ON THE USE OF CERTAIN ANTISEPTIC SUBSTANCES IN THE TREATMENT OF INFECTED WOUNDS.*

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IN order to make a judicious choice of the antiseptic most likely to give useful results in the treatment of infected wounds many different factors have to be considered in addition to germicidal activity, including the irritating properties of the substances, their toxicity, solubility, ability to penetrate tissues and to be absorbed, and their chemical reactions with proteins and other tissue constituents.

The killing of bacteria by ordinary antiseptic substances is essentially a chemical reaction between the antiseptic on the one hand and the proteins and other cell constituents of the micro-organism on the other. The destruction by antiseptics of bacteria suspended in water is easily effected, because no proteins are present in the mixture other than those derived from the micro-organism. The destruction by antiseptics of bacteria mixed with blood serum, pus, and other exudate is much more difficult because the antiseptic acts not only on the micro-organisms but on other protein substances as well. Therefore, in judging of the antiseptic action of a substance suitable for the treatment of wounds, it is essential that its germicidal action be tested against micro-organisms mixed with blood serum or similar substances, and not simply tested against bacteria suspended in water.

The germicidal activity of all known antiseptics is greatly reduced by the presence of blood serum or similar substances, and in some cases this reduction is so great that the compound loses all practical antiseptic value.

The following table contains results which illustrate this enormous reduction in germicidal action by blood serum in the case of several common antiseptics. I am greatly indebted to my colleague, Dr. Maurice Daufresne, for all the bacteriological results referred to in this communication.

Antiseptic.	Without Blood Serum.	With Blood Serum.
Phenol	1 : 250– 1 : 500+	1 : 50– 1 : 100+
Salicylic acid	1 : 2,500– 1 : 5,000+	1 : 100– 1 : 250+
Hydrogen peroxide ...	1 : 3,500– 1 : 8,000+	1 : 1,700– 1 : 2,000+
Iodine	1 : 100,000– 1 : 1,000,000+	1 : 1,000– 1 : 2,500+
Mercuric chloride ...	1 : 5,000,000– 1 : 10,000,000+	1 : 25,000– 1 : 50,000+
Silver nitrate	1 : 1,000,000– 1 : 10,000,000+	1 : 10,000– 1 : 25,000+
Sodium hypochlorite...	1 : 500,000– 1 : 1,000,000+	1 : 1,500– 1 : 2,000+
Benzene sodium sulphochloramide	1 : 500,000– 1 : 1,000,000+	1 : 1,000– 1 : 2,000+
Paratoluene sodium sulphochloramide	1 : 750,000– 1 : 1,500,000+	1 : 2,000– 1 : 3,000+
Acetylchloramino-dichlorbenzene	1 : 500,000– 1 : 1,000,000+	1 : 2,500– 1 : 5,000+

The figures indicate the concentration of antiseptic necessary to sterilize one drop of a fresh culture of *Staphylococcus aureus* in a total volume of 5 c.cm. acting for two hours. + indicates growth; – indicates complete sterilization.

But in choosing a suitable antiseptic many other factors than germicidal action need to be considered. Mercuric chloride, which among the substances referred to in the table shows the highest germicidal action, is probably the least useful and most objectionable as an antiseptic for the treatment of infected wounds. It may be of use to consider some of the limitations of the commonly used substances referred to in the above table.

*The work described in this communication was carried out in laboratories at Compiègne supported by the Rockefeller Institute for Medical Research attached to Hospital 21 of the French army. For cordial co-operation in the preparation of a large number of chloramines and other substances, upon which a detailed report will be published later, I am indebted to my former teacher, Professor J. B. Cohen, F.R.S., of the University of Leeds, and to Dr. J. Kenyon, who was appointed by the British Medical Research Committee.

Phenol is characterized by very low germicidal power, especially when acting in the presence of serum. When used in sufficiently high concentration for germicidal efficiency it is decidedly destructive of healthy tissue.

Hydrogen peroxide gives encouraging results when tested against bacteria in the test tube, but when used on wounds the substance has little germicidal action, for it is decomposed with the greatest ease by the enzyme catalase present in all tissues and in the blood cells. Hence its action can only be exerted during a trifling interval of time. The mechanical detergent action connected with the rapid disengagement of oxygen gas on infected surfaces is probably of greater value than any antiseptic action exerted by the hydrogen peroxide.

An interesting experiment related to me by Professor E. K. Dunham may be quoted here. A rabbit which had received an intravenous injection of the Welch bacillus (*B. aërogenes capsulatus* or *B. perfringens*) was killed, and the infected liver was removed and carefully sectioned. It was found that cubes of the infected liver only 1 mm. in size could be immersed in and incubated with hydrogen peroxide of moderate concentration without destruction of the micro-organisms.

Hydrogen peroxide, as regards its antiseptic action, must be regarded as of slight value, even against anaërobic organisms.

Mercuric chloride readily loses most of its antiseptic action in presence of many tissue constituents, and, as is well known, is irritating even in dilute solution. It is useless for the sterilization of pus when employed at any reasonable concentration.

Silver nitrate is of greater value than mercuric chloride, but when used in sufficiently high concentration is irritating. Many tissue constituents inhibit its action markedly. The photo-sensitiveness of the silver compounds formed is objectionable.

Iodine, which has proved so valuable for the disinfection of skin, has given much less satisfactory results when used for deep wounds owing to protein coagulation and irritation of the tissues. The penetrating power of iodine is slight, and wounds which have been freely treated with it are apt to cicatrize more slowly than others.

Sodium hypochlorite has high germicidal action, and has many other desirable properties. But sodium hypochlorite as ordinarily prepared is of extremely variable composition, contains free alkali and sometimes free chlorine, and is consequently irritating when applied to wounds. By a simple process, which will now be described, it has been possible to render the hypochlorites much less irritating while retaining their antiseptic action unchanged.

PRINCIPLES INVOLVED IN THE PREPARATION OF THE HYPOCHLORITE SOLUTION.

Solutions of sodium hypochlorite always contain free alkali even when prepared with the greatest care. A so-called "neutral" solution of sodium hypochlorite has an alkaline reaction. This is due not only to free alkali which may remain from the process of preparation, but also to the fact that the hypochlorite in solution undergoes hydrolytic dissociation giving free sodium hydroxide and hypochlorous acid.

$$NaClO + H_2O = NaOH + HClO$$

The extent of this dissociation has been measured by Duyk, and quantitatively it is very considerable. The irritating action of ordinary hypochlorites is largely due to this formation of free alkali. The extent of this hydrolytic dissociation increases with dilution, so that practically hypochlorites cannot be effectively rendered non-irritating by simply reducing the concentration, for a point is soon reached at which germicidal action is impaired while the irritating properties of the solution persist. In addition to the above sources of free alkali, it must not be forgotten that alkali may be liberated by the action of sodium hypochlorite on proteins, a reaction in which the chlorine of the hypochlorite is attached to nitrogen in the proteins, as will be shown later.

Now it is well known that certain fluids, such as blood and some other body fluids, also certain artificial salt solutions containing mixture of salts of polybasic acids—for example, phosphoric acid—are able to retain their essential neutrality even after the addition of limited quantities of acid or alkali. This is due to the fact that the addition of acid or alkali simply changes the relative proportion of two or more salts of the polybasic acid present in the solution.

Starting with this idea, and employing the feeble polybasic acid, boric acid, it has been possible to prepare a simple hypochlorite mixture which maintains approximate neutrality under all conditions, is practically non-irritating, and which, when properly applied, has given most encouraging results in the antiseptic treatment of wounds. It must be understood that the insignificant antiseptic action of boric acid has nothing to do with the employment of this acid; nor is the boric acid employed for the purpose of liberating hypochlorous acid, as in Lumière's or Lorrain Smith's preparations.

The principle of the preparation is as follows: Chloride of lime (bleaching powder) is decomposed with a solution of sodium carbonate and the filtered solution containing sodium hypochlorite together with a slight excess of alkali is mixed with boric acid in such quantity that the solution is acid to phenolphthalein suspended in water but still alkaline to litmus. The resultant solution contains a balanced mixture of hypochlorite and polyborates of sodium with small amounts of free hypochlorous and boric acids. Thus the irritating action of free caustic alkali is avoided, for even if momentarily formed it would be at once neutralized by the boric acid or acid borates present in the solution.

Preparation of Solutions.

The preparation of a solution of suitable concentration for direct application, containing 0.5 to 0.6 per cent. of sodium hypochlorite, may be carried out very simply as follows:

One hundred and forty grams of *dry* sodium carbonate (Na_2CO_3), or 400 grams of the crystallized salt (washing soda), is dissolved in 10 litres of tap water, and 200 grams of chloride of lime (chlorinated lime) of good quality is added. The mixture is well shaken, and, after half an hour, the clear liquid is siphoned off from the precipitate of calcium carbonate and filtered through a plug of cotton; 40 grams of boric acid are added to the clear filtrate, and the resulting solution is ready for use. A slight additional precipitate of calcium salts may slowly occur, but it is of no significance. The solution should not be kept longer than one week. *The boric acid must not be added to the mixture before filtering, but afterwards.*

A stronger solution may be prepared by decomposing chloride of lime with sodium carbonate in the proportion of 150 grams of the former to 105 grams of the latter dissolved in a litre of water. The mixture is filtered and a measured portion of it (20 c.cm.) is rapidly titrated with a boric acid solution of known strength (31 grams per litre), using phenolphthalein suspended in water as indicator, in order to determine the amount of solid boric acid to be added to the rest of the filtrate. An excess of boric acid should be avoided, so that it is best to add slightly less than the calculated amount. An ordinary alcoholic solution of phenolphthalein cannot be used as indicator, as the alcohol is at once attacked.

The concentrated solution thus prepared contains about 4 per cent. of sodium hypochlorite, and should be mixed with six parts of water before use. It can be kept for a month without serious decomposition. Such a solution is now prepared by Poulenc Frères, 122, Boulevard St. Germain, Paris, but it can easily be made at a negligible cost by any competent chemist, and I hope that it may be so made generally.

APPLICATION AND RESULTS.

To obtain the best results it is essential to commence the antiseptic treatment of the wound at the earliest moment possible, and to bring fresh quantities of the antiseptic solution in contact with all parts of the wound as frequently as possible for a considerable period of time. This is naturally a difficult problem, requiring different methods for various types of wound. The methods of applying the solution which have been found useful at Compiègne will be described by Dr. Carrel. But to give some idea of the quantities of solution employed it may be mentioned that 5 to 10 c.cm. may be introduced every two hours by means of rubber tubes into small wounds, using a pipette or syringe, while for the irrigation of such wounds as fractured femurs, accompanied by much destruction of tissue, as much as 1, or even 2, litres a day may be employed. The dilute solution, prepared as described, may be used in large quantities for the continued irrigation or instillation of wounds for more than a week without producing visible irritation. It is extremely rare for slight irritation of the skin to occur, and this may be guarded against by the application of vaseline to the skin adjacent to the wound. As a wet dressing the solution may be used almost indefinitely. A few comparative tests on similar surface wounds do not indicate that cicatrization is delayed, even by its continued use.

The solution has the valuable property of assisting in the rapid dissolution of necrosed tissue, this being doubtless due to the ability of hypochlorites to attack the (NH) groups present in proteins with formation of soluble products. It has a certain haemostatic action as well but is actively haemolytic, and should not be injected intravenously.

It is difficult in a printed communication to produce simple convincing evidence of the usefulness of an antiseptic. Records of a few individual cases treated with brilliant results are, of course, of no great value, for many infected wounds do well with a minimum amount of treatment, but the clinical results obtained during six months' use of the solution by a number of observers in different hospitals warrant the belief that the solution is of genuine value. By far the most striking results are seen in ambulances, where treatment can be commenced a few hours after the wound has been received. Among these cases the proportion of cases which at no time show a significant rise in temperature and in which healing without suppuration occurs is very large. In many cases it has been possible to make comparative tests, with and without antiseptic, on similar wounds with striking results. Records obtained by means of serial coloured photographs of the gradual changes in wounds of the most varied kind under different conditions show definite differences in favour of the solution, and in no case has any objectionable after-effect been traced to the action of the antiseptic. It should be stated that most of the cases treated with the antiseptic were kept under observation for several weeks until discharged as convalescent. This is, of course, important for judging of the ultimate value of the treatment.

An idea of the antiseptic properties of the solution may be gathered from the following figures: Staphylococci suspended in water are killed in two hours at a concentration of hypochlorite between 1 : 500,000 and 1 : 1,000,000, while in the presence of serum the necessary concentration is between 1 : 1,500 and 1 : 2,000. Streptococci are more readily killed, while *pyocyaneus* suspended in water is killed in two hours at a concentration between 1 : 100,000 and 1 : 1,000,000, while in serum between 1 : 2,500 and 1 : 5,000 is necessary.

Hypochlorites are extremely active substances chemically, and they should not be used in conjunction with other antiseptics nor with alcohol or ether. Wounds which have been previously treated with much iodine may take on a dark colour, due to the re-liberation of iodine, but this is of no importance.

Many other preparations of hypochlorites have been employed at various times by different workers. The more commonly recommended preparations are the ordinary alkaline solutions of the hypochlorites of sodium, potassium (eau de Javelle), or calcium; while mixtures of powdered chloride of lime with boric acid have been employed by Vincent, Lumière, and by Lorrain Smith and others. It is believed that the solution previously described, when properly applied to all parts of the wound, gives better results than can possibly be obtained from powdered preparations of partially soluble materials. The local production of hypochlorites, hypochlorous acid, or chlorine in high concentration, such as results from the use of the powdered mixture, is much more dangerous for healthy tissue than is the continued application of a weak neutral solution of sodium hypochlorite. Generally speaking, our experiments with powdered substances have given much less good clinical results than have aqueous solutions. It is true, however, that aqueous solutions need more care for their successful application, for it is essential that they reach every part of the infected area, and that the antiseptic should be renewed from time to time.

MODE OF ACTION OF HYPOCHLORITES.

When a solution of a hypochlorite or of free hypochlorous acid acts upon organic substances containing the

=NH group the first reaction almost always consists in the replacement of hydrogen by chlorine with formation of substances of the group known as chloramines. All protein substances contain an abundance of these groups, and they readily react with hypochlorites:

$$\begin{matrix} R^1 \\ R^2 \end{matrix}\!\!>NH + HClO = \begin{matrix} R^1 \\ R_2 \end{matrix}\!\!>NCl + H_2O$$

The antiseptic action of hypochlorites doubtless depends upon reactions of this type. It was therefore interesting to examine many different varieties of the large group of chloramines in order to study their antiseptic actions. In this work I have enjoyed the co-operation of Professor J. B. Cohen of the University of Leeds.

In the first place, it may be stated that all substances containing the =NCl group were found to be strongly antiseptic, and some of them will probably be found to have practical value. Proteins, such as blood serum, egg white, casein, etc., when treated with hypochlorites, give products of high antiseptic value, and undoubtedly compounds of this type are formed *in situ* when wounds are treated with hypochlorites. This is doubtless an advantage, as in this way a certain antiseptic action may be expected to persist even after the free hypochlorite has disappeared.

Substances such as acetanilide when treated with hypochlorous acid under appropriate conditions, carefully studied by Chattaway, give chloramines—for example, acetylchloraminodichlorbenzene — which are sparingly soluble in water, but which may be dissolved in vaseline or lanoline. Although the germicidal power of these compounds is very high indeed, the action on infected wounds of strong solutions of them in vaseline or lanoline was not markedly superior to that of plain vaseline. It appears that, generally speaking, active germicidal action can hardly be hoped for from sparingly soluble antiseptics mixed with fatty substances. Anaërobic organisms can readily grow under the fatty film covering the surface of the infected area.

On the other hand, certain aromatic chloramines which form soluble sodium salts have given most encouraging clinical results. The best of these compounds are the benzene or paratoluene sodium sulphochloramides, both of which have been described by Chattaway.

Benzene sodium sulphochloramide. Paratoluene sodium sulphochloramide.

These substances are extremely powerful antiseptics, are practically non-irritating, and can be used in much higher concentration than can the hypochlorites. A 2 to 4 per cent. solution may be conveniently employed. In general, the action of these substances is similar to that of the hypochlorites, but more powerfully antiseptic. They have, however, no special solvent action on necrosed tissue, this being doubtless due to the fact that the active chlorine in these compounds is already attached to nitrogen. While the number of cases thus far treated with these antiseptics is smaller than those treated with the hypochlorite mixture, excellent results have been obtained in a number of badly infected wounds, notably compound fractures of the femur. It appears probable that these chloramides, which are relatively easily prepared at low cost, and which have the advantage of being stable solids, may be found useful for other purposes than for the treatment of infected wounds. Their possible applications will be the subject of further study.

Benzene sodium sulphochloramide kills staphylococci suspended in water in two hours at a concentration of 1 : 500,000, and the toluene derivative kills at 1 : 1,000,000. In the presence of serum the necessary concentrations are about 1 : 1,500 and 1 : 2,500 respectively. *Bacillus pyocyaneus*, *B. typhosus*, and *B. coli* are slightly more resistant than staphylococci, while *B. aërogenes capsulatus* and streptococci are more readily killed. The concentrations refer to the weight of the crystallized salts. It will be seen that the molecular concentration of toluene sodium sulphochloramide necessary to kill staphylococci in the presence of serum is only about one-fifth of the correspondingly active molecular concentration of sodium hypochlorite.

ON THE USE OF A SLEEVE OF VEIN IN NERVE SUTURE.

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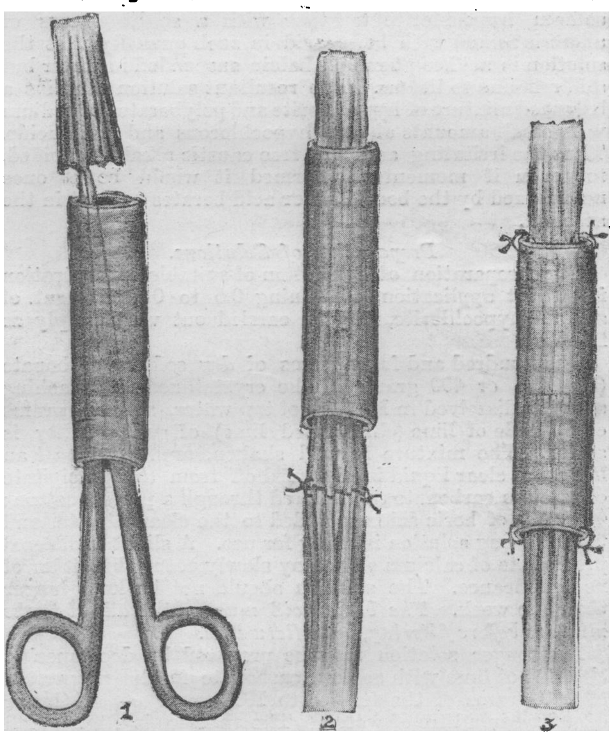
During the present war many cases of nerve injury have been recorded. The injuries are produced for the most part by rifle bullets, fragments of shell, and shrapnel. Primary suture is frequently out of the question, and the wounds are allowed to heal without any attempt being made to suture the divided nerves. Later, secondary suture is required, and often the divided ends have to be



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sought for in a large amount of scar tissue. It is essential in cases of this sort to protect the junction so as to avoid ingrowing of scar tissue between the nerve ends, and consequent failure of the operation. To prevent this various substances have been used, including decalcified bone tubes, gelatine tubes, animal's artery, paraffin wax, Cargile membrane,* and human vein. Sherren (*Injuries of Nerves*) prefers chromicized Cargile membrane.

For some years I have been using portions of vein in the manner here illustrated. The most suitable vein for nerves of the upper extremity—as, for instance, the musculo-spiral, the median, and the ulnar—is the basilic vein at a spot between its commencement and the point at which it pierces the deep fascia of the upper arm. A segment of the vein about 1½ in. or 2 in. in length is excised and threaded on a sinus forceps as in Fig. 1. One end of the nerve is then caught by the forceps, and the sleeve pulled over as in Fig. 2. The ends of the nerve are then freshened with a sharp scalpel and sutured with fine catgut. When the suture is complete the sleeve is pulled over the junction, as in Fig. 3, and fastened to the nerve sheath by a few points of suture. The vein thus applied is intended to form an aseptic sheath for the nerve, to keep the ends in secure apposition, to direct the growth of the new axis cylinders, and to prevent the ingrowth of scar tissue from the outside. Any vein of suitable size will, of course, do, and in the lower extremity a portion of the internal or external saphenous will probably be the most convenient.

The sleeve must be pulled over the first nerve end before trimming so as to avoid damage to the freshly cut end.

Possibly this method has been in use by others, but I have not seen it used or described up to the present.

* Peritoneum of the ox.