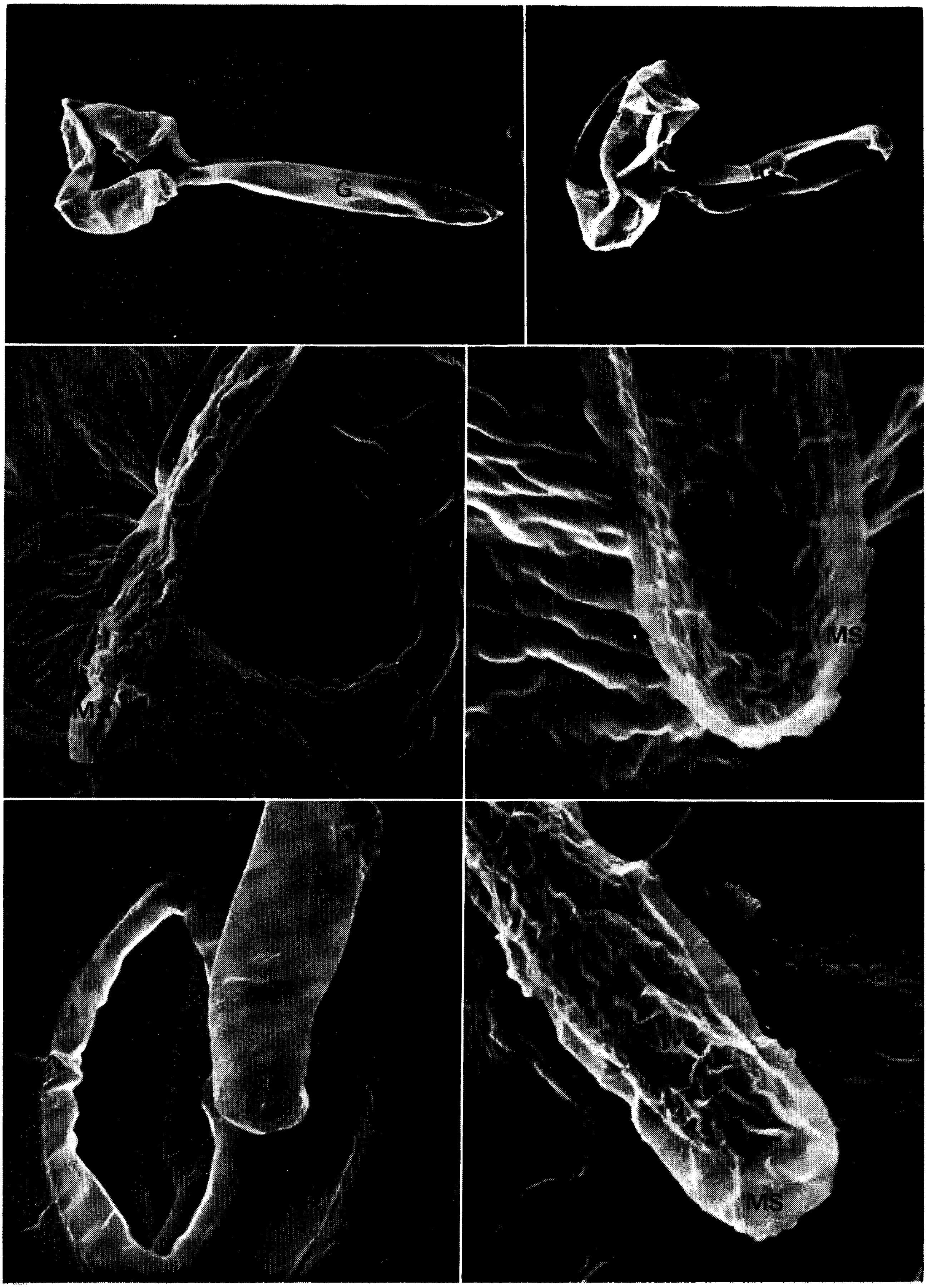
13 A conidiophore on the control surface 13 days after inoculation. Cp: Conidiophore. Bar: 10 μm.

14 A conidiophore on the lecithin (2000 ppm) treated surface 13 days after inoculation. Cp: Conidiophore. Bar: 10 μm.

15 A conidiospore on the control surface 13 days after inoculation. C: Conidiospore. Bar: 10 μm.

16 A conidiospore on the lecithin (2000 ppm) treated surface 13 days after inoculation. C: Conidiospore. Bar: 5 μm.

Plate I



Pate II

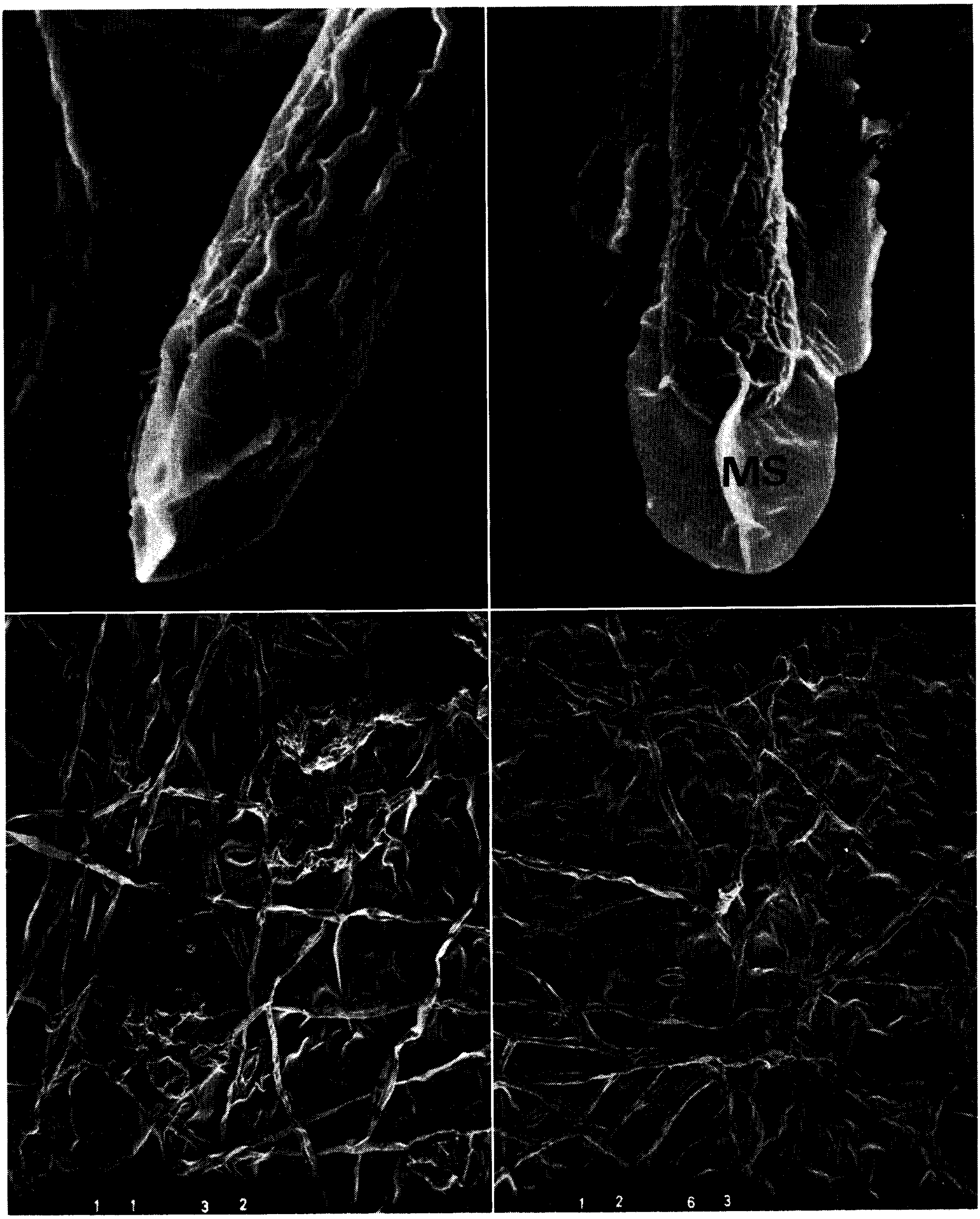


Plate III