Fig. 10. Inclusion in *N. crassa* stained with lead citrate only. Compare with lead citrate and uranyl acetate stained inclusion in Pl. 1, fig. 2.
Fig. 11. Typical Woronin bodies of *A. nidulans*, stained with lead citrate and uranyl acetate.
Fig. 12. Typical Woronin bodies of *A. nidulans* stained with lead citrate only. Compare with fig. 11.
Fig. 13. Inclusion in *A. repens* treated with sodium methoxide.
Fig. 14. Inclusion in *N. crassa* treated with sodium methoxide.
Fig. 15. Inclusion in *N. crassa* treated with trypsin for 25 min.
Fig. 16. Inclusion in *A. repens* treated with trypsin for 1 h.
Fig. 17. Woronin bodies of *A. nidulans* treated with trypsin for 1 h.

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PRODUCTION OF *COPRINUS* PROTOPLASTS BY USE OF CHITINASE OR HELICASE

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Removal of the wall from bacterial, fungal or plant cells releases the naked protoplast. Protoplasts (called sphaeroplasts if remnants of the wall remain) are stable in suspending medium isotonic with the cytoplasm and vacuolar contents, and provide experimental material for many studies (Villanueva & Garcia-Acha, 1971). We have developed methods for the preparation of protoplasts of the basidiomycete *Coprinus cinereus* (=*Coprinus lagopus sensu* Lewis) for studies of sugar transport (Heredia, Sols & De la Fuente, 1968; Moore & Stewart, 1971; Moore, 1972). The technique is described here as it may be of wider interest.

Iten & Matile (1970) reported that protoplasts were released from young gills (but not vegetative mycelium) after incubation in the liquor from autolysing sporophores. They further demonstrated that the major hydrolytic activity responsible for autolysis was due to chitinases formed shortly before spore release. Commercial chitinase (Koch-Light Laboratories) was assessed for protoplast induction with young hyphae and found to be very effective. Helicase ('snail digestive juice' obtained from Koch-Light Laboratories) was also found to release large numbers of protoplasts although ten times less effective, at the concentrations used, than chitinase. The two enzymes are compared in Table 1. The reactions were carried out in SNC medium (Stewart & Moore, 1974) containing an appropriate osmotic stabilizer. The effect of the osmotic stabilizer on protoplast formation is summarized in Table 2.

Ungerminated oidiospores yielded very few protoplasts. The most effective starting material was prepared from a suspension of oidia which had been incubated overnight (at 37°) in maltose-CM medium (Stewart & Moore, 1974). Incubation was carried out in McCartney bottles mounted on an angled turntable (60° to the horizontal) rotated at 27 rpm. This procedure yielded a uniform suspension of hyphae and microcolonies. Static culture was ineffective because of the formation of a single mat of

Table 1. *Effect of enzyme and initial quantity of tissue on yield of protoplasts*

(Entries in the table are protoplast yields expressed as number of protoplasts produced ($\times 10^{-6}$)/mg dry weight of tissue.)

Treatment	Amount of tissue treated (mg dry weight/ml)		
	2·1	4·2	8·4
Chitinase (2 mg/ml)	1·02	0·95	0·67
Helicase (50 µl/ml)	0·21	—	—
Helicase (100 µl/ml)	0·18	—	—

All treatments carried out in 1·0 M-$MgSO_4$ and for 24 h at 37 °C.

Table 2. *Effect of the osmotic stabilizer on protoplast yield*

(Entries in the table are protoplast yields expressed as number of protoplasts produced ($\times 10^{-6}$)/mg dry weight of tissue.)

Identity of stabilizer	Molarity of stabilizer			
	0·6	0·8	1·0	1·2
Sucrose	0·60	0·86	1·40	0·89
$MgSO_4$	0·48	0·68	1·14	0·74
NaCl	0·38	0·22	None	None

All treatments carried out with 2 mg/ml chitinase for 24 h at 37 °C.

interwoven hyphae, while an orbital shaker promoted formation of a few spherical pellets unsuitable for protoplast production. Hyphae were separated from ungerminated spores by centrifugation at 300 ***g*** for 2 min, to give a soft, delicate pellet of mycelium. The supernatant was carefully removed and replaced by the osmotic stabilizer. Three or four cycles of this gentle centrifugation were sufficient to free the hyphae from ungerminated spores and to replace the germination medium with the osmotic stabilizer. Addition of helicase (50 µl/ml of hyphal suspension) or chitinase (2 mg/ml) initiated cell wall digestion, which continued for 24 h at 37°. Hyphal debris was removed by centrifugation as before and the protoplasts then precipitated by centrifugation for 15 min at 4000 ***g***, washed with fresh stabilizer and finally resuspended to the required volume. With this procedure 10^7 protoplasts could be obtained from 120 mg (fresh weight) of mycelium using 2 mg of chitinase in a total volume of 1 ml.

Protoplasts came from the young hyphae as a result of enzymic removal of the wall at the extreme tip. The rest of the wall seemed to remain intact so it is assumed that true (wall-free) protoplasts were formed by extrusion of cytoplasm through the open tip. The youngest hyphae gave protoplasts containing a few small vacuoles while older material gave protoplasts containing a single large vacuole. Both types were osmotically fragile but the latter often left an intact vacuole after rupture of the plasmalemma (Garcia-Acha, Lopez-Belmonte & Villanueva, 1967). Regeneration

occurred during 24 h incubation in nutrient-supplemented stabilizer. The protoplasts lost their osmotic fragility and eventually produced hyphal outgrowths which developed into normal mycelium.

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ACHAETOMIELLA MEGASPORA, THE CORRECT NAME FOR *A. FUSISPORA*

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Achaetomiella megaspora (Sörgel) D. Hawksw. comb.nov.

Chaetomium megasporum Sörgel in Seth, *Beih. Nova Hedwigia* **37**: 82 (1972).

Achaetomiella fusispora Calviello, *Darwiniana* **18**: 558 (1974).

Achaetomiella Arx is a small genus of the Chaetomiaceae Wint. closely related to *Chaetomium* Kunze ex Fr. from which it is distinguished primarily by the presence of pale to rather greenish perithecia which possess lateral hairs but lack terminal hairs.

An examination of the type material of *Chaetomium megasporum* showed that this was in fact a species of *Achaetomiella* which proves to be conspecific with the recently described *A. fusispora.* As the epithet *megasporum* predates *fusispora* a new combination based on it is required as the name of this species.

As an excellent detailed description and illustrations of this species are provided by Calviello (1974) it is not redescribed here. It is of interest to note, however, that to judge from light microscopical observations, the ornamentation on the hairs of *A. megaspora* and *A. macrospora* (Rai *et al.*) Arx appears to be similar to that found in the type species of the genus, *A. virescens* Arx (Hawksworth & Wells, 1973).