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Action of Stains on Living Bacteria.

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In studies on the streptococci and lactobacilli now in progress it became necessary to carefully examine the methods used in staining bacteria. The staining technique of the bacteriologist, for the most part, has advanced very little beyond that employed by the early workers. Perhaps Dobell[1] did more to point out certain fallacies of these methods than any other, yet his work is rarely quoted. The seemingly accepted technique is to dry on a slide the film of liquid containing the organism. After flaming, aqueous solutions of dye are flooded over the films. The delicate cytoplasm of the cell must necessarily be somewhat distorted by such methods. There are some descriptions of vital staining but generally little has been accomplished. We attempted to study the subject, using, as suggested by Dobell, a very large cell. In this case a Gram-positive organism from the gut of a guinea pig was used.

The method of staining was to add a water solution of the stain directly to either a broth or saline suspension of the organisms. The stained bacteria were examined in both wet and dry preparations. Certain stains revealed granules of varying arrangement and size within the cell. From the work of Guillermond[2] these were in all probability metachromatic granules. (Table I.) Dobell's results would agree since he says, "In my experience, only non-living structures in the cells (metachromatic granules, etc.) can be stained during life. But doubtless much depends on the stain itself!"

Gay and Clark[3] suggested that "vital" staining may really only represent a stage in a process of injury by the stain. In an attempt to test the possibility of injury to the cell experiments were designed to determine the toxicity of the various stains. The method employed generally for this test was to mount the suspension of organisms under a cover-slip and allow the stain to diffuse through from one side. This permitted the worker to follow the effect of the stain on the organism. The majority of the basic stains under examination were found to be toxic for the particular bacterium

[1] Dobell, C. Clifford, *Quart. J. Micro. Sci.*, 1910-11, **56**, 396.

[2] Guillermond, A., *Bull. Inst. Pasteur*, 1906, **4**, 145.

[3] Gay, F. P., and Clark, A. R., *J. Bact.*, 1934, **27**, 175.

under observation. (Table I.) Methylene blue was apparently least toxic of the basic stains. Acid dyes proved to be non-toxic and also failed to stain the organism.

TABLE I.

Stain	pH	Intensity of Stain	Metachromatic Granules	Toxicity of Stain
Thionin	6.6	3+	—	2+
Malachite Green	"	3+	—	4+
Methylene Blue	"	3+	+	+
Bismarck Brown	"	2+	—	3+
Neutral Red	"	2+	+	+±
Nile Blue Sulfate	"	3+	2+	3+
Brilliant Green	"	2+	±	3+
Basic Fuchsin	"	3+	—	2+
Diazine Green	"	3+	—	2+
Safranin O	"	2+	—	2+
Rose Bengal	"	2+	—	2+
Gentian Violet	"	4+	—	3+
Orange G	"	—	—	—
Acid Fuchsin	"	—	—	—
Eosin B	"	—	—	—
" Y	"	—	—	—
Congo Red	"	—	—	—

Churchman[4] was able to stain *S. marcescens* with gentian violet and still preserve motility. We were unable to find motility when either marcescens or the Gram-positive organism was stained with gentian violet. When methylene blue was used it was possible to find a few organisms (both Gram-negative and positive) which were definitely stained and motile. This motility soon ceased, usually lasting only a minute or two after staining took place. There were, however, two variables which must be considered (1) time and (2) concentration of the stain. Thus if one were able to carefully regulate the stain to the desired concentration it is possible the time might be increased. There was no evidence that the Gram-negative organism remained motile longer after staining than the larger Gram-positive one. It did, however, remain unstained in a concentration of the dye which stained the positive organism and caused the cessation of motility. There would seem to be every reason to believe that "vital" staining was not obtained with any of the above stains, since in every case there was definite evidence of injury to the cell.

In all probability there is no direct relationship between loss of motility and death of the organism. To test this point the organisms were exposed to the stain until they were completely stained and had lost their motility. They were then centrifuged out and washed

[4] Churchman, J. W., *J. Exp. Med.*, 1912, **16**, 221.

with sterile distilled water. After all the excess stain had been removed they were centrifuged down once more and a large number added to broth. One was then able to watch the recovery of the organisms under the microscope. It was found that the bacteria gradually lost their stain, then after an interval of about 10 hours most of them had again regained their motility.

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Acute Toxicity of Ethyl Chaulmoograte.*

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At the suggestion of Dr. H. W. Wade we studied the toxicity in rats of single subcutaneous doses of a number of representative types of ethyl esters of the fatty acids of chaulmoogra oil. Preparations included were: (1) a purified ethyl chaulmoograte supplied by Dr. R. Wrenshall, fractionated by redistillation *in vacuo* at 10 mm. Hg., containing a small amount of ethyl hydnocarpate as indicated by the iodine number; (2) mixed ethyl esters of fatty acids of *Hydnocarpus wightiana* oil prepared at Culion, containing more than 80% ethyl chaulmoograte as indicated by polariscopic measurements; (3) a similar preparation containing 0.5% iodine, prepared by Cole's method[1]; (4) crude ethyl chaulmoograte containing 4% creosote, a preparation used in India, supplied by Dr. E. Muir from clinical supplies at Calcutta; and (5) "Chaulmestrol" (Winthrop), a proprietary preparation of mixed ethyl esters of fatty acids of chaulmoogra oil. The pertinent findings summarized in Table I agree with Martins' report[2] of 40 cc./kg. as being lethal for *Leptodactylus ocelatus*.

Examination of the tabulated data indicate that: (1) purified ethyl chaulmoograte preparations are more toxic than the ethyl esters of the total fatty acids of chaulmoogra oil, presumably because of

* Part of a cooperative study of the chemotherapy of leprosy conducted by the Pacific Institute of Tropical Medicine within the Hooper Foundation for Medical Research, and the Pharmacological Laboratory of the University of California Medical School, San Francisco.

[1] Cole, H. I., *Philipp. J. Sci.*, 1929, **40**, 503.

[2] Martins, T., *Compt. rend. Soc. Biol.*, 1927, **96**, 474.