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Acceleration of Formol Detoxification of Staphylococcus Toxin by Adsorption.

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The writer has heretofore presented data[1, 2, 3] which demonstrate the manner in which various biological products are attenuated by interfacial adsorption. The adsorption agents chosen have been such as are relatively inert and can easily be removed after any desired degree of change has been effected. The present paper includes data which demonstrate the acceleration of the formol detoxification of staphylococcus toxin† by adsorption processes under various conditions. Johlin and Rigdon[4] have demonstrated the relative effect produced on staphylococcus toxin by emulsions of chloroform and ether as adsorption agents. In the present paper a comparison is made of the action of chloroform and of emulsified gases, oxygen and nitrogen, on staphylococcus toxin, both in the presence and in the absence of formaldehyde.

The attenuation of staphylococcus toxin by emulsified gases is very much more effective than that of other toxins, for example, ricin, whose detoxification by emulsified gases has been attempted in this laboratory.

The emulsification of the staphylococcus toxin and the adsorption agent was brought about by shaking in sealed pyrex glass tubes by a mechanical shaking device. When chloroform was used, all noticeable traces of this substance were removed by subsequent evaporation under reduced pressure at a temperature below 40°C. To determine the relative hemolytic activity of treated and untreated toxin, decreasing amounts of toxin, 0.5 cc to 0.0001 cc, were diluted to 1 cc with normal saline in a series of test tubes and mixed with 1 cc of a 2% suspension of the thoroughly washed red blood cells of rabbits. The relative degree of hemolysis was observed after standing over night.

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1 Johlin, J. M., *J. Biol. Chem.*, 1929, **81**, 99.

2 Johlin, J. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 523.

3 Johlin, J. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 568.

4 Johlin, J. M., and Rigdon, R. H., to be published.

TABLE I.

The relative hemolysis of red blood cells by staphylococcus toxin after treatment of variable periods of time with (a) emulsified chloroform, (b) emulsified chloroform in the presence of .25% formaldehyde, (c) emulsified nitrogen, and (d) emulsified nitrogen in the presence of .25% formaldehyde. 4 indicates complete hemolysis, 0 indicates no hemolysis, and 1, 2, and 3 indicate intermediary stages.

Time of Treatment	Emulsifying Agent	cc of toxin diluted to 1 cc															
		.5	.3	.2	.1	.05	.03	.02	.01	.005	.003	.002	.001	.0005	.0003	.0002	.0001
15 min	Chloroform in an	4	4	4	4	4	4	4	4	3	3	2	1	1	0	0	—
30 "	atmosphere of	4	4	4	4	4	4	4	4	3	3	2	2	1	1	—	—
1 hr	nitrogen without	4	4	4	4	4	4	4	4	3	3	2	1	1	0	—	—
2 "	formaldehyde	4	4	4	4	4	4	4	4	3	2	2	1	1	—	—	—
3 "		4	4	4	4	4	4	4	3	2	2	1	1	—	—	—	—
6 "		4	4	4	4	4	4	4	3	2	2	1	1	—	—	—	—
12 "		4	4	4	4	4	4	3	3	2	2	1	1	—	—	—	—
24 "		4	4	4	4	4	3	3	2	2	1	1	1	—	—	—	—
15 min	Chloroform in an	4	4	4	4	3	3	2	2	1	1	1	0	0	0	0	—
30 "	atmosphere of	4	4	4	4	4	3	3	2	1	1	1	1	0	0	—	—
1 hr	nitrogen in the	2	2	2	1	1	1	1	0	0	0	0	0	0	0	—	—
2 "	presence of .25%	1	1	1	0	0	0	0	0	0	0	0	0	—	—	—	—
3 "	formaldehyde	0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—
6 "		0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—
12 "		0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—
24 "		0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—
30 min	Nitrogen without	4	4	4	4	4	4	4	4	4	4	4	3	2	2	—	—
1 hr	formaldehyde	4	4	4	4	4	4	4	4	3	3	2	2	1	1	—	—
2 "		4	4	4	4	4	4	4	4	3	3	2	1	—	—	—	—
3 "		4	4	4	4	4	4	4	3	2	1	1	1	—	—	—	—
6 "		4	4	4	4	4	3	3	2	2	1	1	0	—	—	—	—
12 "		4	4	4	4	3	3	2	2	1	1	1	0	—	—	—	—
24 "		4	4	4	3	3	2	2	1	1	0	0	0	—	—	—	—
30 min	Nitrogen in the	4	4	4	4	4	4	4	4	4	3	3	2	1	0	—	—
1 hr	presence of .25%	4	4	4	4	3	3	3	2	2	1	1	1	0	0	—	—
2 "	formaldehyde	2	2	2	2	1	1	1	0	0	0	0	0	—	—	—	—
3 "		1	1	0	0	0	0	0	0	0	0	0	0	—	—	—	—
6 "		0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—
12 "		0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—
24 "		0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—
None	Control (untreated toxin)	—	—	—	—	4	4	4	4	4	4	4	3	3	2	2	1
12 hr	Standing with	—	—	—	—	4	4	3	3	2	2	1	1	1	1	1	0
24 "	.25% formalde-hyde without emulsification	4	3	3	3	2	2	2	2	1	1	1	1	0	0	0	0

TABLE II

A Comparison of the Detoxifying Action of Emulsified Gases, Oxygen and Nitrogen, with Each Other and with Emulsified Chloroform Both in the Presence and Absence of .25% Formaldehyde.

Time of Treatment	Emulsifying Agent	cc toxin diluted to 1 cc															
		.5	.3	.2	.1	.05	.03	.02	.01	.005	.003	.002	.001	.0005	.0003	.0002	.0001
1 hr	Nitrogen	—	—	—	—	4	4	4	3	3	2	1	1	0	0	0	0
2 "		—	—	—	—	4	4	3	2	2	1	1	0	0	0	0	0
1 "	Oxygen	—	—	—	—	4	4	4	3	2	2	1	1	0	0	0	0
2 "		—	—	—	—	4	4	3	2	1	1	1	0	0	0	0	0
1 "	Nitrogen in the	4	4	4	4	4	4	3	3	2	2	2	1	—	—	—	—
2 "	presence of .25% formaldehyde	2	2	2	2	1	1	0	0	0	0	0	0	—	—	—	—
1 "	Oxygen in the	4	4	4	3	3	2	2	1	0	0	0	0	—	—	—	—
2 "	presence of .25% formaldehyde	2	2	1	1	1	0	0	0	0	0	0	0	—	—	—	—
1 "	Chloroform in an	—	—	—	—	4	4	3	3	2	2	1	1	0	0	0	0
2 "	atmosphere of nitrogen	—	—	—	—	4	4	3	2	2	1	1	0	0	0	0	0
1 "	Chloroform in an	—	—	—	—	4	4	3	2	2	1	1	1	0	0	0	0
2 "	atmosphere of oxygen	—	—	—	—	4	4	3	2	2	1	1	0	0	0	0	0
1 "	Chloroform in an	2	1	1	1	0	0	0	0	0	0	0	0	—	—	—	—
2 "	atmosphere of nitrogen in the presence of .25% formaldehyde	0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—
1 "	Chloroform in an	1	1	1	1	0	0	0	0	0	0	0	0	—	—	—	—
2 "	atmosphere of oxygen in the presence of .25% formaldehyde	0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—
None	Control (untreated toxin)	—	—	—	—	4	4	4	4	4	4	4	3	2	2	1	1

TABLE III.

A Comparison of the Relative Detoxification of Staphylococcus Toxin, Containing Varying Amounts of Formaldehyde, by Emulsification with Nitrogen for Different Periods of Time.

Time of Treatment	% Formaldehyde	cc of toxin diluted to 1 cc															
		.5	.3	.2	.1	.05	.03	.02	.01	.005	.003	.002	.001	.0005	.0003	.0002	.0001
2 hr	.25	3	3	2	2	1	1	1	1	1	0	0	0	—	—	—	—
,,	.10	4	4	4	4	3	2	2	2	1	1	1	1	—	—	—	—
,,	.05	4	4	4	4	4	4	4	4	4	3	2	1	—	—	—	—
,,	.01	4	4	4	4	4	4	4	3	2	2	1	1	—	—	—	—
4 hr	.25	1	1	1	0	0	0	0	0	0	0	0	0	—	—	—	—
,,	.10	3	3	3	2	2	1	1	1	0	0	0	0	—	—	—	—
,,	.05	4	4	4	3	3	2	2	2	1	0	0	0	—	—	—	—
,,	.01	4	4	4	4	3	3	2	2	2	1	1	0	—	—	—	—
6 hr	.25	0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—
,,	.10	1	1	1	1	0	0	0	0	0	0	0	0	—	—	—	—
,,	.05	4	3	3	2	2	1	1	0	0	0	0	0	—	—	—	—
,,	.01	4	4	4	3	3	2	2	1	1	0	0	0	—	—	—	—
Control	Untreated toxin containing no formaldehyde. Not emulsified	—	—	—	—	4	4	4	4	4	4	4	4	3	3	2	1

The relative efficiencies of emulsions of chloroform, oxygen and nitrogen, in detoxifying staphylococcus toxin, and their relative acceleration of the process of formol detoxification of this toxin are illustrated in the accompanying tables. These tables also compare the effect produced by adsorption with that of the slower action of formaldehyde when the toxin is allowed to stand in its presence without emulsification. Staphylococcus toxin is completely detoxified by emulsification with chloroform, in the presence of 0.25% formaldehyde, in about 2 hours and almost equally fast with emulsified gases, as compared with the days required for a similar detoxification when the toxin is allowed to stand with formaldehyde without emulsification.

The fact that emulsified nitrogen acts like chloroform as an adsorption agent in the attenuation of this toxin furnishes proof that the action of emulsified chloroform is that of adsorption and is not due to its solvent action. A comparison of the action of emulsified nitrogen and of emulsified oxygen, both in the presence and in the absence of formaldehyde, indicates that the attenuation of the toxin by oxidation need not be considered as a probability.

A group of rabbits was immunized, in accordance with the procedure of Bulletin B1199 of the National Institute of Health, with staphylococcus toxin which had been shaken with nitrogen in the presence of 0.25% formaldehyde for 8 hours. This material was found to be entirely non-hemolytic and did not produce any observable symptoms of any kind when 0.5 cc was injected intraperitoneally into each of 4 mice. The hemolytic test dose of the original toxin was found to be 0.059 cc when tested with standard staphylococcus antitoxin.

Three rabbits were found to contain less than 0.02 units of antitoxin per cc of serum before immunization. After immunization the number of units of antitoxin per cc of serum was as follows: Rabbit No. 80, 1.6 units; Rabbit No. 81, 3.0 units; Rabbit No. 91, 1.0 unit.