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Antigenicity of Diphtheric Toxin Subjected to Photodynamic Action of Methylene Blue.

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Although the inactivating effect of methylene blue in the presence of light on bacteriophage, viruses, toxins and bacteria has been generally agreed upon, the antigenicity of substances so treated is still disputed. Some reports have been positive,[1] others, negative.[2] Our observations indicate that definite antigenic property is possessed by photodynamically inactivated diphtheric toxin.

A single batch of toxin containing 400 M.L.D. per cc. (original titre 2000 M.L.D. per cc.) and one of toxoid (20 Lf. per cc.) obtained from the National Epidemic Prevention Bureau were used in this study. Before exposure to the photodynamic action of methylene blue (concentration of dye 4×10^{-5}) in sunlight, the toxin was diluted 1:25 with sterile saline; when electric light[3] was used, the dilution was 1:100. Normal guinea pigs of approximately the same weight were divided into 3 groups of 7 each. All animals of the first group received 8 biweekly subcutaneous injections of 5 cc. of diluted detoxified toxin subjected to the action of sunlight and dye; those of the second group, 8 injections of 5 cc. of diluted detoxified toxin treated with electric light and dye; those of the third group, 8 injections of 0.2 cc. toxoid. One month after the last injection, different amounts of diphtheric toxin were given to all animals in these 3 groups. It was found that the animals of the first and second groups could withstand the toxin up to 640*-960 M.L.D, while those of the third up to 160-320 M.L.D. It is obvious that the toxin after treatment with light and dye still possessed the power of provoking active immunity to a degree even higher than that produced by injections of toxoid.

The production of circulating antitoxin was studied in 6 rabbits divided in 3 groups. Each group was given injections either of

1 Perdrau, J. R., and Todd, C., *J. Comp. Path. and Ther.*, 1933, **46**, 76; Galloway, I. A., *Brit. J. Exp. Path.*, 1934, **15**, 97.

2 Shortt, H. E., and Brooks, A. G., *Indian J. M. Res.*, 1935, **22**, 557; Shortt, H. E., and Mallick, S. M. K., *Indian J. M. Res.*, 1935, **22**, 529; Lippert, K. M., *J. Immun.*, 1935, **28**, 193.

3 Lin, F. C., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 337.

* M.L.D. was calculated according to the individual body-weight.

sunlight- and dye-treated toxin, electric light- and dye-treated toxin or of toxoid in doses and intervals similar to those described above for guinea pigs. Ten days after the last injection it was found that the average amount of toxin that could be neutralized by 1 cc. of serum in 30 minutes at room temperature, was 10 M.L.D. for the animals of the first group, 25 M.L.D. for those of the second group, and 4 M.L.D. for those of the third group.

Traces of residual toxin in the photodynamically treated material were demonstrated by the development of late paralysis in some guinea pigs after having received repeated large doses of the treated toxin. The possibility of this residual toxin being partially responsible for the antigenicity of the detoxified toxin can not be excluded. But in view of the high immunity produced the result is comparable to that obtained by the use of toxoid with the addition of sublethal doses of active toxin.

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Comparison of the Antigenicity of Three Forms of Staphylococcal Toxoid.

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In the preceding report it was shown that staphylococcal toxin after complete detoxification by the photodynamic action of methylene blue still retains its antigenic property. This note records the results of a preliminary study of the antigenic value of this "methylene blue toxoid" in comparison with 2 other common forms.

Materials and methods. (a) A single lot of toxin, No. 9669F, was used for the preparation of the 3 forms of toxoid. Formol-toxoid was prepared with 0.3% formalin. The alum-precipitated formol-toxoid was made by the method of Wells, Graham and Havens.[1] A third portion of toxin was treated with methylene blue, 1:50,000, and exposed to direct sunlight for 3 hours. The 3 toxoids did not hemolyze rabbit-erythrocytes. Immunization and estimation of antigenic response were done as described in the preceding paper.

[1] Wells, Graham and Havens, *Am. J. Pub. Health*, 1932, **22**, 648.