NOTES

GROWTH OF MYCOBACTERIUM TUBERCULOSIS VAR. HOMINIS IN AGITATED CULTURE

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It has been the experience of many investigators that agitation or aeration of freshly inoculated *Mycobacterium tuberculosis* in liquid culture media prevented growth of this organism. In an attempt to secure both initial growth and maximal
drop of the virulent H37Rv strain of *M. tuberculosis*, the influence of various nutrient media in a shake culture was investigated. Erlenmeyer flasks containing 130 ml of a modified Kirchner's medium (McKee, Rake, Donovick, and Jambor,
Am. Rev. Tuberc., 60, 90, 1949) or Dubos’ medium (Dubos and Davis, J. Exptl. Med., 83, 409, 1946) were inoculated with 0.6 ml of a 7 day old actively growing H37Rv culture and incubated at 37 C. A portion of the flasks were agitated at 240 oscillations per minute on a constant speed rotary action shaker. Other flasks were shaken manually once daily or not at all; these served as controls. The variation of pH during growth was uniform with all samples tested. At intervals, 10 ml samples of these cultures were transferred to standard tubes and turbidities measured with a “lumetron” colorimeter (580 m). Results are shown in figure 1.

Values read from dry weight standard curves indicated that shake cultures in modified Kirchner’s medium, after 10 days, produced 5 mg of cells per 10 ml medium as compared to 4 mg developed in control flasks in the same time period. Similar results were produced by agitation with a stream of sterile air. No staining differences were observed between the shaken and nonshaken bacteria.

The organisms grew in the shaken cultures with modified Kirchner’s medium in smaller and more compact aggregates, similar to the type of growth usually obtained with various fungi. The stationary cultures showed typical rope-like arrangements. Organisms grown in Dubos’ medium showed less turbidity when shaken than when manually agitated. In both instances growth was much less than in modified Kirchner’s medium. Turbidity readings of cultures grown in modified Proskauer-Beck medium (Youmans, J. Bact., 51, 703, 1946) were not possible because strain H37Rv, regardless of physical environment, assumes a floculent type of growth.

Thus, it has been shown that M. tuberculosis inoculated into modified Kirchner’s medium may be grown in an agitated or aerated environment. Although it seems that growth by these methods is not more rapid, shake cultures yield greater dispersion of cellular aggregates and an increase in the number of cells after seven days in this medium.

UTILIZATION OF PSEUDOVITAMIN B₁₂ BY MUTANTS OF ESCHERICHIA COLI

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Pfiffner and co-workers (J. Am. Chem. Soc., 74, 1108, 1952; Federation Proc., 11, 269, 1952) have recently reported that two compounds, isolated from certain bacteria and designated as pseudovitamins B₁₂ and B₁₂₁, differ from the corresponding vitamins in containing adenine rather than 5,6-dimethylbenzimidazole. We have tested these pseudovitamins, kindly provided by Dr. Pfiffner, as growth factors for mutants of Escherichia coli that respond to either vitamin B₁₂ or