THE ACTION OF ANTISEPTICS ON THE TOXIN OF BACILLUS WELCHII.

A Preliminary Note.

By HERBERT D. TAYLOR, M.D., and J. HAROLD AUSTIN, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, December 15, 1917.)

The chemical sterilization of wounds has resulted in much work on the bactericidal action of the antiseptics in general use.[1,2] In a former paper we reported[3] on the solvent action of some of the chlorinated antiseptics on necrotic tissue, pus, blood clot, and plasma clot. The only evidence that these antiseptics may have a destructive action on bacterial toxins is, first, the clinical observations of Carrel and Dehelly,[4] who noted that in patients with infected wounds treated with hypochlorite there seemed to be an amelioration of the general symptoms which they thought might be due to a reduction in the amount of toxin absorbed, and, second, some experiments by Lumière,[5] who found that pus containing virulent organisms, *B. tetani*, *B. welchii*, streptococci, and staphylococci, and presumably also bacterial toxins, became innocuous after the addition of hypochlorite solution and did not cause symptoms or death in animals injected with the mixture. Control animals, injected with the untreated pus, showed typical lesions. A second series of animals was injected with candle-filtered specimens of pus before and after treatment with hypochlorite solution. The animals that received filtrate from untreated pus showed symptoms of toxemia, while those in which the filtrate from the pus-hypochlorite mixture was injected showed no toxic effect.

It seemed desirable to perform a series of experiments with a definite toxin which could be quantitatively measured and a suitable, susceptible animal as an indicator. Bull and Pritchett[6] have demonstrated

[1] Dakin, H. D., Cohen, J. B., and Kenyon, J., *Brit. Med. J.*, 1916, i, 160. Dakin, H. D., and Dunham, E. K., *ibid.*, 1917, ii, 641.

[2] Dakin, H. D., Cohen, J. B., Daufresne, M., and Kenyon, J., *Proc. Roy. Soc. London, Series B*, 1916, lxxxix, 232.

[3] Taylor, H. D., and Austin, J. H., *J. Exp. Med.*, 1918, xxvii, 155.

[4] Carrel, A., and Dehelly, G., The treatment of infected wounds, New York, 1917, 31.

[5] Lumière, A., *Compt. rend. Acad.*, 1916, i, 365.

[6] Bull, C. G., and Pritchett, I. W., *J. Exp. Med.*, 1917, xxvi, 119.

a toxin for *Bacillus welchii* which fulfills all the requirements of the so called group of soluble or exotoxins. They have standardized the virulence of this toxin, and confirmed the unpublished observation of Flexner[7] that the pigeon is highly susceptible to the toxin and that the lesions produced in this animal are similar to those observed in human cases of gas gangrene. As wounds infected with *Bacillus welchii* are frequently encountered in military surgery today, and as the antiseptics studied are used extensively on wounds of this character, it was decided to use the toxin of Bull and Pritchett and the pigeon as a very sensitive indicator of the relative toxicity of the various toxin-antiseptic mixtures in the series of experiments recorded here. Ten experiments were performed. Comparable results were obtained in all, and the three series recorded below are in every way typical.

Method.

Production of Toxin.—Virulent strains of *Bacillus welchii* were grown for 18 hours in the culture medium described by Bull and Pritchett,[6] which, briefly, is made as follows: To 10 cc. of 0.2 per cent glucose broth are added a few fragments of sterile rabbit muscle. Inoculations are made into this medium under a layer of sterile paraffin oil and the cultures incubated in a vacuum jar from which the air has been exhausted. After incubation, the fluid is centrifuged for 20 minutes at high speed and filtered through a Berkefeld N candle. The different lots of toxin produced in this way are found to differ considerably in potency. For example, 0.3 cc. of the filtrate used in Experiment 1 contained one fatal dose of toxin, while it required 1 cc. of the filtrate used in Experiments 2 and 3 to produce a similar effect. In all cases the toxic filtrate was titrated previously to its use to determine the smallest amount which would kill, in 12 hours or less, a pigeon weighing from 300 to 400 gm., and this amount was considered as one lethal dose.

Treatment of Toxin with Antiseptic.—Volumes of filtrate containing the required number of fatal doses were measured into Esmarch dishes. Horse serum, inactivated at 58°C. for 1 hour, was next added to the solutions in which it was used. Sodium chloride solution,

[7] Flexner, S., quoted by Bull and Pritchett.[6]

0.9 per cent, was added to the portions requiring additional volume, and the antiseptic to be tested was added last of all. The volume used for injection was kept constant in each experiment, with the exceptions noted below. The antiseptic was allowed to remain for 5 minutes in contact with the other substances to be injected, and then the entire volume was injected into the pectoral muscles of a pigeon. Before injection, the feathers were removed from the breast and the skin was washed with alcohol. The results of Experiment 1 are shown in Table I.

TABLE I.

Experiment 1.

Pigeon No	Weight.	Fatal doses of toxin.	Antiseptic.	Horse serum.	0.9 per cent sodium chloride solution.	Result.
	gm.			*cc.*	*cc.*	
1	410	1			3	Died in 12 hrs.
2	425	2	3 cc. of Dakin's solution. *			Lived.
3	425	4	3 " " " " *			"
4	425	4	3 " " " " *	1.5		"
5	410	2	3 " " phenol " †			Died in 10 hrs.
6	425	4	3 " " " " †			" " 5 "
7	240		3 " " Dakin's " *			Lived.
8	325		3 " " phenol " †			"

* Dakin's solution titrated 0.5 per cent sodium hypochlorite concentration (made from bleaching powder).

† Phenol solution, 0.25 per cent.

Experiment 1.—One fatal dose of toxin killed Pigeon 1 in 12 hours. 3 cc. of Dakin's hypochlorite solution, titrating 0.5 per cent sodium hypochlorite, protected Pigeon 2 against two fatal doses, and the same amount of the solution protected Pigeon 3 against four fatal doses of toxin. That blood serum will cause hypochlorite solution to decompose is well known, and that it will reduce the effectiveness of this solution, at least as a germicide, is shown by the experiments reported by Dakin and his coworkers.[1, 2] 3 cc. of Dakin's hypochlorite solution protected Pigeon 4 against four fatal doses of toxin, even in the presence of 1.5 cc. of horse serum. Phenol did not exhibit

any protective action. Pigeon 5, injected with a mixture of 3 cc. of 0.25 per cent phenol and two fatal doses of toxin, died in 10 hours. Pigeon 6, receiving the same amount of phenol solution but four fatal doses of toxin, died 5 hours after inoculation. Pigeons 7 and 8, injected with the antiseptics in the same amount and concentration as employed in the toxin-antiseptic mixtures injected into the other pigeons, survived, thus demonstrating that the antiseptics themselves were not toxic and could not have explained the death of Pigeon 5 in 10 hours and that of Pigeon 6 in 5 hours.

Experiment 2.—The results of this experiment, recorded in Table II, confirm those obtained in Experiment 1. Because the toxin available at this time was not so potent as that used in the first experiment, it was necessary to use greater quantities for injection. In order that the total volume of the solutions to be injected should not be increased above 12 cc. and that the relative concentration of the antiseptics should be of a degree comparable with those used in Experiment 1, it was necessary to concentrate them somewhat; therefore a triple strength Dakin's hypochlorite solution, titrating 1.5 per cent sodium hypochlorite concentration, and a 1 per cent phenol solution were used. The final strength of the sodium hypochlorite and of phenol in the injected mixtures was comparable with that of those of Experiment 1, inasmuch as the dilution was considerably greater. The results were the same as those recorded for Experiment 1 in Table I.

Experiment 3.—In this experiment the action of chloramine-T was contrasted with that of Dakin's hypochlorite solution and of phenol. The results shown in Table III were similar to those obtained when the hypochlorite solution was used and confirm those summarized in Tables I and II. They also show that chloramine-T is able to protect pigeons against at least three fatal doses of the toxin and that its action is still demonstrable when serum is previously mixed with the toxin and the antiseptic is required to act on it as well as on the toxin.

TABLE II.

Experiment 2.

Pigeon No.	Weight.	Fatal doses of toxin.	Antiseptic.	Horse serum.	0.9 per cent sodium chloride solution.	Result.
	gm.			*cc.*	*cc.*	
9	270	1			7	Died in 15 hrs.*
10	310	3	2 cc. of Dakin's solution. †		3	Lived.
11	310	6	2 " " " " †			"
12‡	310	6	3 " " " " †	3		"
13	275	3	2 " " phenol " §		3	Died in 15 hrs.*
14	200	6	2 " " " " §			" "15 " *
15	240		2 " " Dakin's " †		6	Lived.
16	410		2 " " phenol " §		6	"

* Over night.

† Dakin's solution titrating 1.5 per cent sodium hypochlorite (triple strength).

‡ In this pigeon the volume injected was 12 cc., in the others 8 cc.

§ 1 per cent.

TABLE III.

Experiment 3.

Pigeon No.	Weight.	Fatal doses of toxin.	Antiseptic.	Horse serum.	0.9 per cent sodium chloride solution.	Result.
	gm.			*cc.*	*cc.*	
17	480	1			7	Died in 12 hrs.
18	470	3	5 cc. of Dakin's solution.*			Lived.
19	430	6	2 " " " " †			"
20‡	460	6	2 " " " " †	4		"
21	420	3	5 " " phenol " §			Died in 5 hrs.
22	500	3	5 " " chloramine-T " ‖			Lived.
23‡	500	3	5 " " " " ‖	4		"
24	320		5 " " Dakin's " †		3	"
25	450		5 " " phenol " §		3	"
26	310		5 " " chloramine-T " ‖			"

* 0.5 per cent sodium hypochlorite titration.

† 1.73 per cent sodium hypochlorite titration.

‡ In this pigeon 12 cc. were injected, in the others 8 cc.

§ 0.25 per cent.

‖ 2 per cent chloramine-T (equivalent to 0.5 per cent sodium hypochlorite).

DISCUSSION.

From the experiments outlined above it is apparent that Dakin's hypochlorite and chloramine-T solutions will destroy the toxin produced by *Bacillus welchii.* It has seemed more precise, for experimental purposes, to make the mixtures of toxin and antiseptic *in vitro,* but from the experiments of Lumière[5] and the clinical observations of Carrel and Dehelly[4] it seems possible that these solutions may exert a similar influence when used in the treatment of infected wounds. The fact that the detoxicating action was still demonstrable when the toxin was treated with serum before the addition of the antiseptic adds to the clinical significance of these observations, because the conditions then closely simulate those encountered when the antiseptic is applied to wounds.

Phenol solutions of a final concentration of 0.25 per cent exhibited no destructive action on the toxin, and all the animals injected with a toxin-phenol mixture succumbed in the 24 hour interval following inoculation.

The control pigeons (Nos. 7 and 8, Table I; Nos. 15 and 16, Table II; and Nos. 24, 25, and 26, Table III) always survived; the antiseptic substances in the quantities and concentrations used, therefore, were not of themselves lethal.

No attempt was made to determine the maximum number of fatal doses of toxin against which a given amount and concentration of antiseptic was able to protect, nor did we go into the question of the length of time that the antiseptic and toxin must be in contact before injection in order that detoxication may occur.

The pathology of the lesions in the pigeons that died in the above experiments was substantially the same as that described by Bull and Pritchett.[6] In those that did not die varying grades of local edema, congestion, swelling, and discoloration of the skin and subcutaneous tissue were observed. These lesions were never marked and in no instance did they approach those observed in the birds receiving injections which resulted fatally. Those receiving phenol alone showed slightly more marked lesions than those receiving the hypochlorite alone.

In no instance did the pigeons recorded "Lived" in the tables die in the interval of observation, which was at least 1 week and in most

instances 2 weeks or longer. Any possibility of retarded deleterious effects, therefore, from the toxin-antiseptic mixtures injected, is practically excluded.

Finally, it seems desirable to add that these observations are not recorded with the purpose of advocating the use of an antiseptic in the place of the specific antitoxin produced by Bull and Pritchett.[6] In human surgery the antiseptic treatment of infected wounds will doubtless be combined with specific serum therapy.[8]

CONCLUSIONS.

1. Dakin's hypochlorite and chloramine-T solutions will protect pigeons against multiple fatal doses of the toxin of *Bacillus welchii* when the antiseptic and the toxin are mixed *in vitro* and allowed to stand in contact for 5 minutes before injection.

2. The detoxicating action of the solutions is demonstrable also in the presence of serum.

3. Phenol solution, 0.25 per cent, has no such action.

We take this opportunity to thank Dr. Bull and Miss Pritchett for their help and advice in the production and use of the toxin.

[8] A comparison of the behavior of these antiseptics enables us to distinguish two groups. In one, the antiseptic while bactericidal possesses little or no destructive action upon the products of bacterial activity; of this group phenol is an example. In the other group, the antiseptic attacks chemically not only the bacteria but also their products and by an alteration or disintegration of the molecules of the latter alters their properties and renders them inert; of this group the chlorinated antiseptics are the most striking examples. This action of these chlorinated antiseptics is to be attributed chiefly, as pointed out by Dakin, to their affinity for the amino group of the protein molecule.