

We have studied the effect of short exposures to light of different dilutions of methylene blue on a number of Gram-positive and Gram-negative microorganisms. A preliminary report is presented at this time.

Saturated aqueous solution of methylene blue was diluted to different concentrations with normal saline. Twenty-four hour broth cultures of different organisms were mixed with these solutions in Petri dishes. The bacteria-methylene-blue mixtures were exposed to an ordinary electric light of 100 candle power at a distance of 10 cm. for a period of half an hour. These Petri dishes were placed on a cooling machine which prevents the plate from getting too warm. Soon after the exposure, the organisms were plated out and incubated. The other portions of the same mixtures were not exposed and were similarly plated to serve as controls.

The experimental results are presented in Table I. The photodynamic action of methylene blue on bacteria varies a great deal. In general, the Gram-negative organisms seem to be more resistant than the Gram-positive ones. This parallelism of the reaction with the Gram stain is in conformity with that of bactericidal action of gentian violet in the absence of light⁴ and the photodynamic action of a number of dyestuffs on bacteria in the presence of ultra-violet rays.⁵

It has also been found that the antigenicity of Pneumococcus Type I so treated was not lost, and that even after repeated exposures, a culture of *B. diphtheriae* did not lose its virulence. These subjects are now under study.

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Effect of Frequency of Stimulation on Tension Response of Muscle.

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The response of nerve, as measured by its heat production or summated action potential changes, has been shown by Bugnard and

⁴ Churchman, J. W., *J. Exp. Med.*, 1929, **16**, 221.

⁵ Passow, A., and Rimpan, W., *Müch. Med. Wochr.*, 1924, **91**, 733.

Hill¹ to decline progressively with increasing frequency of stimulation beyond that which calls forth the maximum response. A similar response-frequency relation in the case of muscle is to be expected, but an attempt to obtain it experimentally with toad's or frog's sartorius stimulated by repetitive condenser charges and discharges from a revolving commutator through a pair of silver wire electrodes lying on the tibial half of the muscle, revealed a very different relation. The curve relating the size of isometric contraction in tetani of any duration from 0.44 to 10 sec. and the frequency of stimulation, was found to be a wavy one with recurrent minima and maxima. In an experiment at 26.3°C. with 0.44 sec. tetani the contraction was at a minimum at the approximate frequencies 380, 750, and 1500 per sec. With longer tetani and lower temperatures, the positions of the minima were shifted to lower frequencies.

Further experiments then showed that this strange result was associated with the fact that the stimulating electrodes were laid on the tibial half of the muscle, and that the muscle was partly stimulated through its intramuscular nerve twigs. For this unexpected result was no longer obtained with curarized muscle or when the muscle was stimulated by electrodes on the nerve-free pelvic end. Conversely it was obtained in a much more striking form when the sartorius was excited indirectly through its nerve. The response-frequency curve of muscle when nerve participation is excluded, is a smooth one, and for short tetani generally very flat after the maximum, unless complicated by fatigue.

An explanation may be offered on the following basis: First, assume that the neuromuscular junction has the property of a narcotized region having a somewhat longer refractory period than the nerve and in which conditions for the development of Wedensky inhibition exist, in particular a condition in which ineffective stimuli falling in the relative refractory period leave an inhibitory after-effect, while those extinguished in the absolute refractory period do not.² Second, assume progressive failure of excitation to occur in the intramuscular nerve twigs with increasing frequency of stimulation beyond the maximum, as shown by Bugnard and Hill in isolated nerve trunks. Referring to the experiment at 26.3°C. quoted above, it may be supposed that when the somewhat subnormal impulses impinge on the junctional tissue at the frequency of 300-400/sec., they will be largely made ineffective in its relative refractory phase, each leaving an inhibitory after-effect, producing

¹ Bugnard, L., and Hill, A. V., *J. Physiol.*, 1935, **83**, 383.

² Kato, G., Nakayama, M., and Ota, T., *Am. J. Physiol.*, 1929, **90**, 406.

Wedensky inhibition, and thus giving rise to the first minimum. As the nerve impulses arrive with frequencies higher than 400/per second, about half of them would be extinguished in the absolute refractory period, leaving no inhibitory after-effect, and the other half coming later in the relative refractory phase would have greater chance of becoming effective. Hence the response-frequency curve rises again. But now as the stimulating frequency is increased beyond 500-600 per second, which is probably the maximum rhythm the nerve can follow, the propagated number of impulses per sec. actually become less and less with further increase of frequency of stimulation. When this number has dropped to 400-300 per second, Wedensky inhibition would again occur, hence a second minimum. With further drop in the impulse frequency the response frequency curve would naturally rise again, and would then fall a third time when impulse frequency becomes smaller than that necessary for calling forth the maximum contraction. The subsequent rise in the curve cannot be understood according to this scheme, but in this region of frequency of stimulation, summation of subliminal stimuli would probably occur and more muscle fibres become directly stimulated.

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Use of Saturated Sodium Chloride Solution for Preservation of Viruses.

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There are very few convenient methods for the preservation of viruses. The use of disinfectants is not permissible because of their deleterious effect upon viruses, while the commonly employed glycerol exerts only a feeble antiseptic action and, therefore, cannot be relied upon when freedom from bacterial contamination is important. The preservation of viruses through the process of drying *in vacuo* at low temperatures is an excellent method but it has the drawback of being somewhat cumbersome.

It has been shown by Ginsburg and Kalinin,¹ Sonnenschein,² and

¹ Ginsburg, S., and Kalinin, W., *Z. f. Immunität.*, 1929, **63**, 107.

² Sonnenschein, C., *Z. f. Immunität.*, 1930, **66**, 330.