BACTERIAL SPORES FOR CLASS WORK.

P. A. TETRAULT, Purdue University.

To demonstrate bacterial spores to a class is one thing; but to bring about production of spores in large quantities and to have the majority of these at the stage where they show the relationship to the parent cell, is quite another thing. This mass production of spores is what I wish to discuss in this paper.

To those familiar with laboratory work in Bacteriology, it is unnecessary to point out the drawbacks of the old method of inoculating agar slant after agar slant at intervals varying anywhere from 12 to 24 hours and extending over a period of two or three weeks immediately preceding the spore exercise in class. Then on the appointed day a frantic examination of scores of cultures for suitable ones and finally a possible disappointment in the laboratory because the culture had not all matured to the stage indicated by the sample.

In the ordinary routine of plating it is frequently observed that surface colonies of spore formers yield uniformly developed spores. Now and then a colony will show long chains of bacilli with apparently 100 per cent spore production. This fact furnished the idea for the following investigations.

The first attempt to produce spores in large enough quantities for class work was done with the plaster paris block, the same method as is used for yeast spore development. This method gives such excellent results with yeast that it was thought it might work out with bacteria. In this case it proved to be a failure. First of all, in the block method there is little or no multiplication preceding spore production. The yield can be little or no greater than the amount inoculated. Secondly, the difficulty of handling on a large scale is a decided disadvantage. And lastly, the deposit when removed is always accompanied by a certain amount of plaster paris which interferes with proper staining.

The next method used was to plate heavy suspensions of the organism. Sterile stock nutrient agar was poured into petri plates and allowed to solidify. A homogeneous suspension of the organism in physiological salt solution was made from a 24-hour agar slant culture. This was drawn up in a Pasteur bulb and a very thin uniform layer deposited on the agar in each of the several plates. These plates were then placed at different temperatures, one set at 37 degrees, one at room temperature and the third in the icebox.

At the end of 18 hours the cultures at 37 degrees showed good development. The surface of the agar was covered with a uniform growth and samples taken from different regions showed equally good development. There was a large percentage of cells producing spores and the mapority of spores were retained within the cells. Between 24 and 30 hours the maximum of development was reached and the spores in the free state were much more in evidence.

"Proc. Ind. Acad. Sci., vol. 34, 1925 (1926)."

At room temperature the same stages of development were reached between 36 and 48 hours. The percentage of spores was never as great at this temperature. In the icebox the cultures were kept for six days without any signs of spore development.

The next method tried was mixing the culture with the agar before pouring the plates. The same technique was carried out as in the previous case with the one difference that the agar was melted in flasks; each flask containing 100 cc. and when cooled to 50 degrees inoculated with a homogeneous physiological salt suspension of the organism.

The time required for the development of the spores was the same as in the previous cases. The results seemed to be slightly better. The surface growth was not as heavy, but the percentage of spores was greater. The plates after development may be placed in the icebox and kept for two or three days without greatly diminishing the number of spores retained within the cells.

It is a very simple procedure to remove the surface growth. A few cubic centimeters of sterile physiological salt solution is added to the growth on the petri dish and by means of a loop wire the growth is loosened from the surface of the agar. Then using a Pasteur bulb, the suspension is drawn up and made homogeneous by shaking and then transferred into sterile tubes for the class.

These experiments were carried out with *B. subtilis*, *B. mycoides* and *B. megatherium*. All of these gave equally good results.

For photographing a slide preparation of the spores, a modified Ziehl Nielsen method was used. The ordinary Ziehl Nielsen stain gives excellent results for classroom work, but the combination of red and blue is such that even with a red filter very little contrast is obtained on the photographic plate. As it turned out, this modified method gives better results for laboratory work. The method consists in treating the slide with carbol-fuchsin and heating for four or five minutes. The preparation is then decolorized in acid alcohol (2% HCl in 96% alcohol). The slide is now allowed to dry and two loopfuls of India ink placed at one end of the slide. Then with a clean slide, and holding this at an angle of 45 degrees, the ink is smeared in a thin film over the preparation. The preparation may then be made permanent with balsam or examined directly with oil. (Fig. 1.)

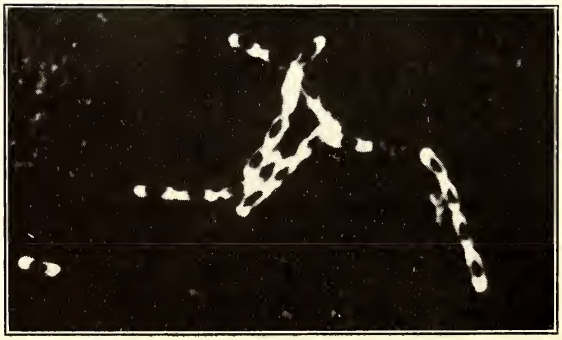

Fig. 1—*B. mycoides* stained with carbol-fuchsin, decolorized with alcohol and smeared with India ink. The cell stands out as a colorless halo surrounding a bright red spore.