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 Samples were withdrawn at 0, 2, 4 and 5 generations after chasing, prepared for autoradiography and the grain distribution was determined.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-