

LOCALIZATION OF  
CHLORTETRACYCLINE AND  
VITAMIN B<sub>12</sub> IN SUBCELLULAR  
FRACTIONS OF *STREPTOMYCES*  
*AUREOFACIENS*

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Up to now no data have been published on the localization of chlortetracycline in subcellular fractions of animal or microbial cells. The distribution of vitamin B<sub>12</sub> in animal cells after oral or parenteral application has been described in several papers<sup>1-8</sup>) and the distribution of added and utilised

vitamin B<sub>12</sub> in subcellular fractions of the cells of microorganisms is also known<sup>9,10</sup>). We are now reporting studies on the localization of chlortetracycline and vitamin B<sub>12</sub> in subcellular fractions of the producing organism *Streptomyces aureofaciens*.

The conditions of fermentation and the dynamics of chlortetracycline and vitamin B<sub>12</sub> synthesis were described in our previous paper<sup>11</sup>), where the methods of assay of both of these substances were also described. The assays of both of these metabolites in all cellular fractions were repeated three times in quintuplicate. For the fractional centrifugation the method of ZAMEČNIK and KELLER<sup>12</sup>) as modified by LINGREL and WEBSTER<sup>13</sup>) was applied, using a cooled blender for homogenization. Samples were taken at the 15 th, 25 th, 35 th and 45 th hour of fermentation and for the centrifugation a Beckman ultracentrifuge, model Spinco L 2 with rotors 19 and 50 Ti at 30,000 × *g* and 105,000 × *g*, respectively, were employed.

The experiment were repeated three times and were fully reproducible. The results are presented in Figs. 1 and 2.

Fig. 1. Content of chlortetracycline in homogenate and subcellular fractions of cells of *Streptomyces aureofaciens* during fermentation. Amounts of homogenized mycelium and of mycelium for the preparation of subcellular fractions were the same.

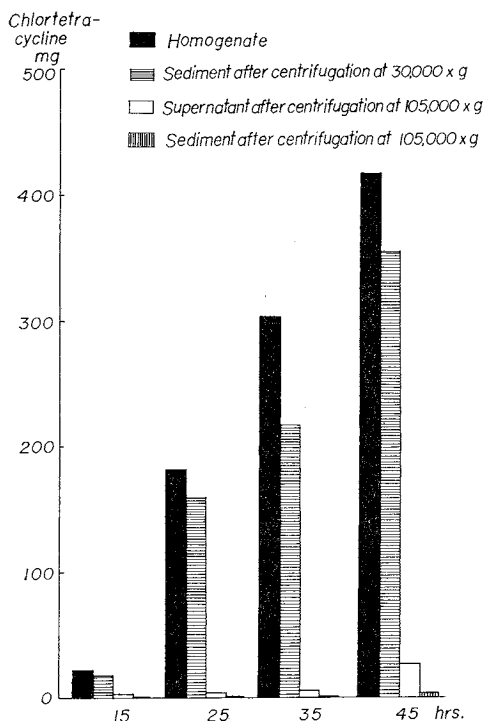
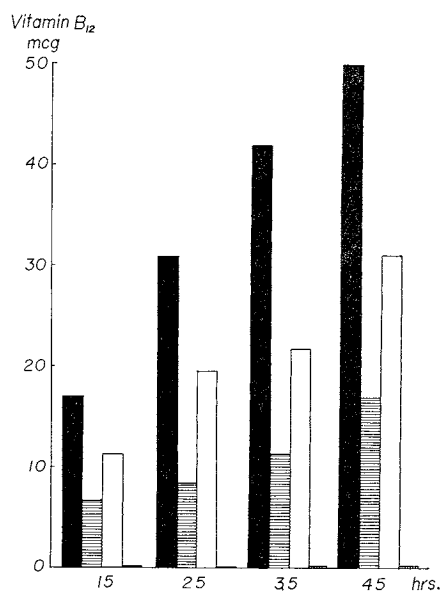


Fig. 2. Content of vitamin B<sub>12</sub> in homogenate and subcellular fractions of cells of *Streptomyces aureofaciens* during fermentation. Explanations as in Fig. 1.



It is evident that a substantial part of the chlortetracycline is localized in the sediment after centrifugation at  $30,000 \times g$ , while a major part of vitamin B<sub>12</sub> is to be found in the supernatant following centrifugation at  $105,000 \times g$ . The localization of the metabolites does not change during fermentation but their amount gradually increases.

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