BACTERIAL GROWTH ON TYGON TUBING

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Keddie, *et al.* (1) reported a biotin requirement for *Arthrobacter atrocyaneus*, but Wolfson and Krulwich (2) reported good growth of *A. atrocyaneus* in the absence of biotin. In our work on the morphogenic cycle of *A. atrocyaneus* **(**CBRI 21001), we initially could not demonstrate any effect of added biotin on the growth or morphogenesis of the culture. Growth was carried out on a medium containing mineral salts with various inducing compounds as carbon sources (3). In order to reduce variation between experiments, the mineral salts solution was prepared in 15-1. quantities and stored in a Pyrex carboy with a glass and Tygon tubing dispensing system. This quantity of solution would last through several weeks of experimentation.

The addition of avidin to the medium caused a significant reduction of growth and a change in the morphogenic cycle, thus revealing that the organism did, indeed, require biotin. Thus the problem arose of tracing the source of biotin in our culture medium. All inorganic chemicals used were analytical reagent quality and the organic components were the purest grade available. No trace of biotin could be found in any of these components. It had been observed that the Tygon section of the dispensing apparatus for the mineral salts solution became cloudy after a week or so, but, owing to the advertised inertness of Tygon, we assumed that this was due to precipitation of some mineral salts in the solution. A more careful examination of the Tygon revealed that bacteria were growing in that section of the apparatus. The bacteria were isolated and examined, and the predominate one was of the genus *Pseudomonas.* Cleaning the apparatus and using new Tygon tubing eliminated the growth and showed that the source of the biotin in our medium was the bacterial growth in the tubing.

Tygon is a polyvinyl chloride with excellent resistance to chemical action. Plasticizers are added to make the PVC more flexible and to give it better working properties. The plasticizers may vary, but all are organic chemicals, most of which can support microbial growth. The *Pseudomonas* species isolated from the Tygon tubing in the dispenser was found to grow well on small pieces of Tygon suspended in the mineral salts solution. As the cells grew, the Tygon became rigid and cloudy, and showed pitting. Thus, although Tygon may be chemically inert, it is not biologically inert.

This report demonstrates how a seemingly safe assumption can lead to false experimental conclusions. It also proves the fallacy of assuming that Tygon tubing is biologically inert. The subject is reviewed by Osmon and Klausmeier (4), who cite references to the mode of microbial action in the degradation of Tygon.

REFERENCES

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