

## TOXICITY OF CERTAIN WIDELY USED ANTISEPTICS.

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In view of the widespread use of certain antiseptics in the treatment of infected wounds, it has seemed desirable to make toxicity tests on animals under conditions in which rapid absorption might be expected. While, as a rule, the antiseptics are employed under conditions that preclude the possibility of much absorption with consequent systemic effect, yet occasionally certain of them have been recommended and in some instances even used for injection into closed cavities. This practice, which would probably lead to considerable absorption, has not been general, the majority of surgeons proceeding with much caution.

A few experiments of Carrel and Dehelly<sup>1</sup> demonstrated that Dakin's hypochlorite solution when injected subcutaneously in the guinea pig was relatively non-toxic, one-fortieth of the body weight of the animal injected being borne without demonstrable ill effect. Bashford<sup>2</sup> has tested the toxic effect of dilute hypochlorite solutions on the living tadpole immersed in Dakin's solution. Inasmuch as these experiments were few and no data for comparing the relative toxicity of a series of antiseptics were given, it was decided to investigate the toxic action of a number of antiseptic substances in common use. The method was to inject increasing doses into mice intraperitoneally and into guinea pigs both subcutaneously and intraperitoneally, and to note and tabulate the results.

### *Method.*

White mice of approximately 20 gm. and guinea pigs of 300 to 600 gm. body weight were employed and the amount of chemical used is

<sup>1</sup> Carrel, A., and Dehelly, G., The treatment of infected wounds, New York, 1917, 32.

<sup>2</sup> Bashford, E. F., *Lancet*, 1917, ii, 595.

based on the weight of each animal. In regard to the chlorinated antiseptics, the lethal dose is calculated in terms of the sodium hypochlorite equivalent and the available chlorine of the antiseptic. The percentage of the total weight of the antiseptic substance represented by the sodium hypochlorite equivalent is given in a foot-note to Table I.

In Table I the results of the intraperitoneal injection in mice of increasing doses of the antiseptics studied are tabulated. In Column A of Table IV will be found a condensed summary of these results, the antiseptics being arranged in the order of their decreasing toxicity for the animals. Control injections of four of the vehicles employed, namely water, isotonic saline solution, sterile paraffin oil, and Dakin and Dunham's bland oil solvent for dichloramine-T (chlorcosane<sup>3</sup>), show these to be well borne in larger doses than those employed in any of the injections with antiseptics into the test animals. Eucalyptol, however, which has been used in combination with paraffin oil as a vehicle for dichloramine-T is so toxic that its use in experiments of the nature of those recorded here is impossible. The diluting vehicle has been water or isotonic saline solution with all antiseptics except dichloramine-T.

In Table I are given in detail the results of the experiments in which mice were used. The nature and strength of the solution injected, the amount of the solution in cubic centimeters, the amount of the drug in actual milligrams administered and in milligrams per 100 gm. of body weight, with the final results, are recorded.

Table II gives in the same way the results obtained with the few guinea pigs that were injected subcutaneously.

In Table III are recorded the results of the experiments in which guinea pigs were injected intraperitoneally. The form of the table is the same as in Tables I and II.

Table IV summarizes the results given in Tables I to III, showing the greatest dose per 100 gm. of body weight that the animals were able to survive and the smallest dose necessary to kill with the antiseptics named.

<sup>3</sup> The chlorcosane was kindly given us by Dr. H. D. Dakin and Dr. E. K. Dunham. Dakin, H. D., and Dunham, E. K., *Brit. Med. J.*, 1918, i, 51.

## DISCUSSION.

It will be seen from the tables that the only antiseptic of which the smallest fatal dose was smaller than the largest survival dose was dichloramine-T. Since two mice survived 4.7 mg. per 100 gm. of body weight, it is probable that 15.5 mg. rather than 1.6 mg. is to be considered the smallest fatal dose for this series. The distribution of the drug in the viscid bland oil used as a vehicle is probably uneven, which may account for the somewhat variable results obtained with this antiseptic both in mice and in guinea pigs.

Of all the substances tested, eucalyptol and brilliant green are the most toxic, the lethal dose of each being 0.1 mg. per 100 gm. of body weight. Mercuraphen,<sup>4</sup> mercuric chloride, and chloramine-T constitute the group with the next highest toxicity, the lethal dose being 1 mg. per 100 gm. of body weight. Dichloramine-T, proflavine,<sup>5</sup> and the four hypochlorite solutions tested follow in the order named with a lethal dose of about 10 to 15 mg. per 100 gm. of body weight. The least toxic chemicals are iodine and phenol, of which the lethal doses are about 50 mg. per 100 gm. of body weight.

In Table II are recorded a few experiments with the antiseptics injected under the skin of the abdomen of guinea pigs. The lethal dose of Dakin's hypochlorite solution per 100 gm. of body weight is the same as that determined intraperitoneally in the mouse. Chloramine-T and dichloramine-T administered in this manner gave rise to local necrosis with extensive sloughing. It is probable that only a small part of the drug injected reached the general system of the animal and in consequence the determination of the lethal dose in this way can hardly be considered satisfactory. It was accordingly abandoned and five of the antiseptics were tested in guinea pigs by intraperitoneal injections. The results are tabulated in Table III and summarized in Column B of Table IV.

Chloramine-T has the same toxicity per unit of body weight for guinea pigs and for mice. The same may be true of dichloramine-T or this substance may be somewhat less toxic for the guinea pig.

<sup>4</sup> The mercuraphen was sent to us for trial through the kindness of Dr. J. F. Schamberg.

<sup>5</sup> The proflavine was obtained from England.

TABLE I.  
Results of Injecting Mice Intraperitoneally.

Animal No.	Solution.	Amount of solution.		Measured in terms of.		Amount of drug.		Amount of drug per 100 gm. of body weight.	Result.
		cc.	mg.	Sodium chloride.	mg.	mg.			
1	Sodium chloride 0.85 per cent.	0.50	4.20	Sodium chloride.	4.20	20.00	Lived.		
2	" " 0.85 "	1.00	8.50	" "	8.50	40.00	"		
3	" " 0.85 "	4.00	34.00	" "	34.00	170.00	"		
4	Distilled water.	0.50					Lived.		
5	" "	2.00					"		
6	" "	4.00					"		
7	Dakin's hypochlorite 0.41 per cent (bleaching powder).	0.10	0.40	Sodium hypochlorite.	0.40	2.00	Lived.		
8	" " 0.41 "	0.50	2.00	" "	2.00	10.00	"		
9	" " 0.47 " (original).	0.50	2.30	" "	2.30	12.00	"		
10	" " 0.49 " (bleaching powder).	0.50	2.40	" "	2.40	12.00	"		
11	" " 0.50 " (neutral)*.	0.50	2.50	" "	2.50	12.00	"		
12	" " 0.47 " (original).	1.00	4.70	" "	4.70	24.00	Died in 15 hrs.†		
13	" " 0.50 " (neutral)*.	1.00	5.00	" "	5.00	25.00	" " 18 " †		
14	" " 0.41 " (bleaching powder).	2.00	8.20	" "	8.20	40.00	" " 3 " †		
15	" " 0.47 " (original).	2.00	9.40	" "	9.40	47.00	" " 15 " †		
16	" " 0.41 " (bleaching powder).	4.00	16.40	" "	16.40	80.00	" " 1½ " †		
17	Hychlorite, 0.5 per cent sodium hypochlorite.	0.33	1.70	Sodium hypochlorite.	1.70	8.00	Lived.		
18	" " 0.5 " "	0.33	1.70	" "	1.70	8.00	"		
19	" " 0.5 " "	0.50	2.50	" "	2.50	12.00	"		
20	" " 0.5 " "	0.50	2.50	" "	2.50	12.00	Died in 6 hrs.		

21	Chloramine-T 0.2 per cent.....	0.10	Sodium hypochlorite.	0.05	0.26	Lived.
22	" 0.2 " ".....	0.30	"	0.16	0.80	"
23	" 2.0 " ".....	0.10	"	0.50	2.60	Died in 19½ hrs.
24	" 2.0 " ".....	0.50	"	2.60	13.00	" " 2 "
25	" 2.0 " ".....	0.50	"	2.60	13.00	" " 1½ "
26	" 2.0 " ".....	0.50	"	2.60	13.00	" " 2½ "
27	Dichloramine-T 0.5 per cent in bland oil.....	0.10	Sodium hypochlorite.	0.30	1.60	Lived.
28	" 0.5 " " ".....	0.10	"	0.30	1.60	Died in 30 hrs.
29	" 0.5 " " ".....	0.30	"	0.90	4.70	Lived.
30	" 0.5 " " ".....	0.30	"	0.90	4.70	"
31	" 5.0 " " ".....	0.10	"	3.10	15.50	Died in 18 hrs.†
32	" 5.0 " " ".....	0.10	"	3.10	15.50	" " 18 "†
33	" 5.0 " " ".....	0.20	"	6.20	31.00	" " 18 "†
34	" 5.0 " " ".....	0.20	"	6.20	31.00	" " 18 "†
35	" 5.0 " " ".....	0.33	"	10.60	50.00	" " 2 "
36	" 5.0 " " ".....	0.50	"	15.50	75.00	" " 1½ "
37	" 5.0 " " ".....	0.50	"	15.50	75.00	" " 1½ "
38	Paraffin oil.....	0.50				Lived.
39	Bland oil.....	0.33				"
40	".....	0.50				"
41	Mercuric chloride 0.01 per cent.....	0.10	Mercuric chloride.	0.01	0.05	Lived.
42	" 0.01 " ".....	0.30	"	0.03	0.15	"
43	" 0.10 " ".....	0.10	"	0.10	0.50	"
44	" 0.10 " ".....	0.50	"	0.50	2.50	Died in 18 hrs.
45	Mercuraphen 0.01 per cent.....	0.50	Mercuraphen.	0.05	0.25	Lived.
46	" 0.10 " ".....	0.10	"	0.10	0.50	"
47	" 0.10 " ".....	0.30	"	0.30	1.50	Died in 48 hrs.
48	" 0.10 " ".....	1.00	"	1.00	5.00	" " 27 "

\* Cullen, G. E., and Austin, J. H., *Proc. Soc. Exp. Biol. and Med.*, 1917-18, xv, 41.

† Died over night.

TABLE I—Continued.

Animal No.	Solution.	Amount of solution.		Measured in terms of.	Amount of drug.		Result.
		cc.	mg.		mg.	per 100 gm. of body weight.	
49	Brilliant green 0.01 per cent.	0.10	0.01	Brilliant green.	0.01	0.05	Lived.
50	" " 0.01 "	0.30	0.03	" "	0.03	0.15	Died in 48 hrs.
51	" " 0.10 "	0.10	0.10	" "	0.10	0.50	" " 6 "
52	" " 0.10 "	0.50	0.50	" "	0.50	2.50	" " 18 "
53	" " 0.10 "	0.50	0.50	" "	0.50	2.50	" " 3½ "
54	Proflavine 0.1 per cent.	0.33	0.33	Proflavine.	0.33	1.70	Lived.
55	" " 1.0 "	0.10	1.00	" "	1.00	5.00	" "
56	" " 1.0 "	0.50	5.00	" "	5.00	25.00	Died in 18 hrs.†
57	" " 1.0 "	0.50	5.00	" "	5.00	25.00	" " 12 "
58	" " 1.0 "	1.00	10.00	" "	10.00	50.00	" " 3 "
59	" " 1.0 "	1.00	10.00	" "	10.00	50.00	" " 18 "
60	Tincture of iodine 7.0 per cent.	0.10	7.00	Iodine.	7.00	35.00	Lived.
61	" " 7.0 "	0.30	21.00	" "	21.00	100.00	Died in 45 min.
62	" " 7.0 "	0.50	35.00	" "	35.00	175.00	" " 10 "
63	" " 7.0 "	1.00	70.00	" "	70.00	350.00	" " 15 "
64	Commercial hypochlorite (Javelle water) 0.5 per cent of sodium hypochlorite.	0.50	2.50	Sodium hypochlorite.	2.50	12.00	Lived.
65	Commercial hypochlorite (Javelle water) 1.0 per cent of sodium hypochlorite.	0.50	5.00	" "	5.00	25.00	Died in 4½ hrs.
66	Commercial hypochlorite (Javelle water) 1.0 per cent of sodium hypochlorite.	1.00	10.00	" "	10.00	50.00	" " 2½ "

	Peroxide of hydrogen 3.0 per cent (commercial)	0.50	Peroxide of hydrogen.	15.00	75.00	Lived.
67	Peroxide of hydrogen 3.0 per cent (commercial)	0.50		15.00	75.00	Lived.
68	Magnesium hypochlorite equivalent to 0.5 per cent sodium hypochlorite	0.50	Sodium hypochlorite.	2.50	12.00	Lived.
69	Magnesium hypochlorite equivalent to 1.0 per cent sodium hypochlorite	0.50	"	5.00	25.00	Died in 25 min.
70	Magnesium hypochlorite equivalent to 1.0 per cent sodium hypochlorite	1.00	"	10.00	50.00	" " 10 "
71	Phenol 0.25 per cent.	0.50	Phenol.	1.25	6.20	Lived.
72	" 1.00 "	0.30	"	3.00	15.00	"
73	" 1.00 "	0.50	"	5.00	25.00	"
74	" 1.00 "	1.00	"	10.00	50.00	"
75	" 1.00 "	1.00	"	10.00	50.00	Died in 5 min.
76	" 1.00 "	1.50	"	15.00	75.00	" " 12 "
77	Eucalyptol 10.0 per cent in paraffin oil	0.10	Eucalyptol.	0.01	0.05	Lived.
78	" 10.0 "	0.30	"	0.03	0.15	Died in 4 hrs.
79	" 100.0 "	0.10	"	0.10	0.50	" " 10 min.
80	" 50.0 " in paraffin oil	0.50	"	0.25	1.25	" " 10 "

Sodium hypochlorite equivalent of

- Dakin's hypochlorite = 100 per cent.
- Commercial hypochlorite (Javelle water) = 100 "
- Hypochlorite = 100 "
- Magnesium hypochlorite = 117.4 "
- Chloramine-T = 26.5 "
- Dichloramine-T = 62.1 "

TABLE II.  
*Results of Injecting Guinea Pigs Subcutaneously.*

Animal No.	Weight.	Solution.	Amount of solution.	Measured in terms of.	Amount of drug.	Amount of drug per 100 gm. of body weight.	Result.
	gm.		cc.		mg.	mg.	
1	400	Dakin's hypochlorite 0.5 per cent.	10.0	Sodium hypochlorite.	50.00	12.00	Lived.
2	550	" " 0.5 "	13.7	" "	68.00	12.00	"
3	425	" " 0.48 "	10.6	" "	51.00	12.00	"
4	475	" " 0.5 "	11.9	" "	60.00	13.00	"
5	450	" " 0.48 "	22.5	" "	108.00	24.00	Died in 12 hrs.
6	350	Hychlorite, 0.5 per cent sodium hypochlorite.	9.0	Sodium hypochlorite.	45.00	13.00	Lived.
7	450	Chloramine-T 2.0 per cent.	11.25	Sodium hypochlorite.	59.00	13.00	Lived (sloughed).
8	550	" " 2.0 "	13.7	" "	73.00	13.00	"
9	450	Eucalyptol 50.0 per cent.	11.25	Eucalyptol.	5,600.00	1,200.00	Died in 12 hrs.
10	350	Dichloramine-T 5.0 per cent in bland oil.	9.0	Sodium hypochlorite.	280.00	83.00	Lived (sloughed).
11	575	Proflavine 0.1 per cent.	7.2	Proflavine.	7.20	1.25	Lived.
12	300	" " 0.1 "	7.5	" "	7.50	2.50	"



TABLE III.  
Results of Injecting Guinea Pigs Intraperitoneally.

Animal No.	Weight.	Solution.	Amount of solution.	Measured in terms of.	Amount of drug.	Amount of drug per 100 gm. of body weight.	Result.
	gm.		cc.		mg.	mg.	
13	507	Dakin's hypochlorite 0.5 per cent. ....	3.10	Sodium hypochlorite.	15.00	3.00	Lived.
14	350	" " 0.5 " " .....	4.37	" "	22.00	6.30	Died in 9½ hrs.
15	375	" " 0.5 " " .....	9.37	" "	47.00	12.50	" " 7½ "
16	566	Hychlorite, 0.5 per cent sodium hypochlorite.	3.50	Sodium hypochlorite.	17.00	3.00	Lived.
17	500	" 0.5 " " " .....	6.25	" "	31.00	6.20	Died in 3¼ hrs.
18	450	" 0.5 " " " .....	11.25	" "	56.00	12.50	" " 7½ "
19	430	Chloramine-T 0.2 per cent. ....	2.70	Sodium hypochlorite.	1.40	0.30	Lived.
20	600	" 2.0 " " " .....	1.20	" "	6.40	1.10	" "
21	502	" 2.0 " " " .....	3.10	" "	16.00	3.20	Died in 3 hrs.
22	425	" 2.0 " " " .....	5.30	" "	29.00	6.70	" " 1½ "
23	450	" 2.0 " " " .....	11.25	" "	59.00	13.30	" " 1 hr.
24	525	Proflavine 0.1 per cent. ....	6.55	Proflavine.	7.00	1.30	Lived.
25	525	" 0.1 " " " .....	13.10	" "	14.00	2.60	" "
26	597	" 0.1 " " " .....	30.00	" "	30.00	5.00	Died in 72 hrs.
27	470	Dichloramine-T 0.5 per cent in bland oil. ....	1.90	Sodium hypochlorite.	5.90	1.30	Lived.
28	470	" 0.5 " " " " .....	5.90	" "	18.30	3.90	Died in 22 hrs.
29	550	" 5.0 " " " " .....	2.20	" "	68.00	12.30	Lived.
30	498	" 5.0 " " " " .....	6.20	" "	193.00	39.00	Died in 1½ hrs.
31	375	" 5.0 " " " " .....	4.70	" "	146.00	39.00	" " 2 "
32	375	" 5.0 " " " " .....	9.37	" "	292.00	78.00	" " 1 hr.

TABLE IV.

*Summary.*

Drug.	A. Mice, injected intraperitoneally.		B. Guinea pigs, injected intraperitoneally.		C. Guinea pigs, injected subcutaneously.	
	Smallest fatal dose per 100 gm. of body weight.	Largest surviving dose per 100 gm. of body weight.	Smallest fatal dose per 100 gm. of body weight.	Largest surviving dose per 100 gm. of body weight.	Smallest fatal dose per 100 gm. of body weight.	Largest surviving dose per 100 gm. of body weight.
	mg.	mg.	mg.	mg.	mg.	mg.
Eucalyptol.....	0.15	0.05				
Brilliant green.....	0.15	0.05				
Mercurphen.....	1.50	0.50				
Mercuric chloride.....	2.50	0.50				
Chloramine-T.....	2.60	0.80	3.2	1.1		
Dichloramine-T.....	1.60 (?)		3.9 (?)			
	15.50	4.70	39.0	12.3		
Proflavine.....	25.00	5.00	5.0	2.6		
Hychlorite.....	12.00	12.00	6.2	3.0		
Dakin's hypochlorite.....	24.00	12.00	6.3	3.0	24.0	13.0
Commercial hypochlorite (Javelle water).....	25.00	12.00				
Magnesium hypochlorite.....	25.00	12.00				
Iodine.....	100.00	35.00				
Phenol.....	50.00	50.00				

All the figures represent milligrams of antiseptic, or in the case of the chlorinated antiseptics, milligrams of sodium hypochlorite equivalent of the antiseptic, per 100 gm. of body weight.

Proflavine, hychlorite,<sup>6</sup> and Dakin's hypochlorite solution given intraperitoneally are all about two or three times as toxic per 100 gm. of body weight for the guinea pig as for the mouse. On the whole, however, the toxicity of the antiseptics follows about the same order in the two species of animal. When the great difference in the body weight of the mouse and the guinea pig is considered, the constancy of the lethal dose per unit of body weight is striking.

While it is, of course, not justifiable to calculate arbitrarily, on the basis of body weight alone, the fatal dose of these substances for man, it is interesting in this connection to note that if such a compu-

<sup>6</sup> Made by General Laboratories, Madison, Wisconsin.

tation could be considered valid the following amounts of certain of the antiseptics under the proper circumstances would constitute a fatal dose for a man weighing 70 kg.

0.14 cc. of equal parts of paraffin oil and eucalyptol (formerly considerably used as a solvent for dichloramine-T).

144 cc. of a 2 per cent solution of chloramine-T.

160 cc. of a 5 per cent solution of dichloramine-T in bland oil.

1,600 cc. of any of the hypochlorite solutions tested, having sodium hypochlorite titration of 0.5 per cent.

However, only a small amount of the antiseptic employed is absorbed from wound surfaces or from an abscess cavity, and little if any danger from constitutional effects would be expected from their employment in this way. When used in closed cavities, in the serous cavities of the body, or when sutured within a wound, these figures should, we believe, be kept in mind. This is especially the case in respect to eucalyptol used as a vehicle.

The drugs are tabulated in Table IV in the order of diminishing toxicity. It is interesting to note that the least toxic drugs that are efficiently bactericidal are the hypochlorite series and iodine.<sup>7,8,9</sup> The only two of the four hypochlorite solutions studied that are suitable for clinical use are Dakin's hypochlorite solution (in this case made from bleaching powder) and hychlorite. Of the other substances which vary somewhat in their greater toxicity for mice and guinea pigs, the most efficient are proflavine, dichloramine-T, chloramine-T, and possibly brilliant green. Mercuraphen, mercuric chloride, and phenol can be disregarded as having too feeble disinfecting powers. Eucalyptol, the most toxic substance included in this study, is not recommended as a bactericidal agent, but merely as a solvent for dichloramine-T. Of these drugs, the only ones having appreciable solvent action on necrotic tissues, pus, etc., are the hypochlo-

<sup>7</sup> Dakin, H. D., and Dunham, E. K., *Handbook of antiseptics*, New York, 2nd edition, 1918.

<sup>8</sup> Dakin, H. D., Cohen, J. B., and Kenyon, J., *Brit. Med. J.*, 1916, i, 160.

<sup>9</sup> Dakin, H. D., Cohen, J. B., Daufresne, M., and Kenyon, J., *Proc. Roy. Soc. London, Series B*, 1916, lxxxix, 232.

rites.<sup>10,11</sup> Chloramine-T and the hypochlorites have also a destructive action on bacterial toxins.<sup>12</sup>

#### CONCLUSIONS.

1. The substances injected intraperitoneally into mice and guinea pigs arranged in the order of their decreasing toxicity are: eucalyptol and brilliant green; mercuriophen; mercuric chloride and chloramine-T; dichloramine-T and proflavine; hychlorite, Dakin's hypochlorite, Javelle water, and magnesium hypochlorite; iodine and phenol.

2. Now that Dakin's bland solvent, chlorcosane, is available as a vehicle for dichloramine-T, eucalyptol should probably be discarded for this purpose because of its much greater toxicity.

3. Inasmuch as experienced surgeons do not approve of the injection of solutions of iodine and phenol into closed cavities, it would seem advisable not to use any of the antiseptics here discussed in this manner inasmuch as all exhibit a greater toxicity for mice and guinea pigs than the two chemicals first named.

4. The method of testing toxicity of antiseptics by subcutaneous injection is not satisfactory because exudation and subsequent sloughing reduce the rate of absorption and make uncertain the amount finally absorbed.

<sup>10</sup> Taylor, H. D., and Austin, J. H., *J. Exp. Med.*, 1918, xxvii, 155.

<sup>11</sup> Austin, J. H., and Taylor, H. D., *J. Exp. Med.*, 1918, xxvii, 627.

<sup>12</sup> Taylor, H. D., and Austin, J. H., *J. Exp. Med.*, 1918, xxvii, 375.