Our previous studies of *Streptococcus faecalis* 9790 have shown that the depletion of an essential amino acid from a highly buffered synthetic medium corresponds to the end of the exponential growth phase (log phase) (2, 3). The end of the log phase is followed by further turbidity changes that are characteristic for the particular amino acid studied. The exhaustion of valine is followed by a slow increase in turbidity of 40 to 50 per cent, in about 11 hours. It was found that this turbidity increase is accompanied by an increase in the dry weight of the culture, most of which can be accounted for by the formation of additional cell wall substance (1, 2, 4).

When growth is limited by the amount of threonine present in the culture medium, the end of log phase (and threonine depletion) is also followed by a turbidity increase. However, instead of leveling off after a gain of 40 to 50 per cent, the turbidity slowly continues to increase for several days. Eventually, a turbidity level that is more than twice that at the end of log phase is reached.

We have now demonstrated that the primary consequence of threonine depletion, like valine depletion, is the synthesis of additional cell wall substance without concurrent cytoplasmic protein synthesis. The extent of wall synthesis appears to be at least twice as large as is the case for valine deprivation.

**RESULTS**

*Analysis of Whole Cell Substance*—Part A of Table I shows the composition of a lyophilized crop of threonine-limited cells (Column 3) compared with that of log phase cells (Column 1).

It can be seen that the percentage content of nitrogen and of three cytoplasmic amino acids, isoleucine, threonine, and valine, is lower in the threonine-limited cells than in log phase cells. The concentration of lysine, a constituent of both cytoplasmic substance and the cell wall, remains fairly constant while the percentage of the sugar rhamnose, which occurs almost exclusively in the cell wall (4, 5), increases considerably.

The total amounts of these substances present in the cells at the end of log phase, or more specifically at the point at which the limiting amount of threonine is depleted from the culture medium (3), are shown in Column 2. The total amounts present in the cells after 41 hours of incubation under the same conditions of threonine restriction are shown in Column 4. From these data the net changes in the total amounts of these substances after exhaustion of the protein essential amino acid, threonine, to the end of the 41 hours of incubation have been calculated (Columns 5 and 6). Thus we find that the 87 per cent increment in turbidity is accompanied by a nearly equal increase in dry weight but that there are only minor changes in the total amounts of the amino acids, isoleucine, threonine, and valine, which are not major components of the cell wall. The lower concentration of these amino acids in the threonine-limited cells (Column 3) is merely the result of dilution by cellular substances from which they are absent. The increment in total amount of bacterial lysine closely follows the increment in dry cell substance and turbidity, whereas the quantity of cellular rhamnose shows a far greater increment since this sugar is essentially absent from the cytoplasm of this organism (4, 5). It may thus serve as a direct indicator of the increase in cell wall substance.

*Cell Wall Substance*—The experiment described in Fig. 2 shows that the time course of mechanical disruption (4, 5) of threonine-limited cells follows the time pattern of exponential and valine-

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1 Optical density adjusted to agree with Beer's law (6).
limited cells (4, 5), but reaches a somewhat lower plateau of soluble nitrogen. Several quantitative disruptions of threonine-limited cells gave average values of 72 ± 2 per cent for the soluble and 25 ± 2 per cent for the sedimentable portion of the total cellular nitrogen.

Part B of Table I shows the nitrogen content of isolated and purified cell wall preparations that are essentially free from cytoplasmic contamination as determined by the absence of electron dense material in electron micrographs (4). It should be noted that as contrasted to the results obtained on valine-limited cells (2, 4), walls from threonine-limited cells show a significantly higher nitrogen content (6.4 per cent versus 5.7 per cent) than do walls of exponential cells. The fraction of the total cellular nitrogen represented by cell wall is also indicated in the table. When these results are used to calculate the changes occurring after the depletion of threonine (see under the discussion of part A of the table), a net gain in cell wall nitrogen of the same order of magnitude as the gain in cellular rhamnose is obtained. The fraction of cell weight accounted for as cell wall can be calculated from its nitrogen content and the fraction of total cellular nitrogen it represents, and gives an estimate of 44 per cent of the dry weight of the whole cells as contrasted to 25 per cent for cells from the log phase.

No qualitative difference in the composition of the cell wall preparations from exponential or threonine-limited cells was revealed by paper chromatography for amino acids, amino sugars, and sugars. The increase in nitrogen content and a similar gain (averaging about 12 per cent) in the concentration of the 5 principal wall amino acids, D- and L-alanine, L-lysine, D-aspartic, and D-glutamic acid (details to be published) indicate that there may be quantitative differences in composition.

It should be noted that the increase in turbidity, dry weight, and cell wall substance which result from threonine depletion are approximately twice as large as those which follow the depletion of valine. All of the gain in nitrogen and 70 per cent of the gain in cell weight was accounted for as cell wall substance in the case of valine limitation (2, 4). In this instance, about 70 per cent of both increases (nitrogen and weight) represent wall substance.

Our results indicate that the events that follow the termination of exponential growth by the exhaustion of the available supply of threonine or valine, and presumably other amino acids...
(3) are characterized by the synthesis of additional cell wall substance. Significant assimilation of amino acids essential to cytoplasmic proteins, but not to the cell wall seems to be absent. The increments after threonine depletion are of a larger order of magnitude than those obtained after valine depletion and have revealed not only a further increase in the ratio of cell wall to cytoplasm but have also indicated that changes in the cytoplasmic portion of the cell may occur. The significance of the changes in relation to the differential effects of the depletion of different amino acids (3) remains to be explored.

Cells grown under conditions of threonine limitation, like valine-limited cells are not as subject to lysis, as are cells resulting from lysine depletion or as cells taken directly from the exponential growth phase (2, 4, 7). It seems reasonable to assume that the amount of cell wall is an important factor in this behavior. The incorporation of radioactive amino acids into the cell wall of Staphylococcus aureus during the inhibition of protein synthesis but not of cell wall synthesis by chloramphenicol (8, 9) seems to resemble the post exponential formation of additional cell wall substance by S. faecalis when cytoplasmic synthesis is prevented by the deprivation of a protein component. The addition of chloramphenicol to S. faecalis cells concurrent with the depletion of threonine is without significant effect on the subsequent turbidity changes.

**SUMMARY**

Analyses of cells of Streptococcus faecalis 9790, indicate that after the depletion of threonine from the growth medium, cell wall synthesis continues. The increases in turbidity, dry weight of the cells, rhamnose, and cell wall substance, which follow the depletion of threonine are about twice as large as those that follow the depletion of valine. Additional cell wall substance accounts for about two-thirds of the gain in weight and nitrogen. Indications of changes in the cytoplasmic portion of the cell have been obtained. Cells resulting from growth under conditions of threonine limitation, like valine-limited cells, are resistant to the type of autolysis shown by log phase or lysine-limited cells.

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