less antitoxin. Objections may be raised in that pure cultures are necessary, thereby causing possible delay. This is only of theoretical interest, because usually it is only in doubtful and convalescent cases of diphtheria that this test is required, and a delay of one day does not limit its usefulness. Furthermore, one must remember that in making the conventional test on guinea pigs a delay of 24 hours is also necessary after the control guinea pig has received antitoxin.

As a result of these studies it has been shown that Chinese hamsters are suitable animals for the routine determination of the virulence of C. *diphtheriae*. The use of these animals instead of guinea pigs is very much less expensive and in certain respects gives more reliable information.

## 8365 P

## Photodynamic Action of Methylene Blue on Diphtheria Toxin.

## F. C. LIN. (Introduced by Samuel H. Zia.)

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

The effect of photodynamic action of certain dyes on various substances, among which may be mentioned different types of animal cells, viruses<sup>1, 2, 3</sup> and soluble toxins of both bacterial<sup>4</sup> and animal origin,<sup>5</sup> is a well-known phenomenon. Workers generally agree as to the extreme susceptibility of these substances to the photodynamic action of dyes, the optimal concentration of the dye employed, and the relatively short time required for exposure, but disagree with regard to the antigenicity of substances so treated. The present study aims to investigate the reaction of diphtheria toxin to the photodynamic action of methylene blue upon which subject little study has been made. Diphtheria toxin is looked upon as a more suitable material than either tetanus toxin or viruses on account of its greater stability and ease with which quantities given can be accurately measured and properly controlled.

The technique employed is essentially similar to those of other workers.<sup>1, 5</sup> Methylene blue is selected since it has been more thor-

<sup>1</sup> Perdrau and Todd, Proc. Roy. Soc. Bull., 1933, 112, 277, 288.

<sup>&</sup>lt;sup>2</sup> Perdrau and Todd, J. Comp. Path. and Therap., 1933, 46, 78.

<sup>&</sup>lt;sup>3</sup> Shortt and Brooks, Indian J. Med. Res., 1934, 21, 581.

<sup>4</sup> Lippert, J. Immunol., 1935, 28, 193.

<sup>&</sup>lt;sup>5</sup> Shortt and Mallick, Indian Med. J. Res., 1935, 22, 529.

oughly studied than other dyes. A single batch of toxin containing 400 M.L.D. per cc. for the guinea pig, or 2000 M.L.D per cc. for Chinese hamsters weighing 20-25 gm., was used throughout the experiment, employing as diluent 0.9% salt solution for both dye and toxin. As source of radiation, both direct sunlight during summer and early autumn and electric light of 100 watts, 110 volts at 16.5 inches distance were used. In a preliminary study, the minimal time for exposure was found to lie between 20-30 minutes. In order to obtain full manifestation of combined action of light and dye, it was subsequently fixed at 60 minutes in all instances. Table I shows the maximal M.L.D. for hamsters which may be destroyed by the optimal concentration of methylene blue when exposed to sunlight and to artificial light. The number of animals in each group varied from 3 to 9.

		IADDE I.		
Methylene Toxin in M.L.D. for hamsters per cc.	blue 1:1	00 1:1,0	00 1:10,00	00 1:100,000
	Exposure	to Sunlight, 6	0 Min.	
10	' s	Ś	S	S
25		- S	S	S
50	D	S	S	S
100	D	S or I	D S	S
200	D	D	S	D
400	D	D	D	D
Ex	posure to	Electric Light	, 60 Min.	
10	·	· Ď	Ś S	S
20		- D	S	S
30		- D	S	S
40		- D	S or !	D S
50		- D	D	D

TABLE I.

D = death within 3 days after inoculation.

S = survived.

- = not done.

Control hamsters receiving unexposed toxin plus dye or exposed toxin without dye invariably succumbed in 3 days or less. From the table it is seen that the combined action of dye and sunlight is greater than that of dye and artificial light. Subsequently it was found, however, that the photodynamic action of dye did not inactivate toxin completely, since it was possible to detect by both intracutaneous test on guinea pigs and subcutaneous inoculation of increased quantities in hamsters, traces of toxin after exposure either to sunlight or artificial light in the presence of methylene blue.