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THE REVERSAL OF THE IODINE DETOXIFICATION OF RICIN

ROY C. AVERY AND FERRIN B. MORELAND

Departments of Pathology and Biochemistry, School of Medicine, Vanderbilt University

The detoxification of bacterial toxins is a relatively simple procedure, a wide variety of physical and chemical agents being effective in bringing about the transformation. The reversal of detoxification, however, is not easily accomplished. The action of some detoxifying agents appears to be quite irreversible, while the effect of others can be overcome. The inactivation by soaps is one example of the reversible process (Larson, Halvorson, Evans, and Green (1925); Wells (1929)). Further, Neill (1926), Neill and Mallory (1926), and Neill and Fleming (1926) have shown that the hemotoxins of certain bacteria after being inactivated by oxidation brought about by exposure to the atmosphere, can be reactivated by reduction, either biologically by anaerobic bacterial action or chemically by $Na_2S_2O_4$. Detoxification with formaldehyde, carbon disulfide, or iodine, on the other hand, is stated to be irreversible (Wells, 1929). Carmichael (1929) has shown that the plant toxin, ricin, can be detoxified by means of iodine. In view of the foregoing work, it seemed of interest to attempt the reversal of the iodine detoxification of ricin using sodium thiosulfate as the reducing agent.

EXPERIMENTAL

The ricin used was Eimer and Amend's "C.P." product. To one gram of ricin were added 100 ml. of 0.9 per cent NaCl solution and the mixture was allowed to stand overnight. Solution was incomplete. The mixture was filtered through paper and then through a Berkefeld candle for sterilization. The sterile solution was kept on ice when not in use. The stock iodine solution was 0.1 N in 3 per cent KI.

The toxicity of the solutions under investigation was determined by means of skin tests. The local reaction resulting from the intracutaneous injection of 0.2 ml. of solution into an adult rabbit was read

after 48 hours. The presence of ricin in a solution so tested leads to the formation of an indurated area consisting of a central necrosis surrounded by erythema, the severity of the reaction depending on the concentration of the ricin. Positive skin reactions could be obtained with a solution containing ricin in as high a dilution as one to a billion; that is, the ricin content of the 0.2 ml. injected was by weight only two ten-thousandths of a microgram. Of the ricin preparation used in this work, a fatal dose was about 4 micrograms per kilogram of body weight. All solutions for injection were made up with 0.9 per cent NaCl.

The reversal of detoxification can be demonstrated either by restoring toxicity to a solution which has been rendered completely nontoxic or by increasing the toxicity of a solution which has been partially inactivated. Both of these methods were used in the present study. In the first procedure, ricin was treated with sufficient iodine to detoxify it completely; the addition of sodium thiosulfate was found to make the solution toxic again. Complete detoxification, however, requires the use of a relatively high concentration of iodine so that in some cases a skin reaction was produced by this substance. This skin reaction to iodine could be distinguished from the ricin reaction by the appearance of a black ring without induration. To avoid the iodine reaction, the second, or incomplete detoxification method was used. From the partially detoxified and retoxified solutions various dilutions were made and injected. It was found that the retoxified solution had to be diluted further than the detoxified before a negative result followed injection. Since the addition of iodine to a solution of ricin causes the formation of a precipitate which remains after the addition of thiosulfate, the mixtures were thoroughly agitated before dilution and injection. The solution plus the precipitate was used. The precipitate when agitated remained in uniform suspension for a considerable period of time.

In the case of the incomplete detoxification technic, a solution was prepared containing ricin in a concentration of 1:200 and iodine 0.005 normal. This solution was allowed to stand in the dark for two hours, after which it was shaken and diluted out and 0.2 ml. of each dilution injected intracutaneously in the side of an adult rabbit. A second solution of ricin 1:200 and iodine 0.005 N was prepared and allowed to stand in the dark for two hours. At the end of this time, sodium thiosulfate was added to a concentration of 0.01 N and the solution allowed to stand for another two hours, when it was diluted out and injected as above. Dilutions of untreated ricin were injected as soon as prepared. Control injections were also made of iodine and of iodine plus excess thiosulfate. All the injections were made at the same time. Table 1 shows the composition of the various dilutions injected and the resulting reactions. The animals used were consistently sensitive to one part of ricin in one hundred million. Treatment of the ricin with iodine lowered the toxicity so that concentrations of from 1:500

TABLE 1

Reversal of incompletely detoxified ricin

Composition of Solutions Injected			Skin Reactions* Rabbit No.			
Ricin	Iodine	$Na_2S_2O_3$	4	5	9	10
	N	N				
1:10,000,000	0	0	═	═	═	═
1:30,000,000	0	0	═	═	═	═
1:100,000,000	0	0	=	=	=	=
1:300,000,000	0	0	—	=	—	—
1:1,000,000,000	0	0	—	=	—	—
1:2,000,000,000	0	0	—	—	—	—
1:200	0.005	0	═	═	═	═
1:500	0.002	0	═	═	=	═
1:1000	0.001	0	—	=	—	=
1:3000	0.00033	0	—	—	—	—
1:10,000	0.0001	0	—	—	—	—
1:100,000	0.00001	0	—	—	—	—
1:200	0.005	0.01	═	═	═	═
1:500	0.002	0.004	═	═	═	═
1:1000	0.001	0.002	=	=	=	=
1:3000	0.00033	0.00067	=	=	=	=
1:10,000	0.0001	0.0002	=	=	=	—
1:100,000	0.00001	0.00002	—	—	—	—
0	0.002	0	=		—	—
0	0.002	0.004	—		—	—
Weight of rabbit in grams			3000	3320	3170	3800

* Reactions: — no reaction; = slight erythema; ═ erythema; ═ erythema = necrosis.

to 1:1,000 were required to produce a positive reaction. On the other hand, the subsequent treatment of the iodized ricin with thiosulfate increased the toxicity to the extent that a one to ten thousand dilution produced a positive skin reaction.

For the method involving complete detoxification, solutions were prepared in a manner similar to that described above, but no dilutions were made except in the case of the untreated ricin.

Table 2 shows the composition of the solutions injected and the reactions obtained. It is seen that iodine can detoxify a solution of ricin as strong as 1:300 and that thiosulfate can reverse this detoxification even after the iodine has been allowed to act for two days.

TABLE 2. *Reversal of completely detoxified ricin*

Composition of Solutions Injected*			Skin Reaction†	Interpretation
Ricin	Iodine	$Na_2S_2O_3$		
A Series	N	N	Rabbit 7 (2970 g.)	
1:300	0.013	0	Necrosis surrounded by black ring, no erythema, no induration	Ricin detoxified, excess iodine toxic
1:300	0.027	0		
1:300	0.013	0.017	Erythema, induration, necrosis	Toxicity of ricin restored
1:300	0.027	0.033	Erythema, induration, necrosis	Toxicity of ricin restored
B Series				
1:300	0.013	0	No reaction	Ricin detoxified
1:300	0.027	0	Necrosis † black ring, no erythema, no induration	Ricin, detoxified excess iodine toxic
1:300	0.013	0.017	Erythema, induration, very slight necrosis	Toxicity of ricin restored
1:300	0.027	0.033		Toxicity of ricin restored
0	0.020	0	Necrosis † black ring, no erythema, no induration	Rabbit sensitive to free iodine
1:100,000,000.	0	0	Erythema, induration, necrosis	.....
1:500,000,000.	0	0	Slight erythema, induration, necrosis	Rabbit sensitive to ricin

A Series: Iodine and $Na_2S_2O_3$ each allowed to act for 2 hours before further treatment or injection.
B Series: Iodine and $Na_2S_2O_3$ each allowed to act for 2 days before further treatment or injection. Negative reaction here probably due to decrease of excess iodine on its standing in dilute aqueous solution.

			Rabbit 3 (3300 g.)	
1:1,000,000....	0	0	Erythema, induration, necrosis	.....
1:10,000,000...	0	0	Erythema, induration	Rabbit sensitive to ricin
1:2000.....	0.0006	0	No reaction	Ricin detoxified
1:2000.....	0.0006	0.001	Slight induration and erythema	Detoxification reversed
0.....	0.0006	0.001	No reaction	Control negative
			Rabbit 6 (2810 g.)	
0.....	0.005	0	No reaction	Rabbit not sensitive to low concentration of free iodine

*All solutions made up in 0.9 per cent NaCl
†Observations made 48 hours after intracutaneous injection of 0.2 ml of solutions

To determine whether or not the restored toxic property was immunologically identical with that of the original ricin, graduated dilutions of partially detoxified and retoxified ricin were mixed with equal volumes of serum obtained from rabbits immunized to ricin. After 30 minutes standing, these mixtures were injected in 0.4 ml. amounts, into the skin of a normal rabbit.

The results of the intradermal tests (Table 3) show that both the partially detoxified and the retoxified ricin could be neutralized by antitoxin obtained from a ricin-immune rabbit.

We may, then, conclude that the detoxification of ricin by means of iodine is a reversible process and that the toxic principle of the recovered ricin is immunologically identical with that of the original sample.

Discussion

The results presented in this paper raise the question: is the alteration of the toxicity chemical or physical? It may be that the ricin is oxidized by the iodine to form a non-toxic substance, and that the

TABLE 3

Neutralization of partially detoxified and retoxified ricin with antiricin rabbit serum

Dilutions	Detoxified Ricin				Retoxified Ricin			
	Normal Serum		Immune Serum		Normal Serum		Immune Serum	
	Nec.* mm.	Ind.† mm.	Nec. mm.	Ind. mm.	Nec. mm.	Ind. mm.	Nec. mm.	Ind. mm.
1:1000	0	8	0	0	9	17	0	5
1:3000	0	3	0	0	0	11	0	0
1:10,000	0	0	0	0	0	8	0	0
1:30,000	0	0	0	0	0	4	0	0

* Necrosis
† Induration and erythema

resulting compound is then reduced by the thiosulfate, with a chemical reaction of the ricin thus involved. Since the detoxification is reversed by the addition of thiosulfate, it is improbable that the iodine produces its effect by addition to a double bond, as Moriyama (1934) considers bromine to do. It is, however, probable, as Moriyama suggests, that the decrease in toxicity is due to an alteration in the colloidal condition of the particle carrying the toxic group rather than to chemical effects on the toxic group itself. It is conceivable that the iodine is adsorbed on the surface of the colloidal ricin micelles, which process would lead to an equilibrium between the adsorbed and the dissolved iodine. Addition of the thiosulfate to the solution would remove free iodine, upset the equilibrium, and finally cause the removal of all the adsorbed iodine. It is also conceivable that the iodine may bring about a change in the degree of dispersion of the ricin with the new (larger or smaller) micelles less toxic than the original ones, this state being again altered by the addition of thiosulfate. Since a precipitate is formed on the addition of iodine to the ricin solution, this latter explanation may very well be the correct one if the ricin is in the precipitate itself. If this is the case, then the iodine causes the precipitation, the thiosulfate subsequently bringing about partial repeptization. Further work is necessary before we can decide what mechanism is responsible for the observed alterations in toxicity.[1]

Summary

The injection of ricin solution into the skin of a rabbit resulted in a typical local reaction. Solutions of ricin treated with iodine in suitable concentration failed to give the ricin reaction on intracutaneous injection or gave it only at higher concentrations of ricin. The treatment of iodized ricin with sodium thiosulfate restored toxicity to these solu-

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