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## Inhibition of sporulation by acid fuchsin.

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Certain members of the tri-phenyl-methane series of dyes are now known to have an inhibitory effect on bacteria. This is particularly true of the basic dyes like gentian violet which are capable of impeding many of the active processes of the Gram-positive bacteria, of preventing their growth in very weak dilution, and even of killing them. These types are less potent in their action on Gram-negative bacteria. The acid dyes of this series (like acid fuchsin) are much weaker in bacteriostatic action than the basic dyes; indeed, in order to bring out their power at all, long exposure to fairly strong solutions (preferably at slight elevations of temperature) are needed. So far as acid fuchsin exhibits selective activity at all, it behaves (at least under experimental conditions in which bacteriostatic and bacteriocidal powers are combined) quite differently from the basic dyes.

In general, four effects of tri-phenyl-methane dyes on bacteria have been observed. These dyes bring moving bacteria to rest, they inhibit fission, they impede sporulation, they bring about a state of suspended animation and they kill. By choosing the dye and the organism used in the experiment, any or all of these results may, within limits, be produced at will.

The present communication has to do with the prevention of sporulation caused by exposures of *B. subtilis* to acid fuchsin. The phenomenon which has been observed appears to be similar to observations made on other dyes by Behring,[1] Roux,[2] Flexner and Noguchi,[3] and Noguchi.[4]

Plants from a 24-hour culture of *B. subtilis* were made on petri dishes, covering the entire surface of the agar, and allowed to grow 3 hours. Smears made at this time showed the growth

[1] Behring, *Ztschr. f. Hyg.*, 1889, vi, 117.

[2] Roux, *Ann. de l'Inst. Pasteur*, 1890, iv, 25.

[3] Flexner and Noguchi, *J. Exp. Med.*, 1906, viii.

[4] Noguchi, *ibid.*, 1908, x, 30.

to consist almost entirely of vegetative forms. A heavy suspension in distilled water was made of the bacteria scraped from the surface of these plates. One cc. of this suspension was placed in one tube for control. To one cc. in a second tube, 10 drops of 1 per cent aqueous solution of acid fuchsin (Grübler) were added. The two tubes were placed in a water-bath and kept at 55° C. for one hour. They were then allowed to stand at room temperature and observations made over a period of 106 days. In the control tube spores began to form promptly; at the end usually of 24 to 48 hours, smears made from this tube consisted entirely of spores. In the acid fuchsin tubes no spores whatever, or only an occasional one, could be observed. The smear from these tubes at the end of 23 days looked exactly as it did at the start of the experiment, that is to say, it consisted almost entirely of vegetative forms. That the dye had been hostile to the organism was shown by the genesistasis which it caused, for bacterial counts showed that no growth whatever had occurred. Indeed, the vegetative forms not only failed to divide, they slowly died. This was proven not only by the decreasing bacterial counts, but also by making single cell transplantations (in order to insure planting only vegetative forms without spores) at various intervals of time. On the 5th day these transplantations were all positive; that is to say, the organisms were alive but had not sporulated. On the 10th day about one half the transplantations were positive. On the 23rd day all of them were negative. The environment was therefore proven to be hostile, yet vegetative forms failed in such an environment to sporulate. This is another bit of evidence against a teleological conception of sporulation as nothing more than a protective mechanism. The process must be ultimately a chemical one. It is true that the chemical stimulus which initiates it is often hostile to the organism and the mechanism then has the appearance of protection, but a chemical stimulus may be hostile to the organism and at the same time inhibit the very sporulation which would protect bacteria against it.