the edges of the material heat-sealed. The mesh bag was placed inside a plastic photographic slide (5 × 5 cm), from which the glass had been removed leaving a window (35 × 22 cm). The 2 halves of the slide were then pressed together and the sides fused by touching them against a hot plate.

The slides provided support for the mesh bags, making handling easy; they did not degrade over the incubation period nor did the plastic appear to inhibit microbial growth.

After incubation in soil the slides were removed and opened and any undegraded keratin azure was carefully removed from the inside surface of the mesh bags and transferred to a centrifuge tube. The substrate was repeatedly washed with deionized water and centrifuged until no further dye release occurred. The water was then removed and 10 ml of NaOH (10% w/v) added. Finally, the tube was heated in a boiling water bath until the keratin azure dissolved when the resultant blue color was determined spectrophotometrically at 595 nm, using NaOH as blank. Samples of keratin azure (0.01 g) were then solubilized in the same way to determine the amount of dye initially present in the substrate, so that the amount of dye lost could be calculated by subtraction. Variation in dye content occurs when different batches of keratin azure are used so dye-loss values should be carefully matched with initial dye values obtained using the same substrate batch. The keratin azure was not sterilized prior to incubation in soil as autoclaving is known to markedly denature keratin proteins[2], while isolation studies showed that the fresh substrate was free of keratinophilic micro-organisms.

Keratin degradation was studied in an agricultural soil (previous crop potatoes; pH, 5.7; total C, 5.5%; total N, 0.7%), and a deciduous woodland soil (pH, 4.9; total C, 9.8%; total N, 0.9%). Slides were placed in 200 g of soil in triplicate, and incubated in sterile polythene bags at 20% v/w water content (maintained throughout) at 25 °C. The polythene bags were closed with an elastic band so as to leave a small hole to allow for gas exchange. Slides were also placed in soil which had been autoclaved on 3 successive occasions at 120 °C for 20 min to act as controls.

Keratin breakdown was much more rapid in the agricultural than in the woodland soil (fig. 1), despite the fact that it had not recently supported livestock, and was thus unlikely to have a high keratin content. Degradation decreased with increasing pH and was optimal at 30 °C (fig. 2), and was negligible in autoclaved soil.

Rapid keratin degradation is generally thought to occur in soils containing large amounts of the substrate[3], although even soils apparently lacking keratin contain keratinophilic fungi[4]. Keratin degradation in the woodland soil was not however, stimulated by the addition of ball-milled keratin; wool or human hair (0.5–200 g soil) to the soil 6 weeks prior to insertion of the slides. Unlike other complex substrates the addition of keratin to soil appears not to rapidly stimulate micro-organisms capable of degrading it.

This technique is currently in use in this laboratory to determine the microbial degradation of keratin in soils frequented by humans (e.g. parks and beaches) and animals, with a view to determining which soils are likely to harbor both the substrates and associated dermatophytes.

1 Acknowledgment. The technical assistance of Mrs Judith Butterworth is gratefully acknowledged.
2 G.E. Mathison, Annls Soc. belge Méd. trop. *44*, 767 (1964).
3 R. Marcantini, R. Marsella, F. Caprilli and G. Dougiallo, Sabouraudia *18*, 123 (1980).
4 D.A. Somerville and M.J. Marples, Sabouraudia *6*, 70 (1967).

## Incorporation of uridine diphospho-N-acetyl-D-glucosamine in the resting sporangium wall of *Synchytrium endobioticum*

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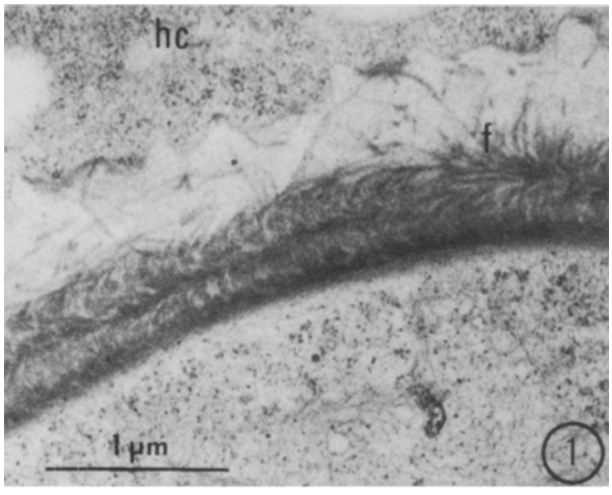
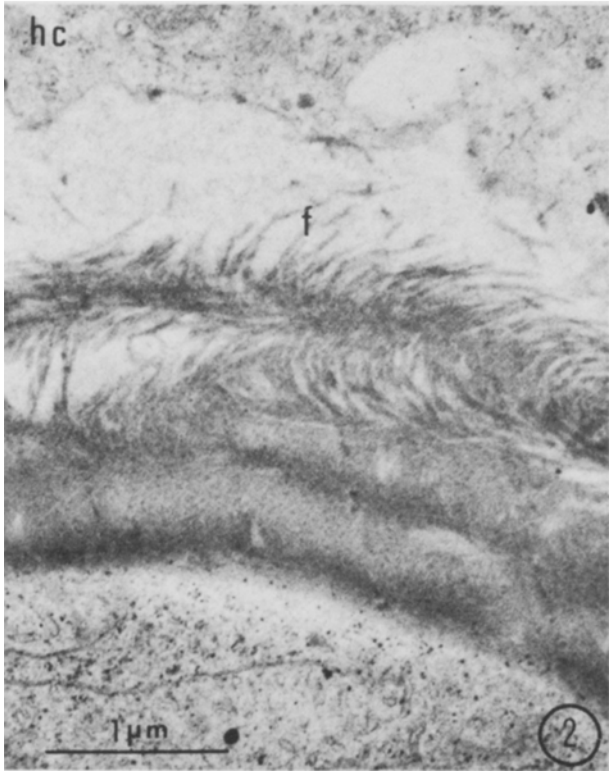
*Summary.* The microfibrils that constitute the walls of *Synchytrium endobioticum* resting sporangia are laid down at the periphery of developing walls and are packed in discrete orientation. Incorporation of uridine diphospho-N-acetyl-D-glucosamine, a precursor of chitin, is restricted to the periphery of the developing wall of the resting sporangium.

A major problem in the control of potato wart disease, incited by *Synchytrium endobioticum* (Schilb.) Perc., is the persistence of resting sporangia (meiosporangia) in the soil[2]. These propagules are known to be viable for 37 years[3]. The basis of such longevity, although not clear, putatively lies in the make up of the sporangium and its wall. Recent observations on the developing walls of sporocysts of a related species, *S. mercuriales* have revealed a complex orientation of microfibrillar material[4] similar to chitin. We report in this short communication a) the presence of microfibrils showing patterns of orientation similar to those observed on other chitinous structures[5], and b) evidence for incorporation of uridine diphospho-N-acetylglucosamine (UDP-GlcNAc), a precursor of chitin, into the walls of developing sporangia.

*Materials and methods.* For electron microscopy, pieces of fresh potato wart tissue were put into a fixative mixture of paraformaldehyde and glutaraldehyde[6] in phosphate buffer pH 7.2 at 23 °C for 1 h. The samples were washed thoroughly in cold (4 °C) buffer and treated with 1% $OsO_4$ in the same buffer. Dehydration was accomplished through an ethanol series for final embedding in Spurr's medium. Ultrathin sections were cut with a Porter-Blume ultramicrotome, stained with uranyl acetate and lead citrate, and viewed with a Zeiss 9S electron microscope.

Small (0.5 $cm^3$) slivers of fresh potato wart tissue cut from field grown potatoes cv. Arran Victory (susceptible to *S. endobioticum* European race 2), were washed in distilled water and treated with UDP-($^3$H) GlcNAc (0.01 mCi/ml). Samples were taken out at 3, 6, 12 and 48 min and fixed in




Figures 1 and 2. Electron micrograph showing successive stages in wall development in the resting sporangium. Note the discrete orientation of microfibrils (f) and their compaction towards the sporangial side. The loose microfibrils in the host cytoplasm (hc) are seen in the upper part of the micrographs.

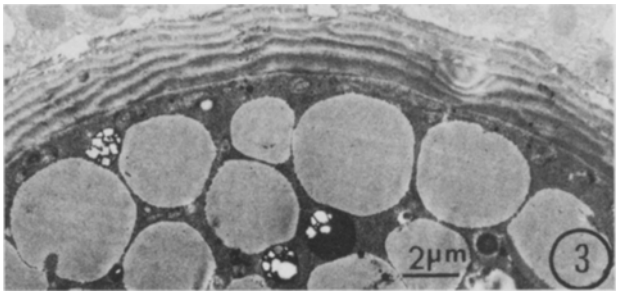



Figure 3. Electron micrograph of an almost mature resting sporangium showing lamellar alternation (dense and light layers).

acetic acid/alcohol (1:3 v/v). The fixative also removed the pool of unincorporated UDP ($^3$H) GlcNAc. A batch of samples was embedded in paraffin, sectioned and processed for autoradiography using Kodak $NTB_2$ liquid emulsion. The slides were exposed for 7 days and then developed with Kodak D19 developer.

Another batch of samples was washed and macerated in a homogenizing tube with distilled water for 1 min. The material was then filtered through polycarbonate filters (Bio-Rad Lab 5 µm pore) using a millipore apparatus. The number of sporangia on the filters was counted under a stereomicroscope at ×60 magnification. The filters, along with the sporangia, were then solubilized in a mixture of 13 ml Aquasol (New England Nuclear) and 0.5 ml 1 N HCl. Counts were made in a Beckman scintillation counter.

*Results and discussion.* Electron microscope observations of developing wall revealed microfibrils with discrete orientation (figs 1 and 2). The microfibrils appear to be laid down towards the host side (periphery) of the wall and exhibited a Bouligand pattern[5]. The microfibrils became progressively more compact (fig. 2) in the matrix of the wall, forming

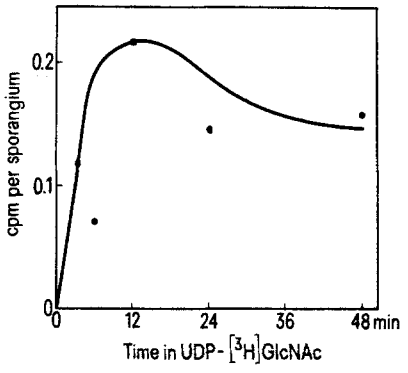



Figure 4. Incorporation of UDP-($^3$H) GlcNAc into the insoluble fraction of resting sporangia.

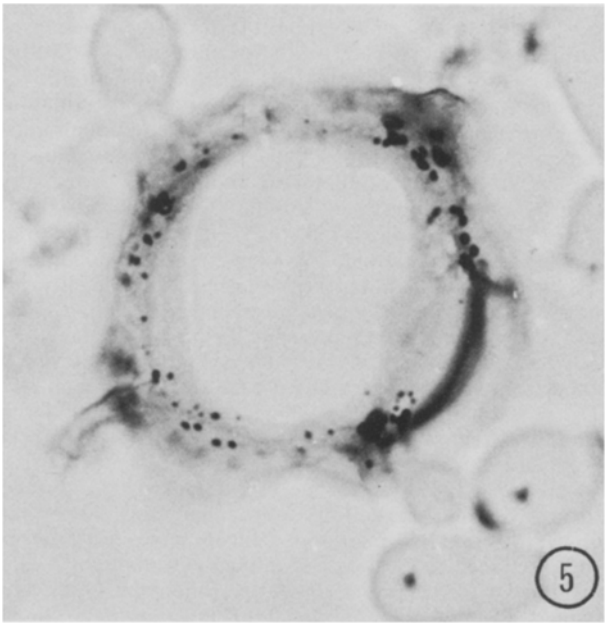



Figure 5. Autoradiograph of a section of a resting sporangium in potato wart tissue showing radioactivity from incorporated UDP-($^3$H) GlcNAc in the wall. Note the distribution of grains along the periphery of the wall. ×500.

lamellae (fig. 3), which exhibited alternate light and dense layers.

Uptake of tritiated UDP-GlcNAc in the insoluble fraction of the sporangia is shown in figure 4. There was an increase in radioactivity up to 12 min after which the uptake levelled off. This was expected in the experiment with cut slivers detached from the plant. In autoradiographs silver grains were localized in the sporangial walls, more towards the periphery (fig. 5). No grains were observed in the cytoplasm or in the host tissue.

Chitin is a polymer of N-acetyl-D-glucosamine and is synthesized from UDP-GlcNAc by chitin synthetase[7]. Incorporation of UDP ($^3$H) GlcNAc into the insoluble fraction of the wall indicates that the microfibrils seen with the electron microscope are chitinous in nature. The labelling pattern showing silver grains towards the periphery of the wall also supports the electron microscopic observation that the microfibrils are laid down towards the periphery of the developing wall. The characteristic Bouligand pattern is similar to that exhibited by chitin microfibrils in insect cuticle. The mature wall of the resting sporangium with compact layers of oriented chitin microfibrils undoubtedly serves as a protective cover, which may be related to its survival characteristics.

1 Reprint request to A.K.B., Department of Biology, Memorial University of Newfoundland, St. John's (Newfoundland, Canada A1B3X9).
2 M.C. Hampson, Can. J. Phytopath. *3* (1981).
3 M.A. Pratt, Conf. Bac. Fung. Dis. Potatoes, Brussels (1975), abstracts p. 8.
4 J. Meyer and P. Michler, Marcellia *39*, 155 (1976).
5 Y. Bouligand, C.r. hebd. Acad. Sci., Paris *261*, 3665 (1965).
6 M.J. Karnovsky, J. Cell Biol. *27*, 137A (1965).
7 L. Glaser and D.H. Brown, J. biol. Chem. *228*, 7729 (1957).

## Stimulation of sporulation of *Clostridium perfringens* by papaverine

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*Summary.* Papaverine induced sporulation in *Clostridium perfringens*, strains FD-1 and PS52; growth was markedly slowed under these conditions. Papaverine induced sporulation in the presence of glucose, a sporulation repressor, although increasing glucose concentrations overcame the papaverine effect. Papaverine induced sporulation of strain FD-1 more effectively than did theophylline.

Bacterial sporulation has been studied extensively as a model system for cell differentiation, but the molecular events leading to sporulation remain obscure. Recently a number of purine analogs were shown to stimulate the sporulation of *Clostridium perfringens*[2,3] and *Bacillus subtilis*[4,5]. In *B. subtilis* these effects occurred in the presence of glucose at concentrations normally repressing sporulation. Interference with some aspect of purine metabolism has been suggested as being responsible for this induction of sporulation[3,4]; more specifically, it has been shown to correlate with decreased levels of guanosine nucleotides[5].

The purine analogs used heretofore to induce sporulation of *C. perfringens* were all methylxanthines, and potent inhibitors of phosphodiesterase. Papaverine, a smooth muscle relaxant used as a vasodilator in humans[6], is also a very powerful phosphodiesterase inhibitor but is a benzyl isoquinoline rather than a purine. Its effects on bacterial growth and sporulation have not been reported previously. This paper demonstrates that papaverine powerfully induces sporulation of *Clostridium perfringens* strains FD-1 and PS52, while markedly reducing their growth rates.

*Materials and methods. C. perfringens* strain PS52 was obtained from the Center for Disease Control, Atlanta. Strain FD-1 was obtained from S.M. Harmon, Food and Drug Administration, Washington, D.C. Spore stocks were prepared and stored as described previously[3]. Inocula were prepared in fluid thioglycolate broth (Difco) as described previously[3]. 16 mm culture tubes containing 13 ml of Duncan-Strong (DS) medium[7], with thioglycolate concentration reduced to 0.05%, or a defined (D) medium[8], were inoculated with 0.5 ml of inoculum when the latter had attained a cell density of about 100 Klett units, determined with a Klett-Summerson colorimeter (No. 66 filter), using a water standard. Inoculated tubes were incubated at 38.5 °C and growth monitored with the Klett-Summerson colorimeter. Heat-resistant spores were estimated after heating at 75 °C for 20 min[3] by 'plating' in oval tubes in a medium containing yeast extract, 0.5%; tryptose, 1.5%; soytone, 0.5%; $NaHSO_3$, 0.1% and agar, 1.5% (Shahidi and Ferguson[9]). Papaverine-HCl and theophylline were obtained from Sigma Chemical Co., 6-mercaptopurine and 6-thioguanine from Cyclo Chemical Corp. Hadacidin was a gift from Merck and Co. Soytone and tryptose were obtained from Difco.

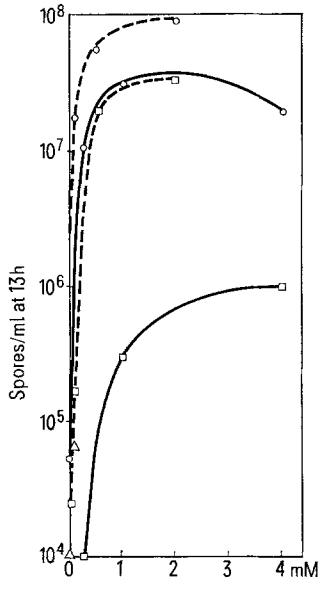


Figure 1. Influence of theophylline and papaverine on sporulation of *C. perfringens* strains PS52 and FD-1 in DS medium. □ FD-1; ○ PS52; —— theophylline; – – – – papaverine.