1127–1138, 1976). Chemical analysis of sodium lauryl sulfate inactivated cell walls isolated from Ni15 and parent cells revealed only minor differences. Lysis of several independent isolates of such walls by autolysate preparations of parent cell walls revealed no reproducibly significant differences in susceptibility between Ni15 and parent walls. Assay, in enzymically active cell wall preparations, for the two autolytic activities shown to be active in wall metabolism in vivo, revealed nearly parent levels of *N*-acetylmuramyl L-alanine amidase in Ni15, whereas the *N*-acetyl glucosaminidase activity was below 5% of that of the parent. 10 min after the addition of chloramphenicol (CAP) to the parent strain, the rate of release of turnover products to the medium became constant and remained so for at least 50 min. Thus CAP addition did not lead to an inhibition of wall degradation. CAP added to a culture 1 generation after a 3 min pulse label almost completely blocked release of labelled turnover products during the following 60 min. It is concluded that continued surface expansion, blocked by CAP, is required for new wall to reach the outer surface of the cell wall layer. These results support the model (see reference above) proposing a continuous enlargement or 'spreading' in the area occupied by old wall during surface expansion. I believe that this model offers a means of reconciling published work reporting a failure to observe segregation of old wall with the apparently contradictory evidence for a discrete segregation pattern which is presented in the following communication.

Autoradiographic Study of Segregation of Labelled Cell Wall in a Mutant of *B. subtilis* with Reduced Wall Turnover

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An autoradiographic study of cell wall precursor insertion was attempted by use of strain Ni15 which has a considerably reduced wall turnover (see previous communications). This has allowed us to largely avoid complications due to wall turnover and follow wall segregation for over 5 generations. Ni15 cells were grown in casein hydrolysate supplemented medium containing *N*-acetyl-D-(1-^{3}H)glucosamine for over 5 generations (under these conditions cells grow in long chains and about 90% of ^{3}H-NAG enters the cell wall fraction (POOLEY, J. Bact. *125*, 1127–1138, 1976). Cells were filtered, washed and resuspended in fresh unlabelled medium. Samples were withdrawn at 0, 2, 4 and 5 generations after chasing, prepared for autoradiography and the grain distribution was determined. At the time of chase, grain distribution was Poissonian (50–70% probability). At 4 and 5 generations, about 10–15% of all the grains were distributed in dense clusters, situated at the ends of the chains, whose number corresponded accurately to the number of septa at time 0. The rest of the grains was distributed in a clearly non-Poissonian fashion (probability lower than 0,1%). Similar results were obtained using ^{3}H-glycerol as wall marker, suggesting comparable behaviour of teichoic acid and mucopeptide. These results are in unambiguous agreement with a discrete mode of insertion of new cell wall materiel, most probably at 3 or more sites per cell under these conditions. In our view, there are at least 2 reasons why previous experiments failed to reveal 'segregation' in gram + rod-shaped bacteria within 2 generations. The resolution by autoradiography may have been insufficient to visualize 3 or more insertion sites per cell. The 'spreading' of the surface area, occupied by old wall, could also obscure segregation within several generations after chase.

Analysis of Thermo-Sensitive Mutants of *B. subtilis* which Lyse at the Non-Permissive Temperature

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Study of cell wall biosynthesis is attempted by isolation of *ts* mutants which lyse at the non-permissive temperature due to defects in wall biosynthesis. A screening aimed to identify such mutants among over 650 *ts* mutants of *B. subtilis* Ni10, *trp*$^-$, *thy*$^-$, *xtl*$^-$, isolated by indirect selection, was performed. In a preliminary screening mutants affected in incorporation of ^{14}C-leucine were identified and further examined. Liquid cultures at relatively low cell density (2×10^7/ml) were shifted to the non-permissive temperature and the O.D. was followed. 32 strains which showed clear lysis were identified, backcrossed into a multiple auxotroph by congression and, by pairwise crossing (transformation), distributed into 8 genetic linkage groups designated A to H. Group A consisted of 15 and the other ones of 1 to 4 mutants. Using PBS1 mediated transduction or transformation groups B to H were mapped on the *B. subtilis* chromosome by 3-points crosses. Their approximate positions are as follows: *xtl*, *met* C, *ts* B; *cys* C, *ura*, *fur*, *ts* C; *ura*, *fur*, *ts* D; *pur* A, *ts* DNA C, *ts* E; *ts* F, *gta* A, *his* A; *ts* G, *his* A, *cys B*; *his* A, *ts* H, *cys* B. Group A has not been mapped so far. It shows no linkage to any of the following markers: *pur A*, *cys* A, *str* A, *pur* B, *arg* C, *met* C, *ura*, *thy* A, *cit* B, *ilv* A, *thy* B, *met* B, *trp*, *lys*, *phe* A, *leu*, *arg* A, *thr*, *cys* B, *his* A. Cell wall biosynthesis of one representative of each group was examined at the non-permissive temperature by ^{14}C-*N*-acetyl-D-glucosamine incorporation (POOLEY, J. Bact. *125*, 1127–1138, 1976). It appears that in groups A to G deficiencies of ^{14}C-NAG incorporation preceed the lysis.

Properties of Lipids in Membranes of the Stable Protoplast L-Form of *Proteus mirabilis*

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Membranes of the cell wall-less stable protoplast L-form of *P. mirabilis* correspond to the cytoplasmic membrane of their parent bacteria. In membranes of L-form strains LVI and LD52 phosphatidyl ethanolamine (PE, 78–80%), phosphatidyl glycerol (PG, 12%), diphosphatidyl glycerol (DPG, 4.5–6%) and lyso-phospholipid (LP, 1%) were found as major phospholipid components. The fatty acid composition of the L-form phospholipids differed from that of the bacterial form by almost complete absence of octadecanoic acid and by a 50% increase of tetradecanoic acid. When horse serum was added to the L-form medium for growth stimulation the protoplasts contained more DPG and LP and less PG. Also, 'foreign' lipids from the serum supplement, phosphatidyl choline and cholesterol were taken up into L-form membranes. However, cholesterol containing L-form cells exhibited no sensitivity to sterol-specific inhibitors, amphotericin B and digitonin. Thus, unlike the sterol-requiring mycoplasmas the *Proteus* protoplast L-form does not incor-

porate cholesterol to fulfill an essential membrane function. L-form membranes retain DD-carboxypeptidase, an enzyme involved in cell wall peptidoglycan synthesis in a tightly membrane-bound form requiring detergent action for solubilization. From this behaviour the enzyme would be expected to belong to the 'intrinsic' membrane proteins whose function normally depends on their association with membrane phospholipids. However, treatment with phospholipase C and/or extraction with acetone/NH_4OH removed up to 90% of the phospholipid from L-form membranes but left DD-carboxypeptidase activity largely intact.

Site of Mannan Synthesis in Yeast

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Thin sections of *Saccharomyces cerevisiae* and *Candida utilis* were floated on colloidal gold (5 nm ∅) labelled with their homologous antimannan antibodies. Mannan was found concentrated in the developing septum, at the periphery of the cell wall and near the plasmalemma. In bud scars, mannan overlaid an area attributed to chitin. Mannan was also located in vesicles near the plasmalemma especially in the bud and in myelinic structures within the cytoplasm which was otherwise almost free of marker. Similar results were obtained with colloidal gold labelled with Concanavalin A. These results indicated that branched mannan is synthesized within the cytoplasm with the same immunochemical specificity as that of cell wall mannan. The experiments confirmed that fully synthesized mannan is present in the plasmalemma, that mannan overlays chitin in bud scars (HORISBERGER and ROSSET, Experientia *32*, 798, 1976, and HORISBERGER et al., Arch. Microbiol., *109* 9, 1976) and that multimembrane bodies could be the sites where polymerization of the mannosyl units takes place (CORTAT et al., Biochem. biophys. Res. Commun. *53*, 482, 1973; KOSINOVA et al., Arch. Microbiol. *99*, 255, 1974).

Papulacandin, a New Antibiotic, Active Especially Against Yeasts

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Papulacandin is produced by a strain of *Papularia sphaerosperma* (Pers.), Höhnel, which belongs to the Fungi Imperfecti. This lipophilic antibiotic was isolated by extracting the culture broth with ethyl acetate and purifying the crude extract using silicagel chromatography. It consists of a mixture of several components. The majority of the activity is present as component B, which contains two unsaturated fatty acids. Papulacandin is highly active against *Candida albicans* and several other yeasts. It shows very slight activity against a number of fungi and is inactive against bacteria. The mode of action is fungicidal but only on budding cells; resting cells are unaffected. The mycelial form of *Candida*, owing to its slower growth rate, seems to be less sensitive to the antibiotic. Papulacandin shows no cross resistance with polyene antibiotics. No reduction of its antibiotic activity was observed in the presence of various sterols. On comparing Papulacandin with two other yeast active antibiotics found in our screening, namely Echinocandin and Conocandin, cross resistance with the former was demonstrated but no cross resistance with the latter. Echinocandin (KELLER et al., Helv. chim. Acta *57*, 2459, 1974) is a polypeptide with a fatty acid residue, whereas Conocandin (pers. communication J. MÜLLER, Ciba-Geigy) is itself a fatty acid of unusual structure. Papulacandin does not cause leakiness in the cell membrane of *Candida albicans*. This could be shown by assaying for nucleic acid release through the cell envelope. There was also no effect on nucleic acid synthesis. A slight inhibition of protein synthesis was found. The new antibiotic inhibits the synthesis of the structural glucan of the cell wall. The mannan component does not seem to be affected. Thus Papulacandin apparently shows a mode of action analogous to that of penicillin on bacteria.

Regulation of Cell Size at Division in the Fission Yeast *Schizosaccharomyces pombe*

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Cell division is regulated by a control which maintains a constant size at division and therefore coordinates division to cellular growth. Mutants altered in that regulation have a normal growth rate but divide at half the size of the wild type. They define two unlinked genes *wee1* (10 mutants) and *wee2* (1 mutant). *Wee1* mutants are semi-dominant, suggesting a gene-dose effect, whereas the *wee2* mutant is almost dominant over wild type. *Wee2* is very close and possibly allelic to *cdc2*$^-$ mutants which are defective in nuclear division. A tentative model for size control over cell division will be presented.

Determination of Enzyme Activities in Permeabilized Cells of Various Microorganisms

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For investigating metabolic pathways in microorganisms, simple methods for determining enzyme activities are required. We have developed a procedure for permeabilizing cells which allows in situ measurement of enzyme activities using minimal cell mass. The method was originally devised for assaying amino acid biosynthetic enzymes in *Saccharomyces cerevisiae* but has also proved to be successful for assaying the analogous enzymes in *Schizosaccharomyces pombe* and *Escherichia coli*. Cells, preferably grown on minimal medium, are harvested during any growth phase by centrifugation. After washing the cells with distilled water and potassium phosphate or *Tris*-HCl buffer (both 0.1 *M*, pH 7.6), they are resuspended in the respective buffer containing 0.05% Triton X-100 to provide a final cell concentration of 100 mg cells (wet weight) per ml buffer. After thorough mixing, the suspension is frozen at −20 °C. The cells could be stored in this state for several weeks without loss of activity for most of the enzymes tested. Prior to the enzyme assays, the cell suspension is thawed carefully in a water bath at 30 °C and then placed in an ice bath. Using this method we tested biosynthetic enzymes of the arginine, histidine, and tryptophan pathways. Enzyme activities in the permeabilized cells proved to be generally higher and significantly more stable than those in crude extracts.

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