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Photodynamic Action of Methylene Blue on Pneumococcus.

T. T'UNG. (Introduced by S. H. Zia.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping, China.

In a previous study on the photodynamic action of methylene blue on bacteria¹ it was observed that such treatment exerted a lethal effect on pneumococci. Further work along this line revealed that, in addition to the lethal effect, this treatment has a preservative action on their morphology and staining property when they were kept at 37°C. Even after a period of 6 months, it was found that at the temperature mentioned, these organisms still retained their Gram positiveness comparable to fresh living cultures. On the other hand, the same organisms treated either with methylene blue alone or with formalin lost their Gram positiveness and showed signs of deterioration within a month. The procedure by which the above results were obtained is described below.

A virulent culture of pneumococcus type III grown in Avery's medium for 18 hours was mixed with a physiological saline solution saturated with methylene blue in a Petri dish in the ratio of 9 to 1. The total volume was 20 cc. The mixture was divided into 2 equal portions, one unexposed, and the other exposed to an electric light of 100 candle power at a distance of 10 cm. for a period of 45 minutes. The dish when exposed was put over a Frigidaire cooling machine which kept the temperature below 20°C. After exposure, the microorganisms were washed in physiological saline to remove the methylene blue. The samples were centrifuged and then resuspended in an equal volume of saline. They were incubated at 37°C. together with a control culture preserved with 0.4% formalin. Smears made from them were treated with alcohol for 2 hours prior to the application of Gram stain for examination giving the results stated above.

In view of the excellent preserving effect of the photodynamic action of methylene blue on pneumococcus, it was considered of interest to see whether or not pneumococci preserved by the photodynamic action of methylene blue would also show a higher antigenic power than those treated with formalin, the latter, according to Tao² preserved type I pneumococci better than heat and possessed a higher antigenic power.

¹ T'ung, T., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 328.

² Tao, S. M., *Chinese M. J.*, 1932, **46**, 12.

One series of 4 rabbits was, therefore, immunized with a pneumococcus type I vaccine subjected to the photodynamic action of methylene blue and another series with vaccine treated with 0.4% formalin. The method of preparation of these vaccines was the same as described above except a 10 times more concentrated culture was used. The vaccines were kept in the ice-chest and used soon after preparation. The rabbits were inoculated intravenously for 6 consecutive days, followed by a week without inoculation. The same course of treatment was repeated twice, making a total of 18 injections representing 9 cc. per animal. Ten days after the last inoculation, the animals were bled and agglutination tests carried out. The 4 rabbits inoculated with pneumococcus vaccine subjected to the photodynamic action of methylene blue yielded sera which gave complete agglutination for 2, to a titer of 1:128, and one each to 1:32 and 1:16, whereas, the other 4 rabbits immunized with formalinized pneumococcus vaccine gave sera that resulted only in partial agglutination at titres not higher than 1:16.

In addition, protection test on white mice was also carried out, using the same methylene blue and formalin treated vaccines of pneumococcus type I. Two series of 30 white mice each were inoculated intraperitoneally with 0.5 cc. each of these 2 vaccines respectively every other day for 4 times. Eighteen days after the last inoculation, they were injected with 0.5 cc. of different dilutions of an 18-hour culture of the same strain of pneumococcus. The animals were kept under observation for 7 days and cultures were made from the heart blood of all those that died. It was found that the control animals inoculated with the test cultures were killed up to a dilution of 1-1,000,000, while the vaccine prepared by the photodynamic action of methylene blue protected a majority of the mice up to a dilution of 1-10 of the virulent culture. On the other hand, the formalinized vaccine protected the mice to a dilution of 1-1,000. This observation shows that type I pneumococci subjected to the photodynamic action of methylene blue are distinctly more antigenic than those treated with formalin. Further work on the protective and antigenic action of this particular vaccine to different types of pneumococcus is under investigation.