cell division. Explants from mid-cortex and pith of apple fruits of similar maturity showed negligible growth. Disks of tissue from relatively mature pear fruits excised so that a vascular bundle ran centrally through each explant showed more active growth than tissue from the cortex, medium *E* giving more rapid growth than medium *A* + coconut milk. Apple vascular tissue (variety Sturmer) requires both 2,4-dichlorophenoxyacetic acid or an equivalent and a cell-division stimulant (coconut milk or kinetin) for active growth in culture[1]. 2,4-Dichlorophenoxyacetic acid or an equivalent is also essential for growth of pear vascular tissue, but this tissue is apparently much less dependent on a cell-division stimulant.

The possibility of replacing 2,4-dichlorophenoxyacetic acid by other compounds has been investigated. Apple and pear vascular tissue did not grow when 2,4-dichlorophenoxyacetic acid (1–5 p.p.m.) was replaced by indole-3-acetic acid (0·3–5 p.p.m.). Higher concentrations (30 p.p.m.) of indole-3-acetic acid produced some growth, but still considerably less than 2,4-dichlorophenoxyacetic acid. Potato-tuber tissue in the presence of coconut milk also shows this unusual dependence on 2,4-dichlorophenoxyacetic acid[3]. Quince cortex was more responsive to indole-3-acetic acid which, although relatively ineffective at 5 p.p.m. with this tissue, at 30 p.p.m. was nearly as effective as 2,4-dichlorophenoxyacetic acid (5 p.p.m.). For the growth of apple and pear vascular and quince cortical tissue, 2,4-dichlorophenoxyacetic acid can be replaced by some related phenoxy acids (for example, 2,4,5-trichlorophenoxypropionic acid).

Tissue has also been cultured from other pome fruits, namely, medlar (*Mespilus germanica* L.), Japanese quince (*Chaenomeles superba* Rehd.) and oriental pear (*Pyrus calleryana* Decne.).

The work reported has been assisted by grants from the Rockefeller Foundation and from the New Zealand Apple and Pear Marketing Board. Thanks are expressed to Mrs. G. E. Mark and Miss D. A. Bridson for skilled assistance and to Dr. H. R. Thompson for statistical analyses.

D. S. Letham

Fruit Research Division,
Department of Scientific and
Industrial Research,
Auckland, New Zealand.

1 Letham, D. S., *Nature*, **182**, 473 (1958).
2 Steward, F. C., and Shantz, E. M., "Ann. Rev. Plant Physiol.", **10**, 399 (1959).
3 Shantz, E. M., Steward, F. C., Smith, M. S., and Wain, R. L., *Ann. Bot.*, N.S., **19**, 49 (1955).

Protoplasts from *Penicillium glaucum*

Bachmann and Bonner[1] were able to obtain free protoplasts from *Neurospora crassa*, if this fungus was cultivated in thick liquid media, such as 20 per cent sucrose and 10 per cent enzyme preparations. Protoplasts emerge through pores in the hyphal walls. The authors showed, by means of a microscopical photo-series, that the protoplasts regenerate to form a complete new fungus mycelium.

If *Penicillium glaucum* was cultivated on thickened serum it was also possible to form protoplasts. Cells of the fungus were inoculated in thickened guinea pig serum between two sterilized glass plates and the upper glass bordered and fastened with paraffin. After some time (7–14 days) those upper plates were loosened, dried and stained with a May-Grünwald-Giemsa solution. (This simple method is very suitable for staining the internal structures of hyphæ in microscopical preparations.) Fig. 1 shows the connexion of the protoplasts with the 'vacuoles' of the interior structures of the hyphæ.

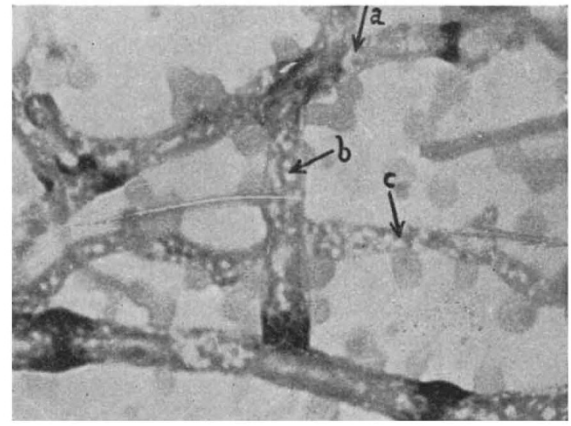

Fig. 1. Formation of protoplasts from *Penicillium glaucum* in a medium of thickened cell-free guinea pig serum. (× *c.* 1,500)

The photomicrograph shows reddish-grey, very tender forms between and on the blue hyphæ. Some of these forms are in direct connexion with the pores and 'vacuoles' of the hyphæ (*a*). Arrows *b* and *c* show these forms flowing out of the vacuoles.

In similar experiments with thick liquid media, such as sera and cell liquids, a wide extension of the hyphæ was observed. Here the typical internal structures of broad hyphæ found continues outside the hyphæ. They are embedded in complexes, the finest structures of which seem to move slowly into the surrounding medium without producing definite contours. The same effect could be seen if *Penicillium glaucum* was embedded into living plants. This, together with some photomicrographs, is published elsewhere[2].

Other experiments have been carried out on soft forms of protoplasts[3]. These results concerning the extension of internal structures may help towards understanding the above phenomenon[4].

In spite of the fact that these observations are different from the growth of fungi in normally used media, which regularly produce a quick and unrestricted growth, the present observed forms are unnatural. Under natural conditions there are many opportunities for fungi to move into living tissues or thick cell liquids and cause fermentation. Therefore it is necessary to pay attention to those alterations in the fungal elements, in order to prevent mistakes of diagnosis. Also for the sake of common biological examinations the study of changes of the cell elements, caused by alterations of the surroundings, is most important[4,5].

Georg Meinecke

Hygiene-Institut,
Hamburg 36,
Germany.

1 Bachmann, B. J., and Bonner, D. N., *J. Bact.*, **78**, 550 (1959).
2 Meinecke, G., *Zbl. Bakt.*, I, **169**, 113 (1957).
3 Meinecke, G., *Medizin. Klinik*, **45**, 772, 803, 1626 (1950); *Photographie und Wissen.*, **3**, 19 (1954); *Zbl. Bakt.*, II, **110**, 184 (1957); I, **177**, 421 (1960).
4 Meinecke, G., *Mikrokosmos*, **41**, 198 (1952); *Virchows Arch.*, **327**, 590 (1955); *Med. Mtschr.*, **12**, 18 (1958).
5 Meinecke, G., *Zbl. Bakt.*, I, **177**, 421 (1960); **180**, H.2 (1960).