8740 P

Antigenicity of Staphylococcal Toxin Detoxified by the Photodynamic Action of Methylene Blue.

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The fact that some organisms, viruses, bacteriophages and toxins may be rendered harmless and inactive by the photodynamic action of methylene blue has been well established. Since Perdrau and Todd[1] reported that their virus of canine distemper so inactivated possessed a high antigenic property, the antigenic value of these products has become an interesting problem. Rivers[2] cautioned workers on the general principle that when the degree of inactivation could not be accurately measured one should be careful in attributing antigenicity to these so-called inactivated agents. Since titrations of minute residual amounts of active virus are still impossible, one naturally turns to bacterial toxins for an answer to this question. Lippert[3] reported failure to demonstrate antigenicity in his inactivated tetanus-toxin. Lin[4] could not completely detoxify diphtheric toxin. In this note, we present the following findings: Staphylococcal toxin can be completely detoxified by the photodynamic action of methylene blue and when so detoxified still possesses antigenic value.

The method of preparing toxins was a modified combination of those recommended by Parker and Dolman.[5] The medium was 0.3% agar with 4% Witte's peptone in beef broth buffered at pH 7.0. After incubation in an atmosphere containing about 20% carbon dioxide, it was filtered through cheesecloth, paper and Berkefeld candles. The final filtrate was our toxin. Only those preparations whose potency equalled or surpassed the minimal standards outlined by Dolman,[7] were employed. Gruebler's "Methylenblau" was used for detoxification.

To measured volumes of toxin, minute quantities of the stock dye-solution were added and thoroughly mixed. These mixtures,

1 Perdrau and Todd, *J. Comp. Path. and Therap.*, 1933, **46**, 78.

2 Rivers, *Am. J. Med. Sc.*, 1935, **190**, 435.

3 Lippert, *J. Immunol.*, 1935, **28**, 193.

4 Lin, PROC. SOC. EXP. BIOL. AND MED.

5 Parker, *J. Exp. Med.*, 1924, **40**, 761; Dolman, *J. Infect. Dis.*, 1934, **55**, 172.

7 Dolman and Kitching, *J. Path. and Bact.*, 1935, **41**, 137.

contained in large covered petri dishes, were placed on ice 20 cm. below a 100 watt lamp. At appropriate intervals, samples of these exposed mixtures and of control mixtures kept in the dark were titrated for hemolysis of rabbit-erythrocytes, dermo-necrosis and killing effect in rabbits.

The completely detoxified product was given to rabbits with known natural antitoxin-titres. At intervals during immunization the animals' sera were titrated for neutralizing property according to the hemolytic method of Hartley and Smith.[6] At the end of the immunizing injections the animals were tested for their cutaneous resistance to staphylococcal toxin according to the method of Dolman.[7]

Results. The photodynamic action of methylene blue can completely remove all the 3 known toxic properties of staphylococcal toxin. Table I illustrates the effect upon the hemolytic property.

TABLE I.
Maximal Dilution of Staphylococcal Toxin which Completely Hemolyzes 1% Rabbit-erythrocytes after 1 hr. in Waterbath at 37° C.
(Undiluted toxin 9669I, pH 7.2, exposed to 100-watt Mazda light at 20 cm.)

Hours Exposed	Concentration of methylene blue 1:10,000	1:50,000	1:500,000
To light			
1	1/1024+	1/1024+	1/1024+
2	1/512	1/1024	1/1024
4	1/256	1/256	1/1024
6	1/32	1/64	1/512
8	None	1/2	1/32
In the dark			
72	1/1024+	1/1024+	1/1024+
240	1/1024+	1/1024+	1/1024+

TABLE II.
Response of Rabbits to Completely Detoxified of Staphylococcal Toxin 9669I.

Mode, am't injected	Rabbit No.	Staphylococcal Antitoxin in Sera (International Standard Units per cc.) Initial titration	1 week after 1st injection	1 week after 2d injection	2 weeks after 2d injection	Dermo-necrotic doses resisted
A. Intravenously, 5 cc.						
	1	>0.05	0.05	0.80	3.20	6.25
	2	0.05	>0.05	Died 3 days after 2d inj.	—	—
	3	0.05	>0.05	0.80	6.420	6.25
B. Subcutaneously, 0.2 cc.						
	4	>0.05	0.40	6.40	25.6	25
	5	0.10	>0.05	3.20	12.8	6.25
	6	0.10	>0.05	0.05	3.20	6.25

[6] Hartley and Smith, League of Nations: *Quart. Bull. of Health Org.*, Special No. Jan., 1935.

It was also found that originally potent toxins became non-skin-necrotizing when injected endermally in undiluted form and non-lethal to rabbits when given intravenously in doses of 3 cc. per kilogram body weight.

Table II shows a good antigenic response in rabbits following intravenous or subcutaneous injections of staphylococcus toxin detoxified by the photodynamic action of methylene blue.

8741 C

Negative Effect on Blood of Normal Rabbits of Inoculation of Killed *Leishmania donovani.*

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Leucopenia is one of the outstanding features in kala-azar. It develops early in the course of the disease and is frequently out of proportion to the anemia. This fact has been well recognized, and its diagnostic value repeatedly emphasized. The mechanism of the development of this phenomenon is, however, by no means understood. By incubating leucocytes with blood serum, Maggiore and Sindoni[1] claimed to have demonstrated the presence in the blood serum of patients with visceral leishmaniasis, of a substance which is leucocytolytic. Arena[2] confirmed this finding. Their results were not at all conclusive, and there has been no further confirmation. Some workers[3, 4] believed that leucopenia as well as anemia in this disease is myelophthisic, the overgrowth of the macrophages ("clasmatocyte tissue") being comparable to the metastatic growths of carcinoma in blood-forming organs. This does not explain satisfactorily the early appearance of leucopenia when the infection is only beginning and the "crowding out" of the myeloid tissue hardly can have taken place.

It has been suggested,[5, 6, 7] in discussing the problem of leucopenia

1 Maggiore, S., and Sindoni, M., *Pediatrica,* 1917, **25**, 81.

2 Arena, G., *Pediatrica,* 1927, **35**, 465.

3 Hu, C. H., and Cash, J. R., *Trans. Far East. Assn. Trop. Med., 7th Congress, British India,* 1927, **3**, 62.

4 Keefer, C. S., Khaw, O. K., and Yang, C. S., *Nat. Med. J. China,* 1929, **15**, 731.

5 Doan, C. A., *J. A. M. A.,* 1932, **99**, 194.

6 Beck, R. C., *Arch. Int. Med.,* 1933, **52**, 239.

7 Beck, R. C., *Am. J. Path.,* 1934, **4**, 189.