

BEHAVIOR OF HYPOCHLORITE AND OF CHLORAMINE-T  
SOLUTIONS IN CONTACT WITH NECROTIC AND  
NORMAL TISSUES IN VIVO.

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(Received for publication, February 13, 1918.)

It has been known for some time that the chlorine content and consequently the potency of hypochlorite of soda solutions diminishes rapidly when in contact with the surface of wounds. This is emphasized by Carrel and Dehelly, and for this reason they advocate a frequent renewal of the antiseptic solution to the wound.<sup>1</sup> This insures that the concentration shall be kept as constant as possible.

It would be difficult to determine the rapidity of the fall in chlorine concentration on an actual wound as encountered in the ward, and almost impossible to parallel such observations with others on an equal quantity of solution in contact with an equal area of normal skin. Inasmuch as exact determinations of the rapidity of the fall in chlorine concentration on pathological and on normal skin, under experimental conditions, might be of value to surgeons using Dakin's hypochlorite and chloramine-T solutions clinically, we chose the following method of investigation.

The left ears of three white rabbits of the same relative size and weight were exposed to the rays emitted by a Coolidge tube. The spark-gap used measured 3 inches; the milliamperage was 10; the distance from the target to the ear was 6 inches; and the time of exposure was 20 minutes.

8 weeks later the x-rayed ears each exhibited a sharply demarcated gangrenous area over which there were considerable crusting of epithelium and secretions and in the lumen there was much thick pus.

The ears of the affected rabbits were each suspended for 20 minutes in a beaker containing 400 cc. of the solution to be tested.

<sup>1</sup> Carrel, A., and Dehelly, G., *The treatment of infected wounds*, New York, 1917, 65.

*Rabbit 1.*—Right and left ears suspended in separate beakers containing Dakin's hypochlorite solution, made from bleaching powder, 10 cc. of which required 13 cc. of 0.1 N sodium thiosulfate solution for reduction (0.48 per cent sodium hypochlorite).

*Rabbit 2.*—Right and left ears suspended separately in beakers containing a solution comparable in alkalinity with properly made Dakin's hypochlorite solution—sodium carbonate 1 gm. and sodium bicarbonate 17 gm. per liter of water.

*Rabbit 3.*—Right and left ears suspended separately in chloramine-T<sup>2</sup> solution which required 12.75 cc. of sodium thiosulfate for reduction (about 2 per cent chloramine-T), and contained approximately the same proportion of available chlorine as the hypochlorite solution used on Rabbit 1.

TABLE I.

Solution.	In contact with.	Before.	Immediately after.	2 hrs. after.	17 hrs. after.
		cc.	cc.	cc.	cc.
Dakin's hypochlorite solution.....	Normal ear.	13.00	12.35	12.15	11.50
“ “ “ .....	Gangrenous ear.	13.00	11.55	10.30	8.65
“ “ “ .....	Control (no tissue).	13.00	13.00	13.05	12.60
Carbonate-bicarbonate “ .....	Normal ear.				
“ “ “ .....	Gangrenous ear.				
Chloramine-T solution.....	Normal ear.	12.75	12.75		12.75
“ “ .....	Gangrenous ear.	12.75	12.75		12.35
“ “ .....	Control (no tissue).	12.75	12.75		12.75

In the tables the figures represent the number of cubic centimeters of 0.1 N sodium thiosulfate solution required to reduce the chlorine in 10 cc. of the solution.

Table I shows the titration figures before, immediately after the 20 minutes' exposure of the normal and necrotic ears to the solutions, 2 hours after the ears had been removed from the solutions, and 17 hours after removal. The solutions were kept in covered vessels at room temperature in the interval between titrations.

The fall in chlorine concentration was more rapid in the Dakin's hypochlorite solution applied to the gangrenous ear than in that applied to the normal ear. The fall in concentration, however, was not complete immediately after the ears were removed from the solution but became more pronounced the longer the interval between

<sup>2</sup> Prepared by the Abbott Laboratories, Chicago.

the removal of the ears and the titration. The titration of the control solution, which had not been exposed to any tissue, demonstrated a fall in the titration figure from 13 to 12.60 cc., and a small proportion of the loss in chlorine of the solutions in contact with the tissues might be explained by this spontaneous deterioration of the unstable hypochlorite solutions. However, this factor is insufficient to account for the fall from 13 cc. before exposure to 8.65 cc. 17 hours later in the gangrenous ear, nor from the same figure before to 11.50 cc. at the end of the 17 hour interval in the normal ear. The fall from 13 cc. before exposure to 11.55 cc. immediately afterward is associated with the erosive action of the hypochlorite solutions, which we have measured quantitatively in a former investigation,<sup>3</sup> but the cause of the further fall to 8.65 cc. is not immediately clear. The fluid in contact with the gangrenous ear was cloudy immediately after the removal of the ear. This cloudiness of the fluid was not so marked at the end of the 2 hour interval when the second titration was made, and the fluid was almost as clear as the control at the end of the 17 hour interval when the last titration gave the lowest chlorine concentration recorded. Close inspection of the fluid immediately after the removal of the ear revealed the presence of small particles of necrotic tissue, flecks of pus, etc., in suspension. These became less noticeable the longer the antiseptic solution was allowed to act. The fall in chlorine concentration exhibited immediately after the removal of the ears was due to the erosive effect of the solution on the necrotic tissue, and to its combination with the products of the tissues *in situ*. However, during this action, appreciable particles of necrotic tissue, agglomerations of pus cells, and little gummy concretions made up of dried serum, epithelial cells, etc., were separated from the necrotic ear and it is the subsequent reaction of the hypochlorite with these which caused the continued fall in the chlorine titer. That the chlorine is directly concerned in this solvent action seems assured from former experiments reported.<sup>3</sup> In the course of the reaction the chlorine probably goes into such stable union with the protein substances that it is not available to the sodium thiosul-

<sup>3</sup> Taylor, H. D., and Austin, J. H., The solvent action of antiseptics on necrotic tissue, *J. Exp. Med.*, 1918, xxvii, 155.

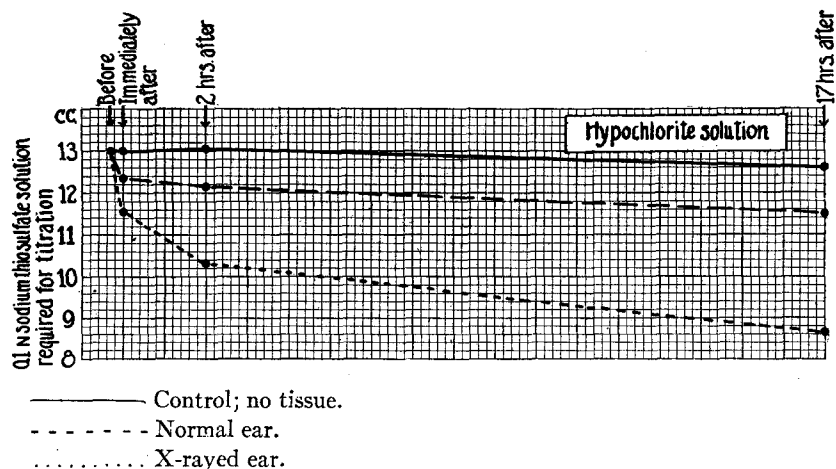
fate solution, and thus the fall in titer measures the exact quantity of chlorine used up in the reaction with the wound products.

The fall in chlorine concentration of the hypochlorite solution in contact with the normal ear, much less pronounced than with the x-rayed ear, was probably due to the erosive action of the solution on the hair and superficial epithelium of the normal ear and the slow digestion of the particles removed caused the slow fall noted over the interval of 17 hours. As there was much less tissue capable of reacting with the solution in the normal than in the gangrenous ear, the titration figures were higher at all observations in the solution exposed to the former. Close observation revealed the erosive effect on the ear itself.

The fall in chlorine concentration noted in the chloramine-T solutions was much less than that observed in the hypochlorite solutions. This corresponds with the greater stability of the former and with their lack of erosive effect on necrotic tissue.<sup>3</sup> It is interesting to note that there was no fall in chlorine concentration in the chloramine-T solution applied to the normal ear, and correspondingly no erosive action on the hair or superficial epithelium was demonstrable. The solution was likewise clear when removed from the ear and throughout the period of observation, in contrast to the hypochlorite solution which was at first cloudy and only late in the experiment became relatively clear. The titration figures after 17 hours were approximately the same as those made immediately after the removal of the ears from the solution. Text-fig. 1 shows graphically the fall in chlorine concentration in the hypochlorite solution applied to the gangrenous ear, in that applied to the normal ear, and in the control solution which was not allowed to act on any tissue. Text-fig. 2 gives comparable curves for the chloramine-T solutions.

A weaker hypochlorite solution, titrating 9 cc. of sodium thiosulfate (0.1 N), was applied to the gangrenous ear and to the normal ear of Rabbit 1, and titration figures before, immediately after removal of the ears from the solution, 2 hours afterward, and 17 hours afterward were compared with a control solution which was never in contact with tissue. These results, shown in Table II and Text-fig. 3, confirm those shown in Table I and Text-fig. 1 for Rabbit 1.

The ears of each rabbit were then suspended in solutions of the same types and concentrations as those shown in Table I for 7 consecutive days, the period of exposure on each day being 20 minutes. At the end of this time it was seen that the gangrenous ear suspended in Dakin's hypochlorite solution had cleared up proportionately more



TEXT-FIG. 1. The fall in chlorine concentration in the hypochlorite solution applied to the gangrenous ear, in that applied to the normal ear, and in the control solution which was not allowed to act on any tissue.

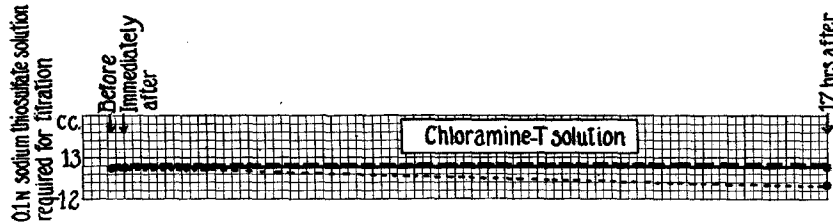
TABLE II.

Solution.	In contact with.	Before.	Immediately after.	17 hrs. after.
		cc.	cc.	cc.
Dakin's hypochlorite solution.....	Normal ear.	9.0	8.5	8.25
" " " .....	Gangrenous ear.	9.0	7.0	5.5
" " " .....	Control (no tissue).	9.0	9.0	8.9

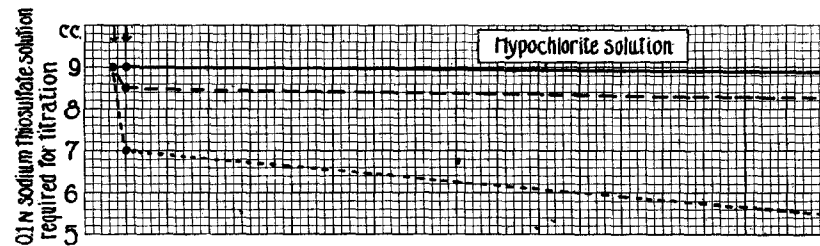
than the gangrenous ears of either of the other rabbits. The scabs were more eroded and the pus present was less in amount than in either of the others. The gangrenous area looked much cleaner. The chloramine-T gangrenous ear, which was not so severe an x-ray burn in the beginning, had not improved appreciably. The gangrenous ear

treated with the control alkaline solution had not changed in appearance.

The normal ears of the chloramine-T and of the alkaline control rabbits were just as they had been before treatment with these substances. The normal ear treated with the hypochlorite solution was



TEXT-FIG. 2.



TEXT-FIG. 3.

TEXT-FIG. 2. The fall in chlorine concentration in the chloramine-T solution applied to the gangrenous ear, in that applied to the normal ear, and in the control solution which was not allowed to act on any tissue.

TEXT-FIG. 3. The fall in chlorine concentration of the second hypochlorite solution, titrating 9 cc. of sodium thiosulfate (0.1 N), applied to the gangrenous ear, in that applied to the normal ear, and in the control which was not allowed to act on any tissue.

intensely inflamed. It was twice as thick as it had been before it was treated, due to intense edema. Congestion was marked and the surface temperature was higher than normal. There was superficial ulceration in places and petechiæ were scattered through the subcutaneous tissues.

## CONCLUSIONS.

1. The fall in chlorine concentration of Dakin's hypochlorite solution is more rapid in contact with necrotic than in contact with normal tissue.
2. The fall in chlorine concentration of chloramine-T solution is very slight when applied to necrotic tissue and is negligible when applied to normal tissue.
3. The action of the hypochlorite solution on tissue results in the separation of particles of necrotic tissue, hair, epithelial scales, coagulated serum, etc., and a gradual digestion of these substances, taking place over a period of at least 17 hours.
4. The fall in the chlorine concentration of the hypochlorite solution is not complete until the particles are completely dissolved.
5. Chloramine-T solution, 2 per cent, has no erosive effect comparable with that exhibited by the hypochlorite solution.
6. Repeated exposures to the three solutions show the hypochlorite solution to be superior in its cleansing ability on necrotic tissue.
7. The hypochlorite solution is much more irritating to normal rabbit skin than chloramine-T solution or the alkaline control solution.
8. Therefore, the irritating effects must be due to the readily available chlorine.