

A NOTE ON THE MORPHOLOGY OF BACTERIA  
SYMBIOTIC IN THE TISSUES OF HIGHER  
ORGANISMS

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In connection with a study of the cytoplasmic relationships of root-nodule bacteria in the common white clover, the author found a stage in the morphogenesis of *Bacillus radicum* that, apparently, is not well known. A careful search was made in textbooks of bacteriology for a description or mention of this form, but in vain. Through the courtesy of Dr. F. Löhnis of the United States Department of Agriculture, I have been able to find references to similar forms. Doctor Löhnis (1921) in his exhaustive review of the literature on the life cycles of the bacteria describes and illustrates round forms of *Bacillus radicum* which appear to be similar to the spherical forms that I found. Löhnis and Smith (1916) were the first investigators to observe the spherical forms. More recently Bewley and Hutchinson (1920) have observed, apparently, the same form in cultural conditions and call this stage in the morphogenesis of the organism the "swarmer" stage.

In relation to the spherical forms that have been described by Löhnis (gonidia, regenerative units) it remains to be decided whether the spherical forms that I have called "senile" are derived from the branched forms (bacteroids) or from the symplasm. The relationship and character of these spherical forms in the root nodule is decidedly interesting and I believe that a study of these forms in sections of the root nodule may be valuable in the interpretation of their nature. It appears to the author that these spherical forms are fragile and are generally destroyed in the ordinary bacteriological technique.

When my specimens were first examined with a low magnification of the microscope I was impressed by what appeared to be three distinct regions or areas in the root nodule. On closer examination with oil immersion lens, the three areas were found to contain three distinct forms of organisms. Each form was more or less limited to a single area. In the part of the nodule next to the plant root, the nodule cells contained no other than the spherical forms. I interpret this part of the nodule to be the older part and on the basis of this interpretation have called the spherical cells "senile" forms. The "bacteroid" forms of the bacillus were likewise limited, almost entirely, to the central portion of the nodule. In what I interpret to be the younger part of the nodule the bacilli were all of the small variety and not nearly so numerous as the other forms.

My interest in these forms is not "bacteriological" and I have no desire to pursue the investigation any further, at least for the present. However, it does occur to me that the technique that I have used may be valuable in bacteriological research. In a recent publication (Wallin, 1922a), I submitted evidence that mitochondrial methods are not specific, but will also stain bacteria. It has since occurred to me, particularly in connection with my study of root-nodule bacteria, that the mitochondrial technique may, at least in some cases, be superior to the usual bacteriological methods, particularly when dealing with symbiotic bacteria. I have tested a number of mitochondrial methods on various kinds of bacteriological material: sputum smears, pus smears and sections, tissue smears, bacterial smears, etc. In the majority of instances the differentiation between bacterium and tissue has been decidedly sharp.

In staining the root nodules, I have used only one method. This consisted in fixation of the entire nodule in a modification of Flemming's fixative: 4 cc. 2 per cent aqueous solution of osmic acid and 6 cc. 1 per cent aqueous solution of chromic acid. (Fix from four to twenty-four hours.) After washing, dehydration, clearing, and embedding in hard paraffin (58°), sections were cut 3 micra in thickness. The sections were mounted on slides and stained by Bensley's (1911) anilin fuchsin-methyl green method:

The staining solutions are:

1. Altmann's acid fuchsin anilin solution:  
Acid fuchsin.....20 grams  
Anilin water.....100 cc.
2. 1 per cent solution of methyl green

The sections after being prepared for the staining process by treatment with permanganate of potassium followed by oxalic acid, are stained for five minutes in the acid fuchsin solution which has been previously warmed to 60°C. Next they are thoroughly washed in distilled water, and dipped for an instant into the solution of methyl green, then washed, rapidly dehydrated in absolute alcohol (alcohol of intermediate strength must be avoided) cleared in toluol, and mounted in balsam.

The author has found that the permanganate and oxalic acid treatment may be omitted if the fixation has not been carried too far. However, if the staining differentiation is not sharp the sections may be treated for a minute or so in 1 per cent permanganate of potassium and followed by a similar treatment in 5 per cent oxalic acid. I have also found a great variation in the quality of various brands of methyl green. I have only been able to get good results with Grüber's methyl green. This method, when carried out successfully, gives excellent differentiation between bacteria and tissues.

There are several mitochondrial methods in use. Benda's crystal violet method, perhaps, gives the sharpest differentiation of all these methods. This is a very long and tedious method, but in some cases the results appear to justify the longer procedure. Other methods are: Altmann's anilin fuchsin picric acid method, Schridde's modification of Altmann's method, Regaud's method, the copper and iron hemotoxylin methods, etc. I have stained bacteria by all these methods with good results.

The mitochondrial staining methods were devised to fix and stain delicate bodies (mitochondria) in the cytoplasm which are not visible after ordinary histological technique. It appears that some symbiotic bacteria, particularly those that have an intracellular relationship, are just as fragile and delicate as mito-

chondria. The author believes that his results on the root-nodule bacteria were due to this technique. For illustrations of the three forms of *Bacillus radicum*, the reader is referred to an article by the author (Wallin, 1922b).

Murray (1919) has recently recommended the use of mitochondrial technique in certain cases where the Gram stain, for example, does not give differential results.

The author also wishes to call attention to the action of janus green when applied to bacteria. All bacteria, apparently, are not stained by janus green, but certain strains are and, apparently, only under certain conditions. This is a vital stain and it is possible that it may be found useful in the study of certain bacteria. The reader is referred to Cowdry (1918) for directions and information regarding the proper brand of janus green.

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