THE ACTION OF CHLORINATED ANTISEPTICS ON BLOOD CLOT.

By HERBERT D. TAYLOR, M.D., and MARIANNE G. STEBBINS.

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

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Experiments recently reported from this laboratory[1] demonstrated that hypochlorite solutions, in the concentration and alkalinity used clinically, exhibit a solvent action on necrotic tissue. Fiessinger and his coworkers[2] have reported a similar action of these solutions on pus cells. The importance of the latter observation is emphasized by the experiments of Rous and Jones,[3] which demonstrated that intact leucocytes may protect bacteria which they have phagocyted from the action of an antiseptic. These bacteria, when liberated after autolysis of the leucocytes, are viable and may proliferate in an apparently normal manner.

As the hypochlorite solutions are extensively used in the treatment of infected wounds, and as their cleansing properties have been emphasized, it seemed important to determine definitely whether these solutions were effective in dissolving or penetrating blood clot. The possibility that clotted blood in wounds might serve as a protective covering for virulent microorganisms, thereby preventing the bactericidal action of the antiseptic employed, seemed worthy of investigation.

As it is generally recognized, and as early experiments seemed to demonstrate conclusively, that solutions of a degree of concentration and alkalinity compatible with clinical use exhibited little solvent action on blood clot, certain modifications were later introduced in an endeavor to influence the solutions in this direction.

[1] Taylor, H. D., and Austin, J. H., *J. Exp. Med.*, 1918, xxvii, 155. Austin, J. H., and Taylor, H. D., *ibid.*, 627.

[2] Fiessinger, N., Moiroud, P., Guillaumin, C.-O., and Vienne, G., *Ann. méd.*, 1916, iii, 133.

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

Method.—Rabbit blood was allowed to clot in test-tubes, and after separation from the serum the remaining cylindrical mass was cut into a number of discs, each 1 cm. in thickness and 1.5 cm. in diameter. Each disc was then placed in 50 cc. of the solution to be tested for varying lengths of time. The appearance and size of the discs were noted before and after exposure to the action of the solution and examined carefully for any evidence of dissolution or penetration by the antiseptics. Careful controls were included in every experiment.

EXPERIMENTAL.

Experiment 1.—The first series of experiments was of a morphological nature, and an effort was made to determine by the appearance of the blood clot discs whether or not any solvent action was demonstrable. Dakin's hypochlorite (0.5 per cent sodium hypochlorite), chloramine-T (2 per cent), and dichloramine-T (5 per cent in chlorcosane) solutions apparently had no solvent, disintegrative, or penetrative action on the discs when allowed to act for periods of time varying from 15 minutes to 12 hours.

Experiment 2.—In the hope of varying the permeability of the blood clots, they were first treated with $\frac{M}{8}$ calcium chloride and $\frac{M}{8}$ sodium chloride. Loeb[4] has already shown that calcium chloride increases and sodium chloride decreases the permeability of masses of finely powdered gelatin. After varying lengths of time in the salt solutions (from 15 minutes to 2 hours), the clots were transferred to the antiseptic solutions, hypochlorite 0.5 per cent, chloramine-T 2 per cent, dichloramine-T 5 per cent in chlorcosane, and in distilled water, and allowed to remain in contact with these solutions for from 15 minutes to 12 hours. No appreciable action was noticeable except all absence of hardening of the surface layer of the clot first treated with calcium chloride and later transferred to Dakin's hypochlorite solution. It was then decided to test the penetration of the solutions in a manner allowing objective analysis, and the following experiments were performed in the hope of getting accurate and comparable figures with regard to the relative solvent powers of certain antiseptics.

Experiment 3.—10 cc. of sterile rabbit blood were placed in a sterile test-tube containing 0.5 cc. of a 24 hour bouillon culture of *Staphylococcus aureus* and shaken thoroughly against a sterile rubber stopper to insure even distribution of the bacteria through the resulting clot. After clotting and separation of the serum the cylindrical mass was cut, with precautions against further contamination, into equal sized discs, 1 cm. in thickness and 1.5 cm. in diameter. The discs were then placed in bottles containing 50 cc. of the following solutions: (1) Dakin's hypochlorite solution 0.5 per cent; (2) chloramine-T solution 2 per cent; and (3)

[4] Loeb, J., *J. Biol. Chem.*, 1917, xxxi, 343.

sterile salt solution, as control. A disc was removed from each bottle after a half hour and a second after a 1 hour interval. The discs were then washed in two changes of sterile saline solution to remove all traces of the test solution; the second saline wash contained a few drops of a $\frac{N}{10}$ sodium thiosulfate solution to neutralize any chlorine remaining. Each disc was thoroughly ground in a sterile mortar with 5 cc. of saline solution, agar tubes were inoculated with two loopfuls of the resulting emulsion, and plates poured. After 24 hours incubation at 37°C. the colonies developing in the plates were counted, and the results are summarized in Table I.

TABLE I.

Time of contact.	Dakin's solution.	Chloramine-T solution.	Salt solution control.
hrs.			
$\frac{1}{2}$	60	100	Confluent.
1	60	100	"

Experiments 4 and 5.—These experiments were similar to Experiment 3, and the results are recorded in Tables II and III.

TABLE II.

Time of contact.	Dakin's solution.	Chloramine-T solution.	Salt solution control.
hrs.			
$\frac{1}{2}$	300	312	Confluent.
1	280	Confluent.	"
2	Confluent.	300	"

TABLE III.

Time of contact.	Dakin's solution.	Chloramine-T solution.	Salt solution control.
hrs.			
1	Confluent.	Confluent.	Confluent.
2	"	"	"

Experiment 6.—Discs prepared as in Experiments 3 to 5 were placed in $\frac{M}{8}$ calcium chloride and $\frac{M}{8}$ sodium chloride for from 1 to 2 hours and later transferred to Dakin's solution to test the effect of the saline solution on the permeability of the clot. The results are given in Table IV.

TABLE IV.

Solution 1.	Time of contact.	Solution 2.	Time of contact.	No. of colonies.
	hrs.		*hrs.*	
M/8 calcium chloride.	1	Dakin's hypochlorite.	2	222
M/8 " "	1		0	Confluent.
Dakin's hypochlorite.	2		0	800
M/8 sodium chloride.	1	Dakin's hypochlorite.	2	545
M/8 " "	1		0	Confluent.

Experiment 7.—The same experiment was repeated, but in one series (A) the clot discs nearest the top of the clotted blood were used and in the other (B) the clot discs nearest the bottom were used. This was done in order to rule out, as far as possible, inaccuracies due to the uneven distribution of the bacteria in the cylindrical clot. The results of both series are given in Table V.

TABLE V.

Solution 1.	Time of contact.	Solution 2.	Time of contact.	No. of colonies.	
				A	B
	hrs.		*hrs.*		
Dakin's hypochlorite.	2			15	20
M/8 calcium chloride.	1	Dakin's hypochlorite.	2	20	40
M/8 sodium "	1	" "	2	10	40
9 per cent sodium chloride (normal).	3			10	

Experiment 8.—To insure still further even distribution of the bacteria through the blood clot the following variation was instituted. A rabbit was injected in-

TABLE VI.

Solution 1.	Time of contact.	Solution 2.	Time of contact.	No. of colonies.
	hrs.		*hrs.*	
M/8 sodium chloride.	2			Confluent.
M/8 calcium "	2			"
Dakin's 0.5 per cent hypochlorite.	2			"
M/8 sodium chloride.	1	Dakin's 0.5 per cent hypochlorite.	2	"
M/8 calcium "	1	Dakin's 0.5 per cent hypochlorite.	2	"

travenously with 15 cc. of a 24 hour bouillon culture of *Staphylococcus aureus* and 1 minute later bled from the heart. The blood was allowed to clot in the usual manner and discs were prepared as described above. The discs were placed in solutions, taken out, ground as described above, inoculated into agar tubes, and later poured in plates, as in previous experiments. The results are shown in Table VI.

Experiments 9 and 10.—The same experiment was repeated on two later occasions with the results shown in Tables VII and VIII.

TABLE VII.

Solution 1.	Time of contact.	Solution 2.	Time of contact.	No. of colonies.
	hrs.		*hrs.*	
M/8 sodium chloride.	2			50
M/8 calcium "	2			200
Dakin's 0.5 per cent hypochlorite.	2			50
M/8 sodium chloride.	1	Dakin's 0.5 per cent hypochlorite.	2	100
M/8 calcium "	1	Dakin's 0.5 per cent hypochlorite.	2	50

TABLE VIII.

Solution 1.	Time of contact.	Solution 2.	Time of contact.	No. of colonies.
	hrs.		*hrs.*	
M/8 sodium chloride.	2			Confluent.
Chloramine-T 2 per cent.	2			"
Dakin's 0.5 per cent hypochlorite.	2			"
Eusol (0.5 per cent hypochlorite).	2			"
M/8 sodium chloride.	1	Chloramine-T 2 per cent.	2	"
M/8 " "	1	Dakin's 0.5 per cent hypochlorite.	2	"
M/8 " "	1	Eusol (0.5 per cent hypochlorite).	2	"

Experiment 11.—Equal sized discs of blood clot, through which *Staphylococcus aureus* had been equally distributed by the *intra vitam*, intravenous injection of the organisms in the usual manner, were placed first in a trypsin solution and later in Dakin's solution to determine whether the trypsin, by partial disruption of the

clot, rendered it more permeable to the antiseptic solution. A 1 per cent solution of commercial trypsin and Dakin's solution with a hypochlorite concentration of 0.5 per cent were used. The results of this experiment are given in Table IX.

TABLE IX.

Solution 1.	Time of contact.	Solution 2.	Time of contact.	No. of colonies.
	hrs.		*hrs.*	
Trypsin 1 per cent.	2	Dakin's solution.	2	Confluent.
" 1 " "	24	" "	2	"
Saline solution.	2			"

Inasmuch as blood clot had been found so resistant to hypochlorite solutions which readily dissolve necrotic tissue, pus cells, and other organic matter, a series of experiments was performed to determine, if possible, the resistant constituent of the clot. The results are summarized in the description of Experiment 12.

Experiment 12.—(*a*) Plasma clot was prepared by centrifuging at high speed freshly drawn rabbit blood. The resulting supernatant, clear fluid quickly coagulated. This clot, without corpuscles, is readily dissolved by Dakin's hypochlorite solution having a sodium hypochlorite concentration of 0.5 per cent. 2 per cent chloramine-T solution is without such action.

(*b*) Red blood corpuscles, prepared by defibrinating freshly drawn rabbit blood and adding sufficient saline solution to make a 5 per cent suspension of cells, are readily dissolved by the hypochlorite solution. The chloramine-T solution merely laked the corpuscles without exhibiting solvent action.

(*c*) Pus cells, prepared from human exudate and from dogs in response to the irritant action of aleuronat, are dissolved by the hypochlorite but not by the chloramine-T solution.

(*d*) It seemed probable that the fibrin of blood clot was more resistant than the fibrin of the plasma clot because the fibrin is held together in closer mechanical mass by the blood cells than in the more loosely formed plasma.[5]

[5] Experiments were undertaken but proved inconclusive and were interrupted by Dr. Taylor's death.

SUMMARY.

This work demonstrates that the chlorinated antiseptics have no power to penetrate blood clots and destroy bacteria therein contained. Correspondingly, blood clots may protect virulent bacteria for a long period of time and the organisms properly planted will be able to proliferate in a normal manner.[6,7]

[6] It seems probable that the fibrin of the blood clot is the resistant substance as plasma and red and white cells are easily dissolved by these antiseptics.

[7] The work on this paper up to this point had been written up by Dr. Taylor before his death. No attempt has been made to discuss his clear-cut and conclusive experiments. The summary given here is taken from the quarterly report rendered to the Director of The Rockefeller Institute.