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.

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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.

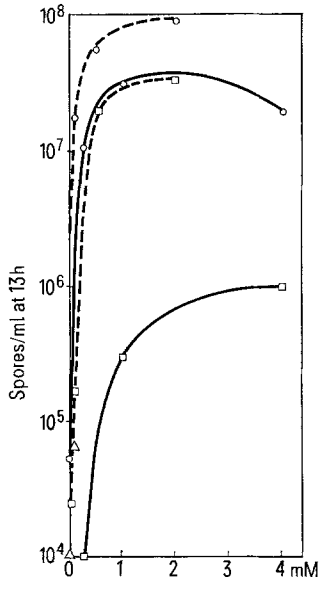

Figure 1. Influence of theophylline and papaverine on sporulation of *C. perfringens* strains PS52 and FD-1 in DS medium. □ FD-1; ○ PS52; —— theophylline; – – – – papaverine.

Results and discussion. Figure 1 shows the sporulation of strains FD-1 and PS52 in DS medium after 13 h; papaverine induced sporulation as well as theophylline for strain PS52 and markedly better for strain FD-1. Glucose is known to repress sporulation and figure 2 shows this effect on strain FD-1, in the presence and absence of papaverine. Figure 2a shows that 0.5 mM papaverine induced very high spore levels at 7 h. (No glucose added) or at 16 h if 0.05% glucose was added; growth rate was greatly reduced in both cases. In the presence of 0.2% glucose little sporulation occurred and growth inhibition was partially reversed.

Increased sporulation at reduced growth rates is well documented[10-12] and reduction of growth rate by sporulation inducing compounds has been noted previously[3,4]. Figure 2b shows that increasing papaverine concentrations could, to some extent, overcome the repressing effect of glucose on total spore yield in DS medium.

Purine analogs inducing sporulation in *B. subtilis*[4,5] (hadacidin, 6-mercaptopurine and 6-thioguanine) also induced sporulation of *C. perfringens* strain FD-1, at levels approximating those induced by theophylline (data not shown); none were as effective as papaverine. Other experiments showed that papaverine also induced sporulation in D medium, a defined medium for sporulation of *C. perfringens*[8].

Methylanthines and papaverine are phosphodiesterase inhibitors and many of the complex effects[6,13] they induce are frequently ascribed to increasing concentration of cyclic nucleotides[14]. Cyclic AMP seems to be absent from sporulating bacteria[15]; cyclic GMP is present, but no relationship to sporulation is apparent[16,17]. Moreover, the purine analogs shown effective in the induction of sporulation[4] are not known to affect cyclic nucleotide levels. A number of reports suggest that many physiological effects occurring in the presence of papaverine and the methylxanthines cannot be attributed to phosphodiesterase inhibitors[13,18-21]. It seems unlikely that the ability of methylxanthines and papaverine to induce sporulation is related to their ability to inhibit phosphodiesterase. Papaverine and caffeine have been reported to inhibit nucleoside uptake[6,13]; and papaverine has been shown to affect nucleotide levels[6]. Such events could lead to reduced guanosine triphosphate levels, postulated as significant in the induction of sporulation[5].

Papaverine may prove useful in studies on sporulation, and sporulation studies may be of value for investigations of certain physiological effects of papaverine, theophylline and caffeine[2,3].

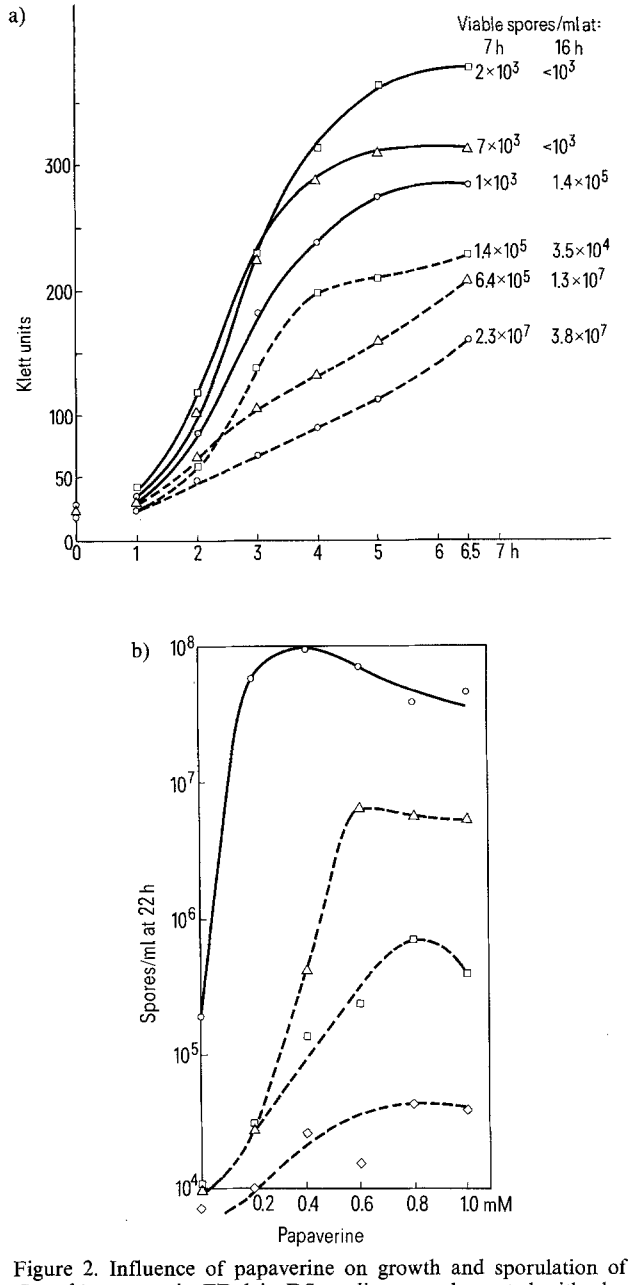

Figure 2. Influence of papaverine on growth and sporulation of *C. perfringens* strain FD-1 in DS medium supplemented with glucose. *a* Effect of added glucose on growth rate in presence of 0.5 mM papaverine. ○ Control, no glucose; △ 0.05% glucose; □ 0.2% glucose added; —— no papaverine; – – – – 0.5 mM papaverine added. *b* Effect of glucose and papaverine on total spore yields. ○ Control, no glucose; △ 0.05% glucose added; □ 0.10% glucose added; ◇ 0.15% glucose added.

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