

$\frac{-M(C_2H_2)}{N(C_2H_2 + N_2)} = 20$. We have also found N_2^+ ions to have a like influence in the polymerization of cyanogen and of hydrogen cyanide where again the values $\frac{M(C_2N_2)}{M(C_2N_2 + N_2)} = 7$ and $\frac{M(HCN)}{N(HCN + N_2)} = 10$ are practically identified with those reported above for cyanogen and hydrogen cyanide alone. This new type of catalysis in the gas phase will be more fully considered in a subsequent paper.

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MORPHOLOGICAL CHANGES IN BACTERIUM TUMEFACIENS

IN determining the location of the organism, *Bacterium tumefaciens*, in the crown gall disease, it has seemed desirable to study daily fixations of tissues inoculated, together with smears of the bacteria from similar cultures used for the inoculation. These studies were made daily for a period of seventy-two days for twelve subcultures.

While I am not ready to report my observations on the tissues studied nor to give in detail my study of the smears examined, I believed there is one point of sufficient interest to students of bacteria, pathogenic to plants and animals to warrant this preliminary statement.

A smear of a bean agar culture of *Bacterium tumefaciens* three months old carefully prepared and stained with Loeffler's methylene blue shows an amorphous mass of jelly-like substance with occasional deeply stained minute spherical bodies. On transferring a needle full of this material to a fresh bean agar media a pearly white growth appears two days later about the streak or point of inoculation. A smear of this young culture shows long rods which not infrequently present a beaded appearance. These bacilli are apparently in the stage Henrici¹ refers to as "embryonic."

The culture grows very rapidly and under favorable conditions covers an area of one and one half cm in diameter in three to five days. Bacteria from the old and young cultures tested separately on young geranium plants were found to produce the crown gall disease equally well.²

¹ Henrici, A. T., *SCIENCE*, N. S. 61, No. 1591, 644-647, 1925.

² Levine, Michael, *Bull. Torrey Bot. Club*, 50: 231-243, 1923.

Daily smears made with care to avoid contamination show that the rods break up and become smaller and smaller as the age of the culture increases until after twenty days or less when the rods are replaced by small faintly staining cocci with occasional slender bacilli or filaments. The zoogloeal mass generally present appears to increase with the increasing age of the culture. The cocci also seem to disappear and the entire field resembles the picture first described. Studies of transfers have been made a great number of times with the same results.

In a large number of my subcultures which were studied separately an abundance of small lenticular bodies appear which are about equal in length but a little wider than the *Bacterium tumefaciens* rod. Both ends of this body stain deeply with a large variety of stains. The center portion of the body is hyaline and fails to color. These bodies are undoubtedly spores. When transferred to fresh media they germinate and give rise to a rod-like body not unlike *Bacterium tumefaciens* in size. In old cultures these spores appear to lose their ability to stain, but may be recognized as the body most abundant in the smear. I have tried to use these spores as a guide in locating the rods of *Bacterium tumefaciens* in the host. Unfortunately they also appear to be lost in old overgrowths. While I am not prepared to state definitely at this time that these spores form another phase in the life of *Bacterium tumefaciens*, they do not interfere with the development of the crown gall disease. Smith³ states definitely that no spores are formed by *Bacterium tumefaciens*.

These observations may, in a measure, explain the difficulty associated with locating the causative organism in old crown gall tissue. It appears that Löhnis's⁴ studies on the life cycle of bacteria find support in my observations of *Bacterium tumefaciens*. It is quite possible that the various sizes ascribed to *Bacterium tumefaciens* by Smith, Robinson and Walkden,⁵ and Riker,⁶ may be due to the fact that the age of the culture studied by these authors was not taken into consideration. The cultures of bacteria I have been studying principally are Smith's hop strain of *Bacterium tumefaciens*.

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³ Smith, E. F., Brown, A. N., and L. McCulloch, U. S. Dept. Agr. Bur. Pl. Ind. Bull. 255, 160, pls. 109, 1912.

⁴ Löhnis, F., *Mem. Nat. Acad. Science*, 16, 1921.

⁵ Robinson, W., and Walkden, H., *Ann. Bot.*, 37, 299-324, pls. 5-6, 1923.

⁶ Riker, A. J., *Jour. Agr. Res.*, 25, 119-132, pls. 1-5, 1923.