THE SOLVENT ACTION OF ANTISEPTICS ON NECROTIC TISSUE.

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

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

Plate 5.

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The recent interest in the chemical sterilization of wounds has led to the introduction of numerous new antiseptics, each of which has in turn been advocated because of some advantage, real or apparent. For many of these compounds, claims have been made which are not always confirmed by carefully controlled experiments. Carrel and Dehelly[1] emphasize that, for the removal of the necrotic tissue that remains after mechanical cleansing, Dakin's hypochlorite solution is the antiseptic of choice because of its solvent action on devitalized tissue, and Dakin and Dunham[2] also recognize the value of the hypochlorite solution for this purpose.

Dakin's solution was shown by Fiessinger and his coworkers[3] to have a disintegrating action on pus cells. Rous and Jones[4] have shown that intact leukocytes may protect virulent bacteria which they have phagocyted from the action of antiseptics, and that subsequently these bacteria may proliferate under suitable conditions. Dakin's solution, by its solvent action on these leukocytes, minimizes the danger of reinfection of the wound from this source. Because of this action on necrotic tissue, pus, and serum clot, Carrel and Dehelly recommend Dakin's hypochlorite solution for the sterili-

[1] Carrel, A., and Dehelly, G., The treatment of infected wounds, New York, 1917, 150, 192.

[2] Dakin, H. D., and Dunham, E. K., A handbook on antiseptics, New York, 1917, 14.

[3] Fiessinger, N., Moiroud, P., Guillaumin, C. O., and Vienne, G., *Ann. med.*, 1916, iii, 133.

[4] Rous, P., and Jones, F. S., *J. Exp. Med.*, 1916, xxiii, 601.

zation of infected wounds.[5] Bashford[6] has demonstrated the ability of Dakin's solution in high dilution to erode the tissues of the tadpole's abdomen. He showed also that this occurred only after the circulation to the part had been interrupted for some time, due to the death of the organism.

As this erosive action of Dakin's solution is an important factor, the following experiments were planned to compare its solvent action with that of certain other chlorinated antiseptics. Fiessinger and his coworkers[3] concluded that the essential factor in the solvent action of the hypochlorites is their alkalinity. Our experiments were therefore designed to determine the importance of three factors: the alkalinity, the nature of the chlorinated antiseptic employed, and the chlorine concentration of the latter.

Method.

The solvent action of the various substances employed was tested by adding 50 cc. of each solution to 5 cc. of an emulsion of macerated liver tissue in a 100 cc. bottle. The mixture was thoroughly shaken every half hour for 2 hours. A 15 cc. portion was then removed to a centrifuge tube and in each case centrifuged at the same high speed for 5 minutes. The volume in cubic centimeters of the sediment thrown down was measured. The solvent action was shown by diminution of the amount of sediment compared with that obtained from inert solutions such as water or normal saline solution.

The liver emulsion was prepared in Experiment 1 from rabbit liver, in the other experiments from cat liver. In Experiments 1, 2, 3, and 4 the liver was purposely infected by handling, placed in the incubator at 37°C. until thoroughly necrotic, cut into small pieces, suspended in saline solution, shaken in a bottle with broken glass to emulsify it, and strained through a single layer of gauze. In Experiment 5 cat liver was similarly emulsified and used after 12 hours' preservation in the ice box. The solutions employed were prepared as follows:

[5] Carrel, A., and Dehelly, G., The treatment of infected wounds, New York, 1917, 109.

[6] Bashford, E. F., *Lancet*, 1917, cxciii, 595.

Control Solutions.—Neutral: water and normal saline solution. Weakly alkaline: a solution of sodium carbonate, 1 gm., and sodium bicarbonate, 17 gm. per liter of water; this solution has approximately the alkalinity of a properly prepared Dakin's solution. Strongly alkaline: 0.1 N sodium hydroxide.

Chloramine-T Solutions.—Chloramine-T solutions were prepared by dissolving the required amount of chlorazene[7] in the appropriate control solution to obtain neutral, weakly alkaline, or strongly alkaline chloramine solutions.

Hypochlorite Solutions.—Weakly alkaline: ordinary Dakin's solution prepared either from bleaching powder or by the action of liquid chlorine on sodium carbonate solution, in either case with careful control of the degree of alkalinity as well as of the hypochlorite content. Neutral: chlorine gas passed through sodium carbonate solution, 28 gm. per liter, until the solution just ceased to give a flash of pink upon the addition of alcoholic solution of phenolphthalein, 1 per cent; the hypochlorite content was determined by titration, and the solution diluted with water to the desired strength. This solution was always prepared immediately before use, as a considerable proportion of the total hypochlorite is present as hypochlorous acid, and the decomposition of the solution is very rapid (Cullen and Austin[8]). Strongly alkaline: A double strength neutral solution of hypochlorite was prepared as just described and immediately added to an equal volume of 0.2 N sodium hydroxide solution.

Chlorinated Oils.—Paraffin oil and eucalyptol were mixed in equal parts, and the same oils chlorinated according to Dakin's method.[9]

Dichloramine-T.—A 15 per cent solution was made in chlorinated eucalyptol and then mixed with an equal volume of chlorinated paraffin oil. In the experiments in which the oils were used (Experiment 1, Tubes 5, 6, and 7; Experiment 2, Tubes 4, 5, and 6; Experiment 3, Tube 9), except in Experiment 3, Tube 10, 5 cc. of liver emulsion were added to 50 cc. of water or of one of the control solutions, and 15 cc. of the oil used were superimposed upon this mixture; the mixture was well shaken every half hour with a rotary motion.

[7] Prepared by the Abbott Laboratories, New York.

[8] Personal communication.

[9] Dakin, H. D., *Brit. Med. J.*, 1915, ii, 318.

At the end of 2 hours, after again being shaken, the oil was allowed to separate from the aqueous suspension and 15 cc. portions were immediately removed from the latter for centrifuging. In Tube 10 of Experiment 3, the 5 cc. of liver emulsion were introduced into 50 cc. of the oil without water and shaken continuously for 2 hours.

The sodium hypochlorite equivalent of the hypochlorite and other chloramine solutions was determined by the addition of potassium iodide solution and glacial acetic acid to 10 cc. samples and titration of the iodine liberated with 0.1 N sodium thiosulfate.

RESULTS.

In Experiment 1, Table I, Solutions 8 and 9 being taken as controls, no solvent effect was noted after the action of chloramine-T solution or of the chlorinated oils, with or without dichloramine-T. Dakin's solution, on the other hand, had a marked solvent action, which was apparent upon inspection of the bottles, even in the first 15 minutes. This was accompanied by pronounced bleaching of the emulsion. A still more rapid and marked action, similar in character, was obtained from the strongly alkaline hypochlorite. Figs. 1 and 2 show the results of this experiment at the end of 2 hours. The reaction of the

TABLE I.

Experiment 1.

Tube No.	5 cc. of liver emulsion in 50 cc. of the following solutions:	Sediment.
		cc.
1	Neutral chloramine-T, 0.5 per cent, equivalent to sodium hypochlorite, 0.12 per cent	0.30
2	Neutral chloramine-T, 0.2 per cent, equivalent to sodium hypochlorite, 0.5 per cent	0.25
3	Weakly alkaline hypochlorite, 0.5 per cent (Dakin's solution)	0.05
4	Strongly " " 0.5 " " " "	0.02
5	Water + paraffin oil and eucalyptol.*	0.27
6	" + chlorinated paraffin oil and eucalyptol.*	0.28
7	" + " " " " " + dichloramine-T, 7.5 per cent	0.27
8	Salt solution	0.28
9	Water	0.27

* 5 cc. of liver emulsion in 50 cc. of water, overlaid with 15 cc. of oil.

solutions used, however, varied, as well as the nature of the antiseptic substance. Experiment 2 was therefore performed with solutions of approximately the same reaction (Table II).

The results of this experiment confirm those observed in Experiment 1. Chloramine-T and dichloramine-T were without solvent action, whereas Dakin's hypochlorite gave marked solution. In Experiment 3, Table III, the effect of diminishing the concentration of the antiseptic in a solution of the same reaction was tested. Both weakly alkaline and strongly alkaline solutions were employed.

TABLE II.

Experiment 2.

Tube No.	5 cc. of liver emulsion in 50 cc. of the following solutions:	Sediment.
		cc.
1	Weakly alkaline carbonate-bicarbonate solution....................	1.27
2	" " " " + chloramine-T, 2 per cent..	1.24
3	Weakly alkaline hypochlorite, stock Dakin's solution (sodium hypochlorite, 0.5 per cent)......................................	0.08
4	Weakly alkaline carbonate-bicarbonate solution + paraffin oil and eucalyptol.*...	1.23
5	Weakly alkaline carbonate-bicarbonate solution + chlorinated paraffin oil and eucalyptol.*......................................	1.24
6	Weakly alkaline carbonate-bicarbonate solution + chlorinated paraffin oil and eucalyptol + dichloramine-T, 7.5 per cent.*.........	1.32

* 5 cc. of liver emulsion in 50 cc. of carbonate-bicarbonate solution, overlaid with 15 cc. of oil.

This experiment showed a loss of the solvent action in weakly alkaline hypochlorite solutions, occurring suddenly between 0.2 and 0.3 per cent sodium hypochlorite concentration. In the strongly alkaline solutions the solvent action was marked, even at the lowest hypochlorite concentration employed. This experiment, taken in conjunction with the well known rapid drop in the sodium hypochlorite titer of Dakin's solution in contact with tissues, indicates that any solvent action resulting from its application clinically may be expected to occur in the first few minutes and emphasizes the importance of frequent flushing of wounds with the solution.

In order to distinguish between the effects of alkalinity and of hypochlorite concentration, Experiment 4 was performed (Table IV). The neutral hypochlorite solutions were prepared as described above. For the neutral control, saline solution was employed. The weakly alkaline hypochlorite solutions in the column headed "Weakly alkaline due to hypochlorite" were prepared as already described, except that the chlorine gas was passed into sodium hydroxide solution instead of into sodium carbonate, thus producing a solution of which

TABLE III.

Experiment 3.

Tube No.	5 cc. of liver emulsion in 50 cc. of the following solutions:	Sediment.
		cc.
1	Water	0.25
2	Neutral chloramine-T, 2 per cent, equivalent to sodium hypochlorite, 0.5 per cent	0.22
3	Weakly alkaline hypochlorite (sodium hypochlorite, 0.5 per cent)	0.01
4	" " " " " 0.4 " "	0.01
5	" " " " " 0.3 " "	0.02
6	" " " " " 0.2 " "	0.22
7	Strongly " " " " 0.5 " "	Tr.
8	" " " " " 0.2 " "	0.08
9	Water + chlorinated paraffin oil and eucalyptol + dichloramine-T, 7.5 per cent.*	0.25
10	Chlorinated paraffin oil and eucalyptol + dichloramine-T, 7.5 per cent, without water.†	0.22

* 5 cc. of liver emulsion in 50 cc. of water, overlaid with 15 cc. of oil. Shaken continuously for 2 hours.

† 5 cc. of liver emulsion in 50 cc. of oil. Shaken continuously for 2 hours.

the alkalinity was due almost entirely to sodium hypochlorite, and which was without buffer substances. The hypochlorite solution in the column headed "Weakly alkaline due to carbonate-bicarbonate" and the strongly alkaline solution were prepared exactly as described above. Table IV shows that in the control solutions solvent action occurred only in the strongly alkaline solution. A somewhat more marked solvent action was obtained when hypochlorite, even in the low concentration of 0.1 per cent, was added to the alkali. No solvent action was obtained in the weakly alkaline control, as compared with the neutral control of normal saline solution. When hypochlorite was added to the weakly alkaline carbonate-bicarbonate solution, or when a weakly alkaline solution of sodium hypochlorite without carbonates was prepared, no solvent action was present at a concentration of 0.1 per cent, but it was marked at a concentration of 0.2 per cent. The change in solvent action resulting from small variations in hypochlorite concentration at about 0.2 per cent was striking, confirming the results of Experiment 3. The absence of solvent action of Dakin's solution below a hypochlorite concentration of 1:500 contrasts sharply with the marked bactericidal action of the same solution in serum to 1:1,500, and in water to 1:500,000 on *Staphylococcus aureus*.[10] In neutral solution at a hypochlorite concentration of 0.2 per cent, solvent action was very slight. At 0.3 per cent it was moderate, and at 0.5 per cent marked.

TABLE IV.

Experiment 4.

Tube No.	Hypochlorite concentration of solutions.	Reaction of solutions.			
		Neutral.	Weakly alkaline.		Strongly alkaline.
			Due to carbonate-bicarbonate.	Due to hypochlorite.	
		Sediment.	Sediment.	Sediment.	Sediment.
	per cent	*cc.*	*cc.*	*cc.*	*cc.*
1	0.5	0.01	0.01	0.01	Tr.
2	0.3	0.04	0.01	0.01	"
3	0.2	0.10	0.01	0.01	"
4	0.1	0.14	0.13	0.12	"
5	Control.	0.12	0.12		0.04

Experiment 5 serves as a final control of the solvent action of alkali alone, of chloramine-T added to neutral, weakly alkaline, and strongly alkaline solutions, and of hypochlorite solutions of the three grades of alkalinity. It is clear that chloramine-T, even in a 2 per cent solution, has no solvent action except that due to the alkalinity of the solution in which it is dissolved, and that therefore it is without this

[10] Dakin, H. D., Cohen, J. B., and Kenyon, J., *Brit. Med. J.*, 1916, i, 160.

action in the grade of alkalinity permissible for clinical use. On the other hand, 0.5 per cent neutral sodium hypochlorite-hypochlorous acid solution (Tube 7) has a marked solvent effect, which must be attributed to the action of the chlorine unaided by alkali. Fig. 3 shows the results of Experiment 5 (Table V) at the end of 2 hours.

TABLE V.

Experiment 5.

Tube No.	5 cc. of liver emulsion in 50 cc. of the following solutions:	Sediment.
		cc.
1	Neutral control (salt solution)	0.25
2	Weakly alkaline control (carbonate-bicarbonate)	0.24
3	Strongly " " (0.1 N sodium hydroxide)	Tr.
4	Neutral chloramine-T, 2 per cent, equivalent to sodium hypochlorite, 0.5 per cent	0.26
5	Weakly alkaline chloramine-T, 2 per cent, equivalent to sodium hypochlorite, 0.5 per cent	0.24
6	Strongly alkaline chloramine-T, 2 per cent, equivalent to sodium hypochlorite, 0.5 per cent	Tr.
7	Neutral hypochlorite (sodium hypochlorite, 0.5 per cent)	"
8	Weakly alkaline hypochlorite (sodium hypochlorite, 0.5 per cent (Dakin's))	"
9	Strongly alkaline hypochlorite (sodium hypochlorite, 0.5 per cent)	"

Experiments upon leukocytes, erythrocytes, and plasma clot in the various solutions employed in the five experiments described gave results practically identical with those obtained with liver emulsion. When, however, discs of blood clot were employed, solvent action could not be demonstrated except, possibly, to a slight degree in the strongly alkaline solutions. Blood clot is the most resistant of the substances studied against the solvent action of the solutions used.

DISCUSSION.

From the results recorded above, it seems justifiable to lay considerable stress on the relatively great solvent action of Dakin's hypochlorite solution as contrasted with the more recent and more stable chloramines of Dakin. It also seems probable that to its greater ability to dissolve necrotic tissue, plasma clot, and leukocytes it owes its chief claim to preference over the chloramines in the treatment of infected wounds. Curves shown by Carrel and Dehelly[11] demonstrate the relative ease with which this solution will sterilize grossly infected wounds in the initial presence of much necrotic tissue and pus.

The results of our experiments show that the solvent action of Dakin's hypochlorite solution in the degree of alkalinity used clinically is due primarily to its hypochlorite content. The slight alkalinity of Dakin's solution, while in itself without solvent action, does, however, increase the effectiveness of the hypochlorite. We are compelled to differ, therefore, from Fiessinger and his coworkers,[3] who attributed this action of the hypochlorite solutions to their alkalinity. In our weakly alkaline solutions, the solvent action of the hypochlorite solution ceased abruptly at about 0.2 per cent sodium hypochlorite concentration. This phenomenon occurs at a lower hypochlorite concentration as the reaction of the solution becomes more alkaline and *vice versa*. Even in neutral solutions, marked solvent action occurs at a hypochlorite-hypochlorous acid concentration of 0.5 per cent. A solution the alkalinity of which is equal to 0.1 N sodium hydroxide exerts a solvent action in the absence of any other factor.[4] Such a solution, however, is not available for clinical use because of its irritating properties.

Chloramine-T failed in these experiments to exhibit any solvent action not explicable as an effect of the alkalinity of the solution in which it was dissolved, and dichloramine-T was also wholly without solvent action. The results of our experimental studies do not, therefore, support the clinical observations of Dakin and his associates,[12] who assert that "the chlorin in dichloramin-T, as in the hypochlorites, has the power of dissolving dead tissues," or similar conclusions reached by Sweet,[13] who states: "The dichloramin-T also possesses to a marked degree the characteristic power of the chlorin solutions in aiding the digestion and removal of necrotic, sloughing

[11] Carrel, A., and Dehelly, G., The treatment of infected wounds, New York, 1917, 162, 170.

[12] Dakin, H. D., Lee, W. E., Sweet, J. E., Hendrik, B. M., and Le Conte, R. G., *J. Am. Med. Assn.*, 1917, lxix, 30.

[13] Sweet, J. E., *J. Am. Med. Assn.*, 1917, lxix, 1076.

tissues. The new solution seems more effective in cleaning up sloughing tissue than the older chlorin compounds." It seems probable that the greater solvent action of hypochlorite solution, as contrasted with the chloramines, is related to the greater instability of the former. We have been unable to demonstrate a solvent action on blood clot from any of the solutions of a reaction available for clinical use.

CONCLUSIONS.

1. Dakin's hypochlorite solution has the power of dissolving necrotic tissue, pus, and plasma clot in the concentration and reaction used clinically.

2. Chloramine-T and dichloramine-T do not exhibit this action.

3. The solvent action of Dakin's hypochlorite solution of the degree of alkalinity used clinically is due primarily to its hypochlorite content, but its slight alkalinity, while in itself without solvent action, enhances the effectiveness of the hypochlorite.

4. In the degree of alkalinity used clinically, the solvent action of hypochlorite is absent below about 0.2 per cent sodium hypochlorite concentration.

5. The hypochlorite concentration at which the solvent action ceases is lower the more alkaline the solution, and *vice versa*.

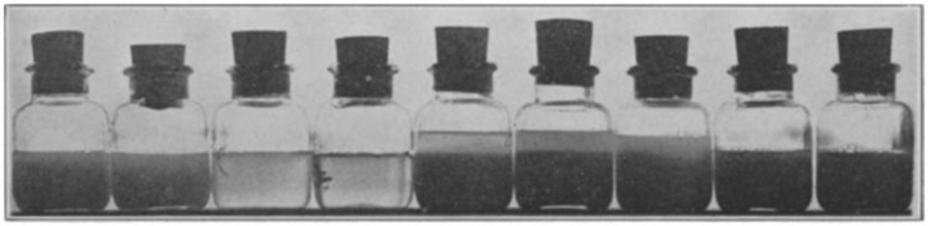
6. None of the antiseptics studied had demonstrable solvent action on blood clot.

EXPLANATION OF PLATE 5.

FIG. 1. Photograph of bottles in Experiment 1 (Table I).

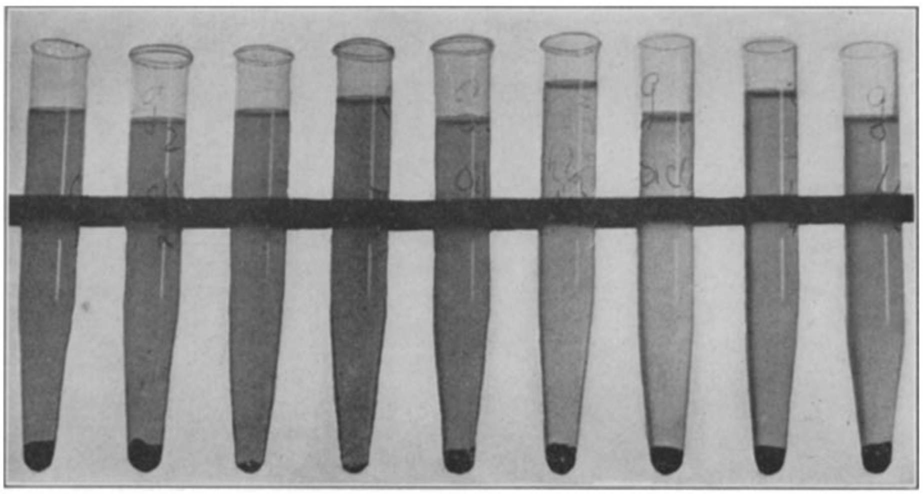
FIG. 2. Photograph of centrifuged samples from Experiment 1 (Table I).

FIG. 3. Photograph of centrifuged samples from Experiment 5 (Table V).



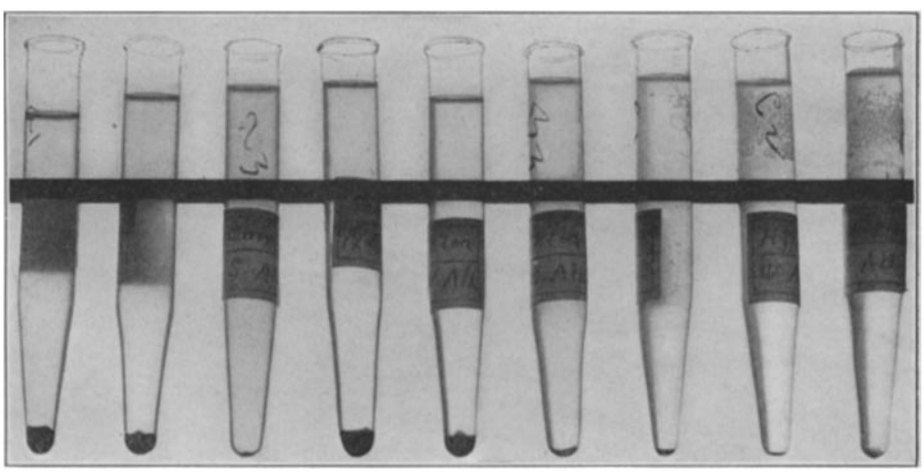
1 2 3 4 5 6 7 8 9

FIG. 1.



1 2 3 4 5 6 7 8 9

FIG. 2.



1 2 3 4 5 6 7 8 9

FIG. 3.

(Taylor and Austin: Solvent action of antiseptics.)