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Effect of Photosensitization on Immunological and Chemical Properties of Antibodies.

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While the photodynamic action of dyes was well known 30 years ago, its application to microbiology was limited to the studies on their lethal action on bacteria and protozoa, and the inactivating action on toxin, antibodies, and complement.[1] It was only in the last few years that the immunological activities of the treated substances were carefully examined. Perdrau and Todd[2] reported on the preservation of antigenicity of canine distemper virus inactivated by photodynamic action of methylene blue. Others have since studied the antigenic action of various photodynamically inactivated bacteria[3] and viruses[4] as well as toxins,[5] and in the majority of instances this has been well preserved. Furthermore, it has been suggested[3] that organisms so treated may actually make better vaccine than those treated with heat or formalin. However, the reason for this better antigenicity is not clear. It might be conceived that the less change is made in the chemical structure of the antigen, the more complete is its antigenicity. It would be difficult, however, to study chemically the change produced by photosensitization on bacteria or viruses, but it is relatively simple to study the change produced on antibodies, particularly with those in a relatively pure state. In such a case, the immunological activity will then act as an index of the photodynamic action. The result of such a study is herewith presented.

The general technic of photosensitization used in this study is essentially the same as that previously reported[3] with the exception that the immune serum was always diluted 40-100 times before exposure. Both methylene blue and eosin were employed and their effect on the immune activity of various antibodies was observed. Furthermore, the action of eosin in the presence of light on the

1 Tappeiner, H., *Erg. Physiol.*, 1909, **8**, 698.

2 Perdrau, J. R., and Todd, C., *J. Comp. Path.*, 1933, **46**, 78.

3 T'ung, T., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 399.

4 Galloway, J. A., *Brit. J. Exp. Path.*, 1934, **15**, 97.

5 Lin, F. C., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 656; Li, K. H., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 659.

chemical properties of a purified antipneumococcus horse precipitin[6] was also examined. It might be mentioned that the action of methylene blue and eosin was essentially similar, and that as in the case of other materials,[7] there was also an optimum for each dye in different concentrations. For instance, after 2 hours, eosin in 1:100 dilution did not exert any change in the typhoid H antibody in the presence of light, while that in 1:10,000 reduced the agglutination titer from 1:5120 to 1:640. In Table I, only the maximum effect of eosin after 120 minutes' exposure is given.

TABLE I.
Effect of Photodynamic Action on Immunological Activities.

Antibody	Immune reaction	Titer of activity	
		Exposed	Control
B. typhosus antiserum	H agglutination	1:640	1:5120
" " "	O "	1:160	1:2560
Pneumococcus type I antiserum	"	1:4	1:16
Diphtheria antitoxin	neutralization for 10 MLD	1:20	1:160
Purified antipneumococcus type I horse precipitin	mg of precipitin N necessary for the protection against 1 MLD of pneumococci	0.0625	0.016

TABLE II.
Effect of Photodynamic Action on Chemical Properties of Immunologically Pure Horse Precipitin (1 mg N/cc) of Pneumococcus Type I Polysaccharide.

	Before exposure	After exposure
Total preciptin nitrogen precipitable by its homologous polysaccharide	85%	25%
Relative viscosity in M/10 phosphate buffer at pH 6.8 at 26.5° C.	1.10	1.09
Saturation of ammonium sulfate at which precipitation of antibody protein starts	38%	20%
Solubility of antibody protein in a normal saline solution after complete precipitation at half saturation of ammonium sulfate	Complete	Complete

The deleterious effect of the photodynamic action of dyes, *e. g.*, eosin and methylene blue, on antibodies was demonstrated. The decrease in activity ranged from 4 to about 10 times. But what appears of greater interest is the relatively mild chemical changes produced on the antibody protein. It remained completely soluble in saline, and its relative viscosity was not appreciably changed from

[6] Chow, B. F., and Wu, H., *Chinese J. Physiol.*, 1937, **11**, 139.

[7] T'ung, T., and Zia, S. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 326.

that of untreated antibody protein. The only noticeable difference lay in its solubility in various saturations of ammonium sulfate solution. The absence of any gross chemical change in spite of the marked immunological change is not surprising. It has now been repeatedly demonstrated that only slight alteration of chemical groups or of their stereo-chemical relationship may destroy all the immunological properties of protein. By analogy, it might be assumed that the protein of microörganisms subjected to the same action may also be little changed. But in this case, the preservation of antigenicity would be more complete. This may explain why organisms treated with photosensitization would make better vaccine.

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Chemical Nature of Component Involved in the Reaction Between Iodine and Complement.

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Chow and Wong have reported[1] that iodine destroys the complement activity of normal sera and that there exists a definite stoichiometric relationship between the amounts of iodine used and complement inactivated. It seemed to be of interest to find out the chemical nature of and the component involved in this process. This may either be an addition of iodine to ethylenic linkages or replacement of hydrogen atoms attached to the carbon atoms, with the result that iodine will be bound to complement. It may also be a simple oxidation of complement by iodine, which in turn, is reduced to free unbound iodide. In order to arrive at some conclusion, we have determined quantitatively first the bound iodine, if any, to the inactivated serum protein, and secondly, the free iodine in the mixture. The present communication gives the result of such a study.

1. Determination of non-dialyzable organic iodide in the inactivated serum protein. To 50 cc of fresh hog serum (having a titer of 21 units per cc) were added 10 to 15 cc of N/10 iodine solution. One-half cc of the resulting solution was found to contain less than

[1] Chow, B. F., and Wong, Sam C., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 120.