A PROCEDURE FOR THE SIMULTANEOUS DEMONSTRATION OF THE CELL WALLS AND CHROMATINIC BODIES OF BACTERIA

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A review of the methods which have been used to demonstrate the chromatinic bodies of bacteria will reveal a failing common to all, namely, that they demonstrate the barriers of the bacterial protoplasm very poorly or not at all.

The transverse cell walls have occasionally been shown by Piekarski's (1937) hydrochloric acid-Giemsa technique, for example, in photographs presented by Robinow (1946) and Flewett (1948). The author has found from his own experiences, and from examining photographs presented by other workers, that with the technique mentioned above the transverse walls appear irregularly and are poorly stained. A method is here presented which corrects this difficulty in at least one group of bacteria, the genus *Bacillus*.

METHODS

Cultures of the various organisms studied were grown on brain heart infusion agar (Difco) plates. The inoculum of the several species of *Bacillus* studied was prepared by suspending in 0.85 per cent sodium chloride the sporulated growth from potato extract agar slants. In the case of *Micrococcus pyogenes* var. *albus* and *Escherichia coli* the inoculum consisted of 18-hour brain heart infusion broth cultures. In all cases, one drop of inoculum was spread over the surface of the medium with a glass rod. *Bacillus* and *Micrococcus* were incubated at 37 C for $3\frac{1}{4}$ hours, and *Escherichia* for 2 hours at 37 C.

Blocks were cut from the plates and fixed in osmium tetroxide vapors, *Bacillus* and *Escherichia* for $1\frac{1}{2}$ minutes, *Micrococcus* for 2 minutes. Other fixatives which were tried did not give as good results as osmium. Following fixation, smears were made on slides which were placed in normal hydrochloric acid at 60 C for 7 minutes, and then washed with tap water. Then the smears were placed in 0.1 per cent basic fuchsin for 5 minutes, washed, and carried to 10 per cent tannic acid[1] for 5 minutes. They were washed again and restained in basic fuchsin for 5 to 10 seconds. After washing away the excess basic fuchsin, the slides were allowed to dry before they were examined.

The stained preparations were examined with critical illumination, using Kodak Wratten B filter # 58 and H filter # 45 in combination. The only medium between the organisms and the objective was immersion oil. The common procedure of mounting in water is not desirable in the author's opinion, since it not

[1] Tannic acid has been employed for cell wall staining by Gutstein (1925), Knaysi (1941), and Robinow (1946).

only creates a false impression that the cells are unusually wide and have very smooth contours, but also causes a decrease in optical resolution.

RESULTS AND DISCUSSION

Bacillus alvei, *B. cereus*, *B. cereus* var. *mycoides*, *B. megatherium*, *B. pumilus*, and *B. subtilis* all gave results similar to those shown in figure 1. The transverse walls are well stained; those which are apparently being newly formed stain less intensely than the completed ones. Positions of some of the apparently newly forming walls are indicated by arrows in figure 1. The free walls are stained well enough to give a clear outline to the cells. They should not be stained too heavily because the cytoplasm stains at the same time and a compromise must be reached in order not to mask the chromatinic bodies by overstaining the cytoplasm.

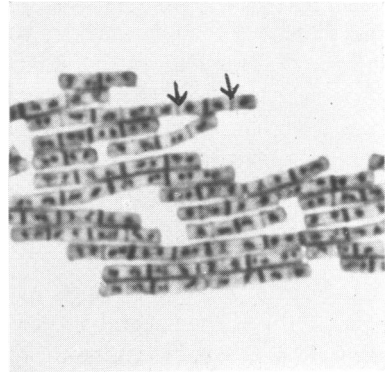

Figure 1. Bacillus cereus X 2,000. Growth 3¼ hours. Stained and mounted as described in the text.

When the staining procedure was modified slightly to allow for the use of the Giemsa stain instead of basic fuchsin, the results were found to be inferior to those obtained with basic fuchsin.

Although reference is made herein to the free surfaces of the cells as walls it is well to keep in mind that these surfaces have been modified, by the hydrochloric acid treatment, from what might be considered as their normal intact condition. According to Knaysi (1949), Dyar (1947) has made electrophoretic studies of the disintegrating action of hydrochloric acid, at elevated temperatures, on the bacterial cell wall. The results showed a slow disintegrating action. Also, the author, in unpublished studies, has shown that a basophilic substance is removed from the wall regions of *B. cereus* and *E. coli*.

The action of the tannic acid, aside from mordanting, is to destain selectively all of the cell except the chromatinic body. The latter, if the second staining is omitted, will be found to stand out in very sharp contrast to the background.

When *E. coli* was used, this method for staining walls and chromatinic body together was not successful. The use of 30 per cent tannic acid as recommended by Gutstein (1925) did not help. Even in preparations stained to show walls

only, according to Robinow's methods (1946), the results were poor. It may be mentioned, incidentally, that Robinow's Bouin-crystal violet method for wall staining also clearly demonstrates plasmodesms in the genus *Bacillus*.

Results with *M. pyogenes* var. *albus* were unsatisfactory. The free walls and chromatinic bodies could frequently be stained together, but never the transverse walls and chromatinic bodies. Other procedures, besides the use of hydrochloric acid, for clearing the cytoplasm did not make possible the desired results. The procedures included Badian's Giemsa-eosin method (1933) and perchloric acid, Cassel (1950).

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SUMMARY

A procedure is presented which makes possible the simultaneous staining of the cell walls and the chromatinic bodies in the genus *Bacillus*. The procedure, judging from limited examinations, does not appear to be useful among other genera.

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