Section of Pathology

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DISCUSSION ON THE EFFECT OF ANTISEPTICS ON WOUNDS

Professor Alexander Fleming : Almost all chemicals, even sodium chloride, are antiseptic if used in sufficient concentration, but it is only those which have an especial power of inhibiting bacterial growth which are classed as antiseptics. There is a great variety of chemicals which fall into this category, and which have been, or are, used in surgery as antiseptics.

Seeing that the definition of an antiseptic is a chemical which will inhibit the growth of bacteria, it is remarkable that after the best part of 100 years of the study of antiseptics the tests which are commonly quoted by the manufacturers of these chemicals relate only to their power to *kill* bacteria, not to inhibit them. This power of killing bacteria in the conditions of these tests has little or no relation to the usefulness of antiseptics in a wound, septic or otherwise.

It is possible that it is because of these tests that in the consideration of local antiseptics, as well as in the study of chemotherapeutic drugs, far too much attention has been paid to the bactericidal power of the chemical, and far too little to the bacteriostatic power. It would be better if, in a consideration of chemicals for use in septic wounds, the bactericidal power were neglected, and the bacteriostatic power were accepted as a criterion. The literature on the sulphonamide drugs is a good example of how the wells of knowledge may be muddied by attempts to show that the action of these drugs was bactericidal, when their beneficial action could easily be accounted for by a consideration of the bacteriostatic power. The natural defensive mechanism of the human body against the common infections is very considerable, and is the most important antiseptic in contaminated wounds. If the scales are weighted against it by inefficient surgery then it appears to be a poor thing. But it is quite easy to weight the scales against chemical antiseptics, and to make it appear that the natural defences are far more potent than are the chemicals commonly used as antiseptics. The following experiment (Fleming, 1928) illustrates this. An ordinary nutrient agar culture plate was taken, and a series of holes were punched in it with a cork borer. Into each of these were placed discs of filter paper which had been soaked in the following substances : (1) Iodine, 1 : 100. (2) Mercurochrome, 1 : 100. (3) Mercuric chloride, 1 : 1,000. (4) Carbolic acid, 1 : 20. (5) Acriflavine, 1 : 1,000. (6) Gentian violet, 1 : 100. (7) Nasal mucus. (8) Tears.

The holes were then filled up with agar, and when that had set the whole surface of the culture plate was thickly planted with a strongly growing coccus, *M. lysodeikticus*, which is specially sensitive to the lysozyme of human secretions. After incubation it was found that there was a large zone of inhibition around the tears and nasal mucus, while over all the chemical antiseptics there was a copious growth of bacteria.

In the conditions of this experiment, therefore, the natural antiseptics, nasal mucus and tears, appeared to be much more potent than carbolic acid, mercuric chloride, mercurochrome, iodine, acriflavine, and gentian violet.

It is essential to scrutinize carefully the conditions of any test of the power of antiseptics for use in the human body.

Antiseptic Action

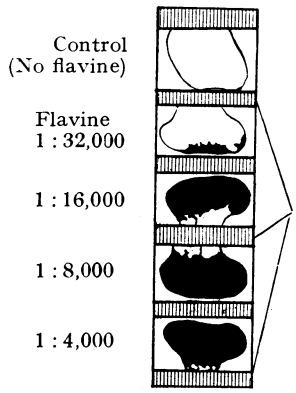
Specificity.—Some chemicals such as carbolic acid act more or less equally on all bacteria, while others, such as gentian violet and some others of the antiseptic dyes, have a specific action on some species only. It seems obvious that, other things being equal, a non-specific chemical which acted on all bacteria would be the most

valuable in a septic or contaminated wound, although it would be quite reasonable to use, if such were available, a chemical which had a powerful specific action on the streptococcus or the gas gangrene bacilli, as these organisms are a special danger in the contaminated war wound.

Quenching by albuminous or cellular media.—Some chemicals have a potent antiseptic action in a non-albuminous medium, such as nutrient broth, but when acting in serum are much less powerful, and still less so in blood or pus. A very good example is mercuric chloride, which inhibits staphylococcus as follows: In broth 1 : 500,000; in serum 1 : 30,000; in blood 1 : 2,000.

Some, such as carbolic acid, are of approximately equal potency in broth, serum, or blood.

Acriflavine has at least as powerful an antibacterial action in serum as in a non-albuminous fluid. Its potency is, however, decreased in a cellular fluid, such as pus or blood.



Imperfectly vaselined paper strips which are wetted by the blood-acriflavine mixtures. Acriflavine absorbed by paper, allowing *B. coli* to grow and hæmolyse blood. Black areas represent unhæmolysed blood. Clear areas represent blood hæmolysed by growth of *B. coli*.

FIG. 1.—Affinity of flavine for paper and for bacteria. Hæmolytic *B. coli* in human blood.

In the last war possibly the most popular antiseptic for use in septic wounds was Dakin's solution (sodium hypochlorite), especially when it was used by Carrel's method of instillation into the wound through a manifold of tubes once every two hours. I have made observations (1919) as to how long Dakin's fluid maintains its antiseptic power in a septic wound. A cup-shaped wound was chosen into which a measured volume of fluid could be introduced and removed. When Dakin's fluid was introduced and removed after ten minutes, it was found on testing the available chlorine that this had been reduced to a point below that which was antiseptic in serum. If the fluid was kept agitated during its sojourn in the wound the strength was reduced below the antiseptic level in five minutes.

Therefore, in Carrel's method, which was generally regarded as the most successful " antiseptic " treatment in the Great War, there was an antiseptic in the wound for, at the most, ten minutes out of every two hours.

In connexion with the antiseptic dyes, also, the affinity of these for the tissues and dressings has to be considered. I have, over twenty years ago, published some experiments on this point. If a piece of gauze is saturated with flavine, 1 : 1,000, and the fluid is then squeezed out, the concentration of flavine in the extruded fluid is about 1 : 5,000, showing that about 80% of the flavine has been immediately absorbed by the gauze. A very simple experiment shows the great affinity of antiseptic dyes, such as gentian violet or acriflavine, for cotton-wool. A test tube is half filled with a solution of one of these dyes, and then a plug of absorbent cotton-wool is slowly pushed down into the fluid so that the fluid has to percolate through the plug. The whole of the dye is retained on the cotton-wool, and only water passes through. It might be said, however, that this has really no bearing on the relative

affinity of the dye for the dressing and for bacteria. I have, however, an experiment which has a direct bearing on this subject.

Blood suitably infected with a hæmolytic *B. coli* was mixed with various dilutions of acriflavine, and placed in slide cells in which the paper partitions had been insufficiently vaselined, so that portions were wetted with the blood-acriflavine mixtures. The results are shown in fig. 1, and it will be seen that where the blood wetted the paper, enough of the antiseptic had been removed by the paper to allow the bacteria to grow out and produce hæmolysis in concentrations of acriflavine which would otherwise have produced complete bacteriostasis.

Similar results were obtained with gentian violet.

These experiments show that paper has such an affinity for these antiseptic dyes that it will successfully compete with bacteria for the dyestuff, and render it inactive as an antibacterial agent. This affinity of dyes for dressings and other things must be taken into account in the consideration of the use of these antiseptic dyes.

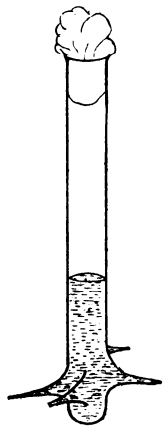


FIG. 2.—"Artificial Wound."

Use of Antiseptics in Wounds

(1) *Is it justifiable for the surgical pessimist who distrusts his own cleanliness to use antiseptics in an attempt to cover up his own deficiencies?*

The only reasonable answer to that is that the surgeon should so improve his methods that he would cease to be a pessimist. All the antiseptics in common use are toxic in some degree to the human organism as well as to bacteria, and are to be avoided wherever possible.

(2) *Is it possible, by the use of an antiseptic, to destroy an infection in a freshly inflicted wound before the bacteria have had time to grow out?*

In the last war at a base hospital in France I examined series of wounds which had been specially treated with carbolic acid, and compared them with others which had not been so treated. So far as the bacterial content went the carbolic-treated wounds were worse than the others.

Later in the war, a series of observations was made in which wounds were surgically cleansed, treated with various chemicals, and then primary suture was performed. It was found that the results were essentially the same whether the usual chemical antiseptics were used or not. They did no good, and apparently little harm.

I made some *in vitro* experiments which bear on the question whether it is easy to *sterilize* a contaminated wound with a chemical antiseptic. I made an "artificial wound" by drawing out a few processes from a glass test tube, so that the tube imitated the cavity of a war wound, except that the processes were less complicated and, of course, the walls could not be invaded by the bacteria (*see* fig. 2).

Serum containing a variety of bacteria from septic wounds was incubated in the tube, and after twenty-four hours it was dressed by inverting it to allow the serum

to run out, and then filling it with the antiseptic. On the first day the antiseptic was allowed to act for twenty minutes, and then it was emptied out, fresh serum replaced, and the tube was incubated for twenty-four hours. The flora was unaltered. On the next day one hour's application of the antiseptic was allowed, but there was no sign of sterilization of the tube. Later the antiseptic was allowed to act for twenty-four hours, but on emptying out the fluid and replacing it with serum there was still a copious growth. This result was obtained with all the antiseptics tested, viz. phenol, eusol, Dakin's solution, brilliant green, and acriflavine.

An experiment of this kind makes it seem unlikely that it would be possible to *sterilize* an infected wound by means of a chemical antiseptic (other than a clean cut, which in war is a rarity). I might here quote from an article which I wrote in the *Journal of Surgery* in 1919 :—

" During last summer I examined the packs removed from 75 cases of fractured femur on arrival of the patients at the base. These packs were mostly soaked in flavine, but there were also a certain number of B.I.P.P. or plain gauze pads. In all cases I found microbes present, and there seemed to be little difference between the bacterial content of the different varieties of pack."

(3) *Is it possible, by the use of antiseptics, to combat the infection in a septic wound in which the bacteria have grown out and invaded the tissues which have, in their turn, reacted to the infection ?*

Such a wound is full of pus, and the walls are infected well below the surface. Pus cells are polynuclear leucocytes, and they have a considerable antibacterial power. This can be shown in a very simple experiment. A drop of pus from a septic wound is placed on an agar plate, and a coverslip is gently placed on it. After incubation no growth of bacteria is seen in the pus, but there may be a few colonies around where, by the weight of the coverslip, infected fluid has been extruded beyond the limit of the cells.

If, however, the pus cells are killed by any method which will not kill the bacteria, an entirely different picture is seen. Multitudes of colonies appear throughout the pus. Simple methods of killing the pus cells without damaging the bacteria are : (1) Heating to 50° C. (2) Freezing and thawing. (3) Applications of a poisonous chemical such as carbolic acid.

The immediate effect of applying a chemical antiseptic to a wound can be easily ascertained by making impression preparations of a septic wound before and after such an application. Such observations were made on relatively clean granulating wounds, and it was found that immediately after washing the wound with an antiseptic there is a more copious growth of bacteria in these impression cultures. This is doubtless due to the pus cells being killed or washed away by the chemical, leaving many bacteria which are free to grow out.

The fact that in a septic wound the pus cells are powerful antibacterial agents makes it important to study the relative potency of the commonly used antiseptics on leucocytes and on bacteria. This can easily be done by using fresh blood as the culture medium, and I have published (Fleming, 1924) the results obtained with the common antiseptics when tested in this manner.

When the chemical was used in a weak concentration there was no action on leucocytes or bacteria, so that the resultant growth was exactly as the control. As the strength of the chemical was increased the leucocytes were interfered with, so that there was an increased growth of bacteria, until a concentration was reached in which every microbe grew out. It was only with stronger concentrations that the bacteria were inhibited.

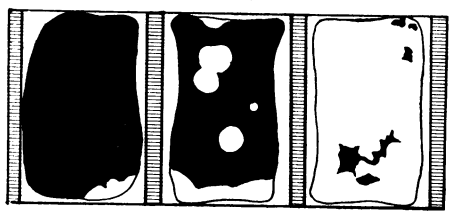
We have, then, with these chemicals, three phases as the concentration increases : (1) Indifferent. (2) Antileucocytic. (3) Antibacterial (only when the leucocytes are destroyed).

Carbolic acid is, if used strong enough, an antiseptic, but if it is added to blood in a concentration of about 1 : 700 it renders that blood a first-class culture medium and might, in certain circumstances, be used with advantage in blood culture.

One exception to the usual result is seen in connexion with the antiseptic dyes. These act slowly on both bacteria and leucocytes, and when the simple experiment alluded to above is made the leucocytes have time to destroy the bacteria before they are themselves destroyed by the chemical. When acriflavine was introduced (Browning *et al.*, 1917) it was shown that it inhibited the growth of bacteria in concentrations of 1 : 20,000 or less, but that when phagocytic experiments were made it required about 1 : 500 of acriflavine to seriously reduce the phagocytic power of the leucocytes. Thus it appeared that acriflavine was much more active on bacteria than on leucocytes.

These results have been widely copied. In these experiments, however, the dye was allowed to act on the leucocytes only for a few minutes.

I have shown (Fleming, 1917) that when acriflavine is allowed to act on leucocytes for a period of a few hours, a concentration of the chemical far lower than that which interferes with bacterial growth will seriously interfere with leucocytic function. This has recently been questioned, so I made a fresh series of experiments, this time testing the effect of acriflavine on the bactericidal power of human blood. Various concentrations of acriflavine were mixed with equal volumes of defibrinated blood, and then after four and a half hours, 50 c.mm. of these mixtures were infected with 2·5 c.mm. of a suitable dilution of a hæmolytic streptococcal culture and incubated in slide cells.



Control No acriflavine | Acriflavine 1 : 810,000 | Acriflavine 1 : 270,000

FIG. 3.—Blood mixed with acriflavine for 4½ hours, then infected with hæmolytic streptococci and incubated in slide cells. Clear areas represent hæmolysis due to growth of streptococci.

The result is shown in fig. 3, from which it is clear that a dilution of 1 : 810,000 damaged leucocytic function to some extent, and that a dilution of 1 : 270,000 acting on blood for four and a half hours rendered it a much better culture medium for a hæmolytic streptococcus.

In my opinion the best simple test for the possible efficacy of an antiseptic for use in a septic wound is that I have described, where the chemical is mixed with defibrinated or deleucocyted blood, suitably infected, and then the mixtures are incubated whenever possible in slide cells.

Human blood is a good fluid to use in the test, as it is very constant in its composition, and any antiseptic used in a wound has to act in a fluid more or less akin to it. Deleucocyted blood has no antibacterial action on the common microbes which infect wounds, so the test in deleucocyted blood gives a simple measure of the bacteriostatic power of the chemical. When defibrinated blood containing its full complement of leucocytes is used, then the action of the chemical on the leucocytes is also manifest.

If in this test it can be shown that the chemical is more strongly antileucocytic than it is antibacterial, it is extremely unlikely that such a chemical will be effective as a direct antiseptic in a septic wound.

It seems clear that, as in a septic wound there is a natural antibacterial element provided by the normal tissue reaction, the ideal antiseptic should be one which would not harm the leucocytes in concentrations which would inhibit the bacteria, so that the pus cells and the antiseptic could exercise a synergic action. This is what

happens with the sulphonamide compounds which are used for their general antibbacterial action, but there are reasons why they should be ineffective when introduced to a *septic* wound as local antiseptics. I would like, however, to draw your attention to the action of mercurial salts in human blood on the hæmolytic streptococcus. (This is a specific reaction which, so far as I know, only occurs with this organism.)

If dilutions of mercuric chloride are mixed with defibrinated human blood suitably infected with hæmolytic streptococci, and incubated in slide cells, there is a zone of concentration (from about 1 : 40,000 to 1 : 160,000) when no growth occurs although there is growth in a 1 : 20,000 concentration. In deleucocyted blood there is copious growth throughout. This experiment has been figured in the *Proceedings*, 1931, **24**, 808.

The result in the deleucocyted blood shows that the chemical by itself cannot inhibit the growth of the streptococci. The control cell with the defibrinated blood shows that the blood itself cannot inhibit, but yet there is complete inhibition by the combination of defibrinated blood and certain weak concentrations of the mercury salt, although when this chemical is used in a concentration of 1 : 20,000, which interferes with the leucocytes, there is no inhibition.

This action of mercury salts resembles the action of the sulphonamides in that it is only evident when a small infection is present, and that it is especially manifest on the hæmolytic streptococcus.

Physiological Action of So-called Antiseptics

I would like to draw attention to the fact that some of the more popular antiseptics —the hypochlorites—have a physiological action quite independent of their direct antiseptic action. Carrel's treatment of a septic wound was probably the most popular in the last war, and I have shown that by this method the chemical remained in antiseptic strength in the wound for not more than ten minutes. However, Dakin's fluid, or chloramine T, induces a greatly increased transudation of fluid from the walls of the wound, and so drains the infected walls of the wound. This is brought out in fig. 4 which shows the rate of transudation from the walls of an infected wound before, during, and after the application of the chemical.

The drainage of the infected walls of a wound seems at least as important as drainage of the wound cavity, but while it is a basic surgical teaching to drain the cavity, little attention seems to have been paid to the drainage of the infected walls.

It seems likely that the benefits obtained by the intermittent instillation of Dakin's fluid into a wound depend more on this physiological effect than on any direct antiseptic action.

Practical Application of Antiseptics.

There does not appear to be a good case for the local use of chemical antiseptics in a really septic wound in which the bacteria have grown out and invaded the walls. In such wounds chemicals such as the hypochlorites might, with advantage, be used for their physiological action in draining the infected walls quite irrespective of their antiseptic action, which has been shown to be transitory.

The experience of the past and the experimental evidence indicate that it is not possible to sterilize serious war wounds by the local application of an antiseptic. It may be, however, that in times of stress, surgical treatment of the recently inflicted wound would have to be postponed, and in such a case the *thorough* application of an antiseptic would, in many cases, delay the growth of the bacteria. If this result could be achieved it would be worth while, even if the chemical did injure some of the tissue cells.

Acriflavine or another member of the same series would be a suitable chemical to employ in these circumstances, as it has a powerful bacteriostatic action on a small infection, and as its deleterious action on cells is slow. The surgeon should, however, never forget that these local applications of chemicals are only a second best, and that at the earliest possible moment the wound should be surgically cleansed and the antiseptic treatment discontinued.

I have already (1940) given reasons why the sulphonamide drugs are unlikely to act as local antiseptics in septic wounds in which the bacteria have grown out, and which are filled with pus. The presence of very large numbers of bacteria or of peptones completely inhibits the action of these compounds. In the freshly inflicted wound, however, bacteria are present only in small numbers, and there is no pus, so it is possible that the local application of the sulphonamide drugs would induce sufficient bacteriostasis to tide over the interval until the wound could be dealt with surgically.

In the sulphonamide drugs the choice lies between sulphanilamide and M & B 693.[1] Sulphanilamide is soluble to the extent of 1 : 100, and M & B 693 1 : 1,000. Sulphanilamide has a bacteriostatic action on a small inoculum of hæmolytic streptococci in a concentration of 1 : 500,000, and M & B 693 in 1 : 4,000,000. There is, therefore,

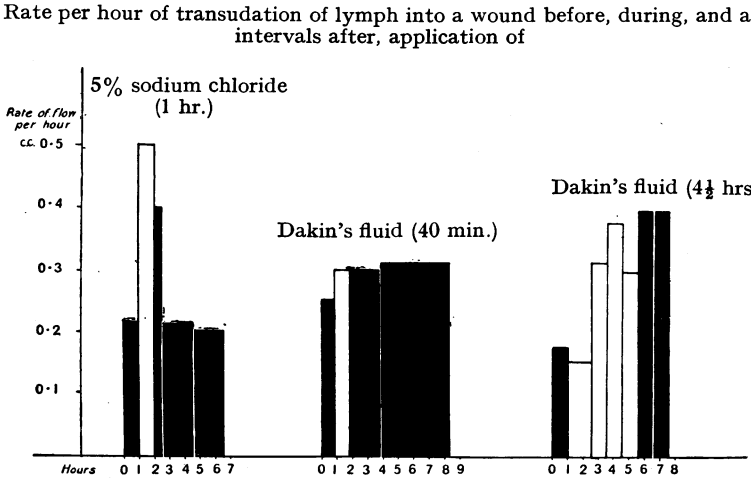


FIG. 4.—Black columns = Before and after. White columns = During application.

little difference in the solubility-bacteriostatic ratio between the two chemicals. As regards their interference with leucocytic function, sulphanilamide has, in blood, an antileucocytic action in a concentration of 1 : 800, whereas semisaturated M & B 693 (1 : 2,000) in blood has no antileucocytic action. Thus the bacteriostatic-antileucocytic ratio is in favour of M & B 693, and if the chemical is applied as a powder or paste it seems probable, for this reason, that M & B 693 is the more suitable. It must not be forgotten, however, that the sulphonamide drugs only have an effective action on some of the bacteria which invade war wounds, and that if sufficient time elapses some of the insensitive ones will grow out till they are present in very large numbers, and will induce the emigration of masses of leucocytes. The action of the chemicals will then be completely inhibited, and their continued application will be useless, except for their general effect after absorption from the wound.

Summary

In the study of antiseptics attention should be given to the bacteriostatic action rather than the bactericidal action.

[1] Since this paper was written I have had the opportunity of making some tests with Sulphathiazole—another of the sulphonamide group of chemicals. This substance is *in vitro* at least as potent as M & B 693 on streptococci, and it is much more active on staphylococci, *B. coli*, *B. pyocyaneus*, *B. influenzæ* and some other organisms which may infect wounds. Reports have been published showing that it also functions as an efficient bacteriostatic agent *in vivo*. Because of its potency and its wide range of activity it would seem that sulphathiazole should be the most effective of the sulphonamide drugs for application to an infected wound with the object of inhibiting the infection until the wound can be surgically cleansed.

By a suitable arrangement of the conditions of an experiment, naturally occurring antibacterial agencies can be made to appear much more powerful than the usual chemical antiseptics.

Dakin's fluid in a wound loses its antiseptic power in less than ten minutes, so that in the Carrel-Dakin method of treatment there was no antiseptic in the wound for one hour and fifty minutes out of every two hours.

The affinity of the antiseptic dyes for dressings is illustrated.

Experiments are cited :—

(1) Indicating that it is unlikely that it is possible to sterilize a war wound with a chemical antiseptic. (2) Illustrating the antibacterial power of pus. (3) Illustrating the antileucocytic power of chemical antiseptics. (4) Showing the synergic action of mercury salts and leucocytes on the hæmolytic streptococcus.

Attention is drawn to the physiological action of hypochlorites in draining the infected walls of a wound.

It is suggested : (1) That antiseptics are not indicated in a septic wound where the bacteria have grown out and invaded the walls. (2) That while efficient surgery is the most effective method of dealing with a contaminated war wound, there might be occasions where surgery would have to be delayed, and then an antiseptic might be useful as a bacteriostatic. The antiseptics whose properties are most suited for such a purpose are members of the acridine series, and the sulphonamides.

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STOKES, A., and TYTLER, W. H. (1918), *Brit. J. Surg.*, **6**, 21.

Dr. Dorothy S. Russell and Mr. Murray A. Falconer[1] : One of the principal criticisms levelled at the use of antiseptics in wounds is the allegation that they are more damaging to the tissues than to the bacteria. This allegation is unfortunately true for most surgical antiseptics. It explains why in the treatment of recently inflicted traumatic wounds most surgeons have rightly come to rely, for the control of infection, more on the thorough cleansing of the wound together with the excision of all contaminated and devitalized tissues than on the use of antiseptics. In traumatic wounds of the brain, however, it is often impossible to perform as complete a débridement as in wounds of other parts of the body, and therefore in cerebral wounds the use of antiseptics for the control of infection is more necessary than in other wounds. In this connexion it is vitally important that the antiseptics which we apply to the brain should not damage it. Comparatively little attention, however, has hitherto been paid to the reactions produced in human tissues by various surgical antiseptics.

At the instigation of Professor Hugh Cairns we have investigated various surgical antiseptics in order to find out which is the least harmful to cerebral tissues. We selected those antiseptics which possess widely acknowledged germicidal properties and which appear to retain these properties when applied to wounds. The method which we adopted was to operate on anæsthetized rabbits with full aseptic precautions and to expose the left cerebral hemisphere. After making a few minute scratches in the arachnoid membrane we applied the antiseptic to the surface of the brain by placing a piece of lintine thoroughly soaked in the antiseptic in contact with it for ten minutes. We also injected a little of the antiseptic into either the left or right

[1] Abstract of paper which will be published in full elsewhere.

hemisphere and then sutured the wound. The animals were killed at intervals ranging from one to eight days later and the brains examined macroscopically and microscopically.

Control Experiments

This method of course inflicted a slight but unavoidable amount of trauma upon the brain. Before we could evaluate the various antiseptics we had to ascertain what were the effects of our operative procedures. We did this by arranging a series of control experiments in which, instead of antiseptics, we used isotonic saline buffered to pH 7·4. Most antiseptics in clinical use are definitely acid or alkaline ; we found that the range of pH in those we tested was from 2 to 12. Moreover, in many hospitals and clinics it is customary to use ordinary water for making up or diluting antiseptics. We therefore controlled our observations by investigating the effects produced by isotonic saline buffered to pH 2·2 or pH 10 and by distilled water.

With neutral saline we observed only minimal degrees of reaction in the brain. Microscopically the leptomeninges showed slight focal hæmorrhage and proportionate inflammatory reaction where they had been exposed at operation, and the site at which saline had been injected was represented by a streak of hæmorrhage surrounded by active microglial cells. A few of the superficial neurones were shrunken and hyperchromatic, but for the most part the cortex presented normal appearances. The results obtained with neutral saline thus afforded a good standard for comparison in the experiments with other solutions. With salines at pH 2·2 and pH 10 we obtained slightly more reaction in the form of slight necrosis and severer leptomeningeal hæmorrhage associated with patchy degeneration and necrosis of the superficial neurones. When distilled water was substituted for saline we observed more extensive necrosis of the cortex. From these experiments it was clear that whereas the brain will withstand fairly acid or alkaline solutions for short periods of time, it is more seriously damaged by grossly hypotonic solutions. The logical deduction from our observations with various salines and distilled water is that all solutions which are to be applied to the brain should be rendered isotonic and as nearly neutral as possible.

Experiments with Antiseptics

We tested a wide range of surgical antiseptics, employing them in the concentrations which are recommended for application to wounds in general (*see* table). In the preparation of our solution we tried to apply as far as possible the principles of isotonicity and neutrality. We found no difficulty in rendering our solutions isotonic, but it was often impossible to achieve neutrality because precipitation of the active principle occurred. We then had to be content with buffering our solutions to a point as near neutrality as was compatible with maintaining a stable solution.

TABLE OF ANTISEPTICS INVESTIGATED.

I. *Acridine series*—			
Acriflavine in distilled water	0·1%	pH 2
Acriflavine in saline	0·1%	pH 2
Acriflavine in saline	0·1%	pH 7·2
Euflavine in saline	0·1%	pH 7·2
Euflavine in saline	0·05%	pH 7·4
Proflavine in saline	0·1%	pH 6·2
2 : 7-diaminoacridine in saline	0·1%	pH 6·2
II. *Coal-tar series*—			
Dettol in distilled water	5%	pH 12
Dettol in saline	5%	pH 7·4
" Modified " dettol in saline	5%	pH 8·4
" Modified " dettol in saline	5%	pH 8·1
Supersan in saline	5%	pH 8·2
III. *Halogen series*—			
Azochloramid in saline	0·03%	pH 7·4
Azochloramid in triacetin	0·2%	pH 4
Eusol		pH 9

IV. *Organical mercurial series*—			
Metaphen in saline	0·1%	pH 10
Metaphen in saline	0·05%	pH 10
Merthiolate in saline	0·1%	pH 10
Merthiolate in saline	0·05%	pH 10
Phenyl mercuric nitrate in saline.. ..	0·08%	pH 10
Phenyl mercuric nitrate in saline.. ..	0·04%	pH 10
V. *Oxidizing agents*—		
Hydrogen peroxide in distilled water ..	3%	pH 4
Hydrogen peroxide in saline	3%	pH 7·4
VI. *Sulphonamide series*—		
Soluseptasine in saline	3·3%	pH 10
Soluseptasine in saline	3·3%	pH 7·4

With a few important exceptions the antiseptics which we investigated caused serious hæmorrhage and necrosis in the brain. The two principal exceptions were proflavine sulphate and 2 : 7-diaminoacridine hydrochloride, both of which, when applied in a concentration of 0·1% in isotonic saline buffered to pH 6·2, proved hardly more harmful than neutral isotonic saline. On the other hand acriflavine and euflavine, two other members of the acridine group, consistently caused intense hæmorrhage and necrosis wherever they came into contact with the brain. We are unable to account for the striking contrast in the effects of such closely related substances. It is probable that differences in chemical constitution are responsible. Moreover, proflavine and 2 : 7-diaminoacridine are pure compounds, whereas acriflavine and euflavine are mixtures of acridine compounds, and may vary in composition from sample to sample. In our view it is unfortunate that the generic term " flavine " has been applied collectively to these antiseptics because it has tended to obscure the differences that exist between individual members of the group.

None of the remaining antiseptics which we tested gave such favourable results as proflavine and 2 : 7-diaminoacridine. The coal-tar derivatives, dettol and supersan, both emulsions of a chlor-xylenol, when applied in buffered isotonic solutions, produced effects which, while not quite as mild as those seen with proflavine and 2 : 7-diaminoacridine, were yet a great improvement on acriflavine and euflavine. The halogen group was unsatisfactory. Azochloramid, an organic chlorine compound held by many to be superior to dichloramine-T, produced very variable results, some batches of solution being mild in action while others caused intense hæmorrhage and necrosis. Despite repeated attempts we were unable to account for or control this variability. Its solution in triacetin caused intense necrosis, as did eusol. The organic mercurial compounds all produced considerable hæmorrhage and necrosis in 0·1% concentrations, although merthiolate (0·04%) was fairly mild in its action. Hydrogen peroxide, which has no appreciable antiseptic action in wounds, but which is widely used in neurosurgery as a cleansing and hæmostatic agent, produced superficial fragmentation of the tissues accompanied by necrosis and hæmorrhage. Soluseptasine, which we selected as a representative of the sulphonamide drugs on account of its high solubility, was mild in its action.

Conclusions

Our experiments indicated that proflavine sulphate and 2 : 7-diaminoacridine hydrochloride are greatly superior to all the other antiseptics that we tested in that the damage which they cause to the brain is minimal. Both of these substances have been shown to be potent bactericidal agents in the concentrations in which we employed them. The latter compound is of recent introduction, but proflavine has been acknowledged for over twenty years. Proflavine as well as acriflavine has been proved to retain its efficiency when applied to wounds. Many investigators, including Neufeld, Schiemann, and Browning, have shown that in a high percentage of experiments in mice and guinea-pigs they are capable of sterilizing wounds which have been inoculated an hour or so previously with cultures of virulent organisms

such as *Streptococcus*, *Pneumococcus*, and *Bacillus diphtheriæ*. We therefore feel that in recommending proflavine sulphate (0·1% in isotonic saline buffered to pH 6·2) for application to wounds of the brain we are supporting an antiseptic which is both potent against bacteria and yet innocuous to the tissues.

Professor L. P. Garrod : Antiseptics can help in cleansing a wound, will deodorize it, prevent the access of further infection, and prevent the transference of infection to other cases, but that they can make all the difference between a wound being septic or not is discredited. This sceptical attitude dates from the Great War, when antiseptics were said to have been well tried and to have failed. There are special reasons for this alleged failure which have been overlooked. In the first place, the average gunshot wound is a most unpromising field for antiseptic treatment ; when it consists of a long track with a foreign body and dirt at the end of it, the deeper part of it cannot be treated at all by an application which, to be effective, must reach the whole of its internal surface. The possibilities of antiseptic treatment can be fairly judged only in reasonably open wounds capable of thorough irrigation. I beg you not to be depressed by the results of Professor Fleming's experiment with a test tube drawn out by blow-pipe into a number of thin lateral spikes. These narrowly tapering branches which prove incapable of disinfection have no equivalent in the body unless in wounds involving bone. They are rigid, whereas the crevices in soft tissues bordering a wound are elastic and mobile. It should not be beyond the wit of the surgeon to devise a means of ensuring that such crevices are penetrated by an antiseptic solution ; its injection under pressure through a fine jet would probably secure this effect. A second and even more serious reason for the failure of antiseptic treatment in the Great War was the inevitable delay in applying it. The period which elapses between the infliction of a wound and the invasion of surrounding tissues by bacteria is two hours or more. An antiseptic applied during this period has only to destroy bacteria in the cavity of the wound ; applied later it has to attack them in the tissues, a vastly more difficult task involving new factors, notably that of tissue penetration. The difference between prophylactic treatment, which is possible only for a few hours, and treatment applied when infection is already developing or even frankly in progress, is therefore immense, and it is astonishing that this distinction is so disregarded. This time factor must always operate against the success of antiseptic treatment in military surgery, where casualties can often not be brought in for several or many hours. The primitive antiseptic methods used in France in the early part of the Great War were in any case not calculated to succeed. If it be granted that for reasons such as these antiseptics did not in fact get a fair trial at that time, the present attitude to them is to that extent unjustified, and a more open mind in inquiring what they can do and how best they can be used is called for. A defeatist attitude is indeed a strange and unnecessary confession of failure at a time when the much more difficult problem of internal antisepsis has been largely overcome. Now that it is possible to destroy bacteria even in the circulation, it is surely not impossible to attack them when they are merely lying in a wound cavity and therefore directly accessible.

What I have further to say concerns the methods by which the suitability of an antiseptic for wound treatment can be judged. Of three principal methods, clinical trial is the most obvious, but in my submission should come last, both because it needs to be directed by information obtained in other ways, and because it is not a method which can easily yield significant results. Accidental wounds are too various in their site and extent and in the nature of their bacterial contamination to afford a proper test of prophylactic action, unless such a test be conducted on a very large scale. The conditions of test can be better regulated in the experimental animal, since wounds of determined extent can be identically contaminated in as many animals as may seem necessary and then treated by different methods. Experiments such as these by several workers in three different countries have agreed in showing that fatal infection by virulent streptococci can be averted by treatment of the wounds with acridine compounds at any time within the first two hours after their

infliction and contamination with these bacteria. Phenol, mercury perchloride, and other commonly used antiseptics consistently fail in such experiments. This is solid evidence that prophylaxis is feasible, and cuts across a multitude of objections that conditions in the body, as distinct from the test tube, militate against antiseptic effect. The third possible method of study is the purely *in vitro* experiment, which can be designed to assess the effect of an antiseptic on bacteria either under quite simple conditions or under conditions more or less resembling those existing in the body. The almost infinite variety of technique employed for this purpose makes even the approximate correlation of different workers' results exceedingly difficult. It is deplorable that no generally recognized *in vitro* method exists for testing the suitability of antiseptics for application to wounds. A simple and appropriate method would be to determine the degree of disinfection in a mixture containing 50% of blood at 37° C. in a fixed time of, say, two hours.

It is also necessary to know something of the action of an antiseptic on body cells. I think the importance of this factor can be exaggerated ; if a wound can really be disinfected by something which also damages leucocytes, this is a small price to pay for such an effect ; leucocytes are easily and quickly replaced, and unless there is demonstrable damage to fixed tissues as well, this degree of " toxicity " is no bar to usefulness in wounds involving connective tissue and muscle. There is the same lack of a generally accepted and appropriate method of test here. Parenteral methods of administration are a test of the action of the antiseptic on sensitive and vital structures with which in ordinary use it does not come into contact at all. Two *in vitro* proceedings are available—application to tissue cultures, and admixture with fresh blood followed by observation of the effect on leucocytes, this being judged either by motility or by phagocytic capacity. Acridine compounds come well out of such tests, and 1 : 2,000 acriflavine, although it reduces leucocytic activity does not abolish it ; this observation is in apparent conflict with that of Professor Fleming, who found that as little as 1 : 274,000 acriflavine interfered with leucocytic activity in the slide cell. The variability in composition of acriflavine underlies some of the inconsistencies in results obtained with it, but this discrepancy is so extreme that it raises the question whether some peculiarity in the conditions existing in the slide cell may account for it. Certainly the most appropriate method of testing toxicity for a particular tissue is *in vivo* application to that tissue and histological study of the results. The facts so elicited by Dr. Dorothy Russell and Mr. Murray Falconer about the effect of antiseptics on the brain are of the highest practical value, and should lead anyone requiring an antiseptic of the highest degree of harmlessness to select proflavine.

Summary

(1) Prevention of infection and treatment of infection already in progress are to be sharply distinguished in considering the place of antiseptics in wound treatment.

(2) Prevention is feasible in open wounds treated within a few hours.

(3) Available evidence points to the acridine compounds as the most suitable agents for this purpose.

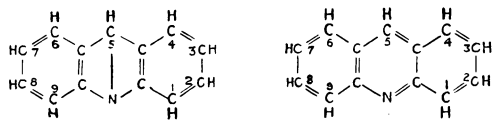
Dr. Malcolm C. Manifold (Department of Biochemistry, Oxford University) : *The Effect of Certain Antiseptics on the Metabolism of Brain Tissues in vitro.*[1]— This preliminary investigation was carried out at the suggestion of Professor R. A. Peters, in order to determine whether brain tissue was damaged from the point of view of normal metabolism when various antiseptics were severally added to the medium in which the brain tissues were respiring.

The respiration experiments were carried out in Dixon-Barcroft manometers at a temperature of 38·2° C. and a pH of 7·3 using air or oxygen according to the conditions required. The brain tissue was used either in the form of slices, minced tissue, or a finely ground dispersion, and in the experiments where slices or minced tissue were used, the medium was a Ringer-phosphate solution of pH 7·3, which gives

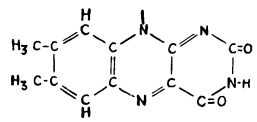
[1] Abstract of a paper which will shortly be published elsewhere.

the same values for the respiratory quotient of brain as a bicarbonate-Ringer solution. In certain experiments the medium contained, in addition, substrates such as glucose or sodium pyruvate. The dispersion medium was a buffered isotonic solution of potassium chloride with the addition of sodium fumarate, which is an essential intermediary in the pyruvate oxidation system of the brain and must be added to systems made in the form of tissue dispersions. To this medium, glucose or sodium pyruvate was added in certain cases.

The main results of this investigation are those appertaining to the use of some derivatives of acridine. These compounds are frequently but erroneously known as flavines, for example acriflavine, proflavine, &c. It is, in passing, very important to realize the fact that acridines do not belong to the true "flavin" series, of which riboflavin, a member of the vitamin B_2 complex, is an example. In order to demonstrate the great difference between the two series of compounds, the following structural formulæ are presented.



The above are alternative formulæ for acridine.



The above formula shows the ring system for riboflavin.

It will be seen that these two sets of formulæ are very different, and the importance of realizing this difference is strongly emphasized. In this, and all other communications on this subject from this Department, the numbering of the acridine ring will be as shown in the above diagrams. This is pointed out because there are alternative ways of numbering such ring systems and it is very important to realize which system is being used in the discussion of any particular problem, as failure to do so would lead to a deal of confusion.

The brain tissue used in these experiments was obtained from either human, rabbit, or pigeon sources, but the results do not differ fundamentally from one source to another.

In the case of the acridine antiseptics, the concentration used was in general 1 : 1,000, and the final pH of the mixture was adjusted to 7·3.

The first experiments deal with the effect of acriflavine on the ability of brain tissue to utilize oxygen. In all the experiments quoted, duplicate determinations were made and were adequately controlled.

Acriflavine as normally prepared is a mixture of the hydrochlorides of 2 : 8-diaminoacridine and 2 : 8-diaminomethyl acridonium chloride, and may thus vary considerably in composition. Hence the results obtained on the physiological effects of such a mixture are of no great value since they cannot be traced to the effect of any one pure compound. The results with acriflavine (1 : 1,000) showed clearly that it was very toxic to brain tissue and caused a 70% inhibition of the respiration of brain slices placed in a medium with or without glucose. This figure of 70% was the average result of six experiments.

Neutral acriflavine or euflavine showed a slightly lower toxicity (though probably not significantly so), than acriflavine, as far as brain slices were concerned, for the average percentage inhibition of the respiration in seven experiments was 62, and the same inhibition occurred using minced brain and euflavine. Three experiments in which euflavine was tested on brain dispersions showed a further increase in

respiratory inhibition to 79%, which increase one might reasonably expect, for in dispersions the rapidity with which a substance can attack the enzyme systems responsible for respiration is greatly increased.

The use of proflavine sulphate (which is 2 : 8-diamino-acridine-sulphate) in a series of six experiments on brain slices, resulted only in an average figure of 36% for the percentage inhibition of the respiration. In a further four experiments on minced tissue this figure increased to 54%, while three experiments on dispersions showed a percentage inhibition of 73. It is thus clear that as far as brain slices are concerned proflavine sulphate is far less toxic than either acriflavine or euflavine. Since the technique involving the use of tissue slices more closely parallels the conditions under which antiseptics are used clinically than that in which minced tissue or dispersions are used, we shall for true comparative purposes stress the results for slice experiments.

The next experiments were carried out using 2 : 7-diamino-acridine-hydrochloride, which proved to be a remarkable compound in that three experiments on slices showed the low figure of 28% for the inhibition of respiration, while three experiments on minced tissue and three on dispersions showed respectively only 34 and 31% inhibitions.

While proflavine sulphate has been known for a number of years, 2 : 7-diamino-acridine-hydrochloride is a relatively new compound synthesized by Linnell in 1936. A survey of the literature relative to the antiseptic values of the various acridine compounds reveals the fact that acriflavine has been extensively used as an antiseptic and has been reported not to cause any extensive damage to the tissues. Earlier results did not, however, include any references to the use of acriflavine on brain tissue. Therefore the results of Mr. M. A. Falconer and Dr. Dorothy Russell, coupled with those reported in this paper, seriously question the advisability of employing acriflavine as an antiseptic for general use, especially since proflavine sulphate is as efficient a bacteriostat as is acriflavine.

Neither acriflavine nor proflavine sulphate are superior in bacteriostatic power to 2 : 7-diaminoacridine-hydrochloride, and while the experiments of Mr. Falconer and Dr. Russell with this compound were not as extensive as those on proflavine sulphate, they did show that the 2 : 7 compound had no deleterious effect on the brain tissues.

It would thus appear that on the evidence from bacteriological, biochemical, and histological sources, proflavine sulphate is definitely more suitable than either acriflavine or euflavine in the treatment of wounds.

The bacteriostatic values of 2 : 7-diaminoacridine have been well demonstrated, and the biochemical evidence, although by no means complete, would suggest that this compound may turn out to be the most suitable of all these derivatives for general use. Further biochemical and histological evidence with regard to this substance is awaited with interest.

Certain other antiseptics were tried out by means of these respiration techniques. Azochloramid, used in the recommended concentration of 1 : 3,300, was found to be very toxic to brain slices, the percentage inhibition of the respiration being of the order of 80. The action of this substance was tested at various concentrations and it was found that at concentrations of 1 : 45,000 the azochloramid no longer interfered with respiration. Many experiments were carried out using this compound, and its behaviour was found to be irregular in that one could not be sure that two experiments carried out in an exactly similar manner would give reproducible results. A full discussion of these results will shortly be published.

Two mercurial antiseptics, merthiolate and metaphen, have been examined, and the results with these were unsatisfactory. Metaphen seemed to be unstable, for in each case where this compound was used, the medium at the end of the experiment was found to be very alkaline, an observation which had not been noted with any of the other antiseptics.

Experiments on soluseptasine, a sulphanilamide derivative, showed that this compound caused a quite high inhibition of the respiration of brain slices. In no

way were the results with these latter three compounds comparable with those obtained with either proflavine sulphate or 2 : 7-diaminoacridine-hydrochloride.

The utilization of oxygen by the tissues is a sensitive process, and is liable to be affected by adverse changes in the cell environment. Probably all such changes would be clinically harmful. The phenomenon of inhibition of respiration by these antiseptics, as set forth in this report, certainly has significance in view of the fact that it may be correlated with pathological changes as instanced by the histological work on this subject.

It would, however, be a mistake to assume that the *in vitro* results can be directly related to changes taking place *in vivo*.

So far, I have not found any reliable method of deciding whether the inhibitory action of these antiseptics is reversible or not. In the absence of evidence on this point it must not be assumed that there is necessarily permanent damage to the tissue concerned. Nevertheless, it is possible that those cells which have been intimately in contact with the antiseptic do not recover their normal metabolic function. In any case the conditions obtaining *in vivo* may not be compared with those of our experiments where thin slices of tissue are surrounded on all sides by the medium and antiseptic, and partially at any rate penetrated by the antiseptic. These tissue slices are not as it were " backed up " by a great mass of actively living tissue as would be the case in a wound. Hence the *in vivo* effects for a given strength of antiseptic might be expected to be less severe.

In conclusion, although the results do not afford a precise index of the extent of the injury which may occur clinically from the use of an antiseptic, they can nevertheless be used to indicate the relative toxicities of various substances to a vital and sensitive mechanism, and in this respect they have been well supported by bacteriological and histological evidence.

I wish to express my appreciation of the kind help and criticism afforded me by Professor R. A. Peters, and to thank the Rockefeller Foundation for a grant in aid of this work.

Colonel E. M. Cowell (*in absentia* read by Dr. Downie) : *Morbid anatomy of gunshot wounds.*—This subject is discussed in the various textbooks on War Surgery and with special reference to air-raid wounds in Mitchiner and Cowell's " Medical Organization and Surgical Practice in Air Raids ".

The factors to consider are :—

(1) Infections, aerobic and anaerobic, which will be present in wounds received in fighting in most of the countries of Europe.

(2) Massive destruction of tissue with comminution of bone in wounds caused by even minute fragments of air bombs.

Principles of treatment.—In the last war, principles of treatment were evolved, and by 1918 were giving good results. Treatment at first consisted in removal of the foreign body and establishment of drainage, together with the following sequence of method :—

(1) Application of pure carbolic.
(2) Iodine.
(3) Salt packs and hypertonic saline lavage.
(4) B.I.P.P. and the coloured antiseptics.
(5) Carrel-Dakin continuous irrigation.
(6) Excision and primary suture.
(7) Excision, vaseline, or soap packs and secondary suture.
(8) Winnet Orr's method of excision and débridement and immobilization in plaster of Paris.

From this list of methods it will be seen that in the early stages surgeons were beaten by the profound sepsis of all wounds ; they failed to rely on careful surgery with the removal of infected tissues and foreign bodies, but attempted to kill bacteria by the use of strong germicides. These methods failed, although the Carrel-Dakin

treatment was an advance. When more surgical teams were available, and organization was better, a greater proportion of wounds dealt with at the casualty clearing station, excision was perfected, and the results enormously improved.

In the Spanish war local antiseptics were hardly used at all. Trueta and other surgeons whose work I saw in Barcelona relied on débridement and immobilization in plaster of Paris.

Constitutional methods.—The use of sera, vaccines, and intravenous therapy may have a subsidiary place in the treatment of war wounds, but in my opinion the best results will be obtained by good organization, rapid evacuation to surgical centres where careful surgery is carried out by trained traumatologists. The operation consists in wide excision of all damaged tissues, removal of foreign bodies, and immobilization in plaster of Paris.

The treatment of wound shock and hæmorrhage by blood transfusion is important.

Evacuation of casualties.—The subject of " The Effect of Antiseptics on Wounds " in war can only be properly discussed when front-line conditions are taken into consideration.

Front-line treatment of gunshot wounds.—The layout of the modern battle-field is very different from that familiar to some present in the last war. The wounded soldier will have a shell dressing or first field dressing applied as early as possible and then probably have to lie under cover till a lull in the fighting occurs or till darkness falls before he can be carried in to the regimental aid post (R.A.P.).

These dressings are only very mildly antiseptic and iodine is no longer used in the Army Medical Services. Under favourable conditions he will soon be got away by field ambulance bearers—say an hour's hand-carry—some distance by wheeled stretcher, and then by ambulance car to the advanced dressing station (A.D.S.) a a few miles back from the R.A.P.

His stay here should be brief if there are enough vehicles and the roads are not being shelled too badly. His dressings are not interfered with unless there is severe unchecked hæmorrhage. Cases are marked in order of urgency and sent on to the main dressing station (M.D.S.) some 7–10 miles further back—a slow journey in the dark, over congested roads with shell fire, shell holes, possibly gas, and a tired driver who does not know the way very well. Very little surgery will be attempted at the M.D.S. and no disinfection of wounds is possible. Casualties will be sent back by cars of the motor ambulance convoy (M.A.C.) as rapidly as possible. This stage of the journey will occupy one to two hours, probably more at night, and ends with admission to the surgical centres casualty clearing station.

The total distance covered from the most forward areas to the C.C.S. may be from 25–30 miles, and the time lag from six to twenty-four hours.

At the C.C.S.—Expert surgeons will be available—six or eight teams, each with two or three tables, will be required to deal with large numbers of casualties. After débridement, irrigation with some mild antiseptic may do no harm, but in my opinion it is safer to rely on conscientious surgery and if lavage soothes the surgeon's conscience, saline does less harm than anything else.

Summary

(1) Strong antiseptics cannot remove germs or dead tissue.

(2) Careful ablation of dead and damaged tissues, together with removal of foreign bodies is the essential principle, followed by immobilization.

(3) Increasing the patient's resistance by blood transfusion and administration of fluids is more useful than relying on chemical measures or the use of strong antiseptics.