THE ACTION OF CHEMICAL AND PHYSIOLOGICAL ANTISEPTICS IN A SEPTIC WOUND.

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Since the war began, much attention has been directed to the treatment of suppurating wounds, for in the earlier years—1914–15–16—practically all the gunshot wounds became infected, and even in 1918 nearly all the wounds which were left open for more than a week became grossly infected with pyogenic cocci and other organisms.

Prior to the war, the surgeon gave most of his attention to aseptic methods, his great object being to exclude microbes from the wound. The question of how to deal with the bacteria after they were in possession was a problem of much less interest to him. I can remember in the days when I was first admitted to the surgical wards as a dresser, there were always a certain number of septic wounds which we were instructed to dress with this or that antiseptic, which stood in jars around the fire, and which we were told possessed great virtue as destroyers of microbes in the wound. These antiseptics were chiefly carbolic acid, mercury salts, and boric acid. The wounds were religiously dressed once or twice a day with these lotions, and although it was obvious that the antiseptic did not kill all the microbes in the wound, we were always told that it would kill many of them, and so the condition would be better than if no antiseptic were used. We were not then in a position to criticize this view.

At the beginning of the war in 1914, all the old antiseptics were used in military hospitals, in just such a manner as when I started surgery. Carbolic acid, perchloride or biniodide of mercury, boric acid, and hydrogen peroxide were poured into septic wounds once or twice a day, either singly or in mixtures of two or more, according to the fancy of the medical officer.

Very soon, however, campaigns were started in favour of particular antiseptics. Early in 1915, the merits of pure carbolic acid and of 2 per cent iodine in spirit were tested. A certain number of cases were treated with these two chemicals at the Front, and the patients were sent down to No. 13 General Hospital, where I had the opportunity of seeing the wounds and examining them bacteriologically. In the carbolic series there was a higher percentage of gas gangrene than in the general run of the wounded, while the iodine cases showed practically no difference from the cases treated in other ways. Soon after this, antiseptic pastes were advocated. The wound was plugged with the paste, but it soon appeared that in France, at any rate, the chief result of this method of treatment was to shut off all drainage, so that gas infections developed in a large number of cases. These pastes, therefore, very soon disappeared from the antiseptic armamentarium.

The first real advance in the 'antiseptic' treatment of wounds was the introduction of the hypochlorite solutions. Lorrain Smith introduced eusol, which is a solution of hypochlorous acid, while Dakin, about the same time, produced a solution of sodium hypochlorite, which has come to be recognized as Dakin's solution. Both of these solutions attained great favour in wound treatment, and I shall have more to say about their action later. About the same time that these solutions came into favour, new surgical procedures were introduced in the treatment of wounds, and thus the actual effects of the antiseptics were rather obscured. It was about this time that the importance of the thorough surgical cleansing of the wound was recognized, and that Carrel introduced his system of intermittent irrigation with the 'antiseptic' solution.

After 'Carrel' treatment came the era of B.I.P.P. This was a paste made by mixing iodoform, bismuth, and paraffin, and it obtained many supporters. It was, however, combined with very careful surgical treatment of the wound, and in connection with its use another factor was introduced into wound treatment, in that after treatment with the paste the wound was not touched for a considerable time, thus obtaining complete rest for the part and so aiding the physiological activities of the body in combating the infection. B.I.P.P. paste is not in itself an antiseptic, and Rutherford Morrison, who introduced it, has given directions for its sterilization before it is put into the wound. It was reported to have an antiseptic action when placed in contact with tissues or tissue fluids ; but this we have never been able to demonstrate, and thus are forced to the conclusion that the paste is not a chemical antiseptic at all, but if it has any action in keeping down infection, it is due to some physical or physiological action.

After B.I.P.P. came the dye-stuffs. The antiseptic action of some of these dyes had been known for a number of years, and some of them had been used prior to the war, without much success, in the treatment of septic wounds. Churchman, Dreyer, and others had investigated the power of a number of the aniline dyes in inhibiting the growth of bacteria, and Browning recommended brilliant-green, and more especially flavine, to which he attributed very remarkable properties as an antiseptic for use in septic wounds. Flavine differed from the other antiseptics in that its bactericidal action was greater in a serous than in a watery medium. Browning maintained also that it killed all the common microbes found in wounds in a very high dilution, while it had little toxic effect on the tissues, as exemplified by leucocytes. I have already published my findings in connection with the antiseptic action of flavine. Briefly, I found that it had a very slight antiseptic action on some of the microbes found in wounds, such as *B. proteus* (which is one of the most common bacteria found in a septic wound). Its action on the pyogenic cocci was not nearly so marked as was maintained by the originators of the method, except where there was a minimal infection. In pus, also, it had little bactericidal action, and it had a very destructive effect on the leucocytes. Browning's findings that it had little action on the leucocyte, while it had a powerful bactericidal action, followed merely from the fact that he tesied the action on leucocytes for a few minutes only, whereas the action on the microbes was tested on a minimal infection for twenty-four hours. Flavine also has a remarkable affinity for the walls of the wound and for the dressing, so that it is rapidly picked up and rendered inert by these walls and by the gauze pack. Its great affinity for the dressing can be shown by the following simple experiment : A test tube is half filled with flavine 1–1000, and a tight-fitting cotton-wool plug is slowly pushed down into it, until fluid escapes above the plug. The first portion of fluid which comes through the plug will be found to be absolutely without the yellow colour of flavine, showing that the cotton-wool has been able to remove the whole of this substance from the solution.

It appears that flavine has the property of combining with and staining a large variety of substances, including bacteria. In a fluid, therefore, where the bacteria form the only stainable objects, the dye can exert its full strength on these ; but in a wound or in pus, where the bacteria form only a very small part of the stainable material, the major portion of the dye is used up in staining the tissues and cells, and in this way the bacteria are largely protected from its action.

It was soon found in practice that when a wound was treated with flavine 1–1000 (the strength recommended) for more than a few days, all the reparative processes stopped, while the flavine did not sterilize the wound, so that for the continued treatment of septic wounds it was found quite unsuitable. In the later stages of the war it has been very largely used as a prophylactic, following the preliminary surgical cleansing of the wound. This use will be considered later, in dealing with antiseptics in primary and delayed primary suture.

There have been, during the war, two schools in the treatment of wounds : the *physiological school*, which concentrated their efforts in aiding the natural protective agencies of the body against infection, and the *antiseptic school*, which aimed at killing the microbes in the wound with some chemical agent.

PHYSIOLOGICAL TREATMENT.

Sir Almroth Wright and his followers have maintained that it is impossible for any of the commonly used antiseptics to have an appreciable effect in sterilizing a wound in which the infection has become established, and that the greatest benefit is to be obtained by aiding the physiological agencies which bring about the natural recovery from infection. Two such agencies especially have to be considered in connection with infected wounds : (1) *Blood and tissue fluids* ; (2) *Leucocytes*.

Blood and Tissue Fluids.—

Reaction.—The normal reaction of the blood fluids corresponds to a N/35 alkaline solution. The reaction of the discharges from a wound varies enormously, according to the microbes with which it is infected, and with their rate of growth.

Sir Almroth Wright has shown that some bacteria commonly found in wounds, e.g. *B. Welchii*, grow very badly in unaltered blood serum, but if the normal alkaline reaction is reduced or abolished, the bacilli will grow luxuriantly. *Fig.* 110 represents

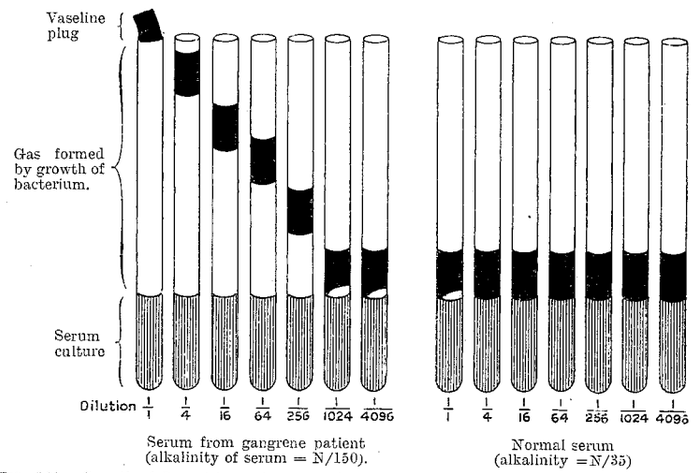


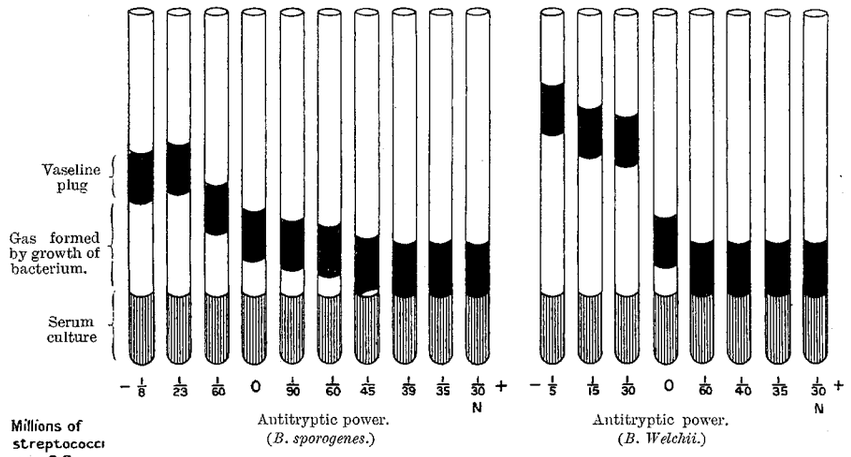
FIG. 110.—Growth of *B. perfringens* in twenty-four hours in serum from gangrene patient with acidosis.

the growth of *B. Welchii* in normal serum, and in serum from a patient with gas gangrene which had lost almost all its alkalinity. Into equal quantities of the serums graduated implantations of *B. Welchii* were made, and it will be seen that in the 'acidosed' serum from the gas-gangrene patient growth takes place with one-thousandth of the implantation necessary to induce growth in the normal serum. The gas-formation in the acidosed serum is also very much greater.

The alkalinity of the blood serum in toxic cases of gas gangrene has been found to be greatly reduced.[1] As an example, the serum used in the experiment illustrated in *Fig.* 110 had an alkalinity of only N/150. Following from this, alkali therapy has been used as an adjunct to surgery in the treatment of cases of severe gas gangrene, often with very marked beneficial results.

Antitryptic Power.—By this term is meant the capacity of the serum or lymph to neutralize tryptic ferments. It has been found that all serum has some antitryptic power, and that in certain conditions this is markedly increased. Sir Almroth Wright has shown that the men who were suffering from severe septic infections had a greatly enhanced antitryptic power in their blood fluids, and he has also pointed out the remarkable effect which the antitryptic power of the serum has on the growth of microbes in such serum. In serum of normal or raised antitryptic power, streptococci are the only microbes which grow at all freely when planted in small number. Staphylococci and some diphtheroid

bacilli will also grow to some extent, but not so well as streptococci. To these microbes, on account of their capacity of growing in unaltered serum, he has given the name of 'serophytes.' The other microbes found in wounds he has called 'sero-saprophytes,' as they grow badly in unaltered serum or lymph but flourish in such fluids when they have become 'corrupted.' It has been demonstrated that leucocytes when they disintegrate liberate a tryptic ferment. The discharges from a wound contain leucocytes in greater or less numbers. As a result of the toxic products produced by the bacteria in a wound, or the chemical substances introduced into the wound, some of these leucocytes are broken down, and tryptic ferments are liberated, which diminish the antitryptic power of the lymph or even render it tryptic, and so make it a good cultivation medium for all the microbes which are to be found in a wound.



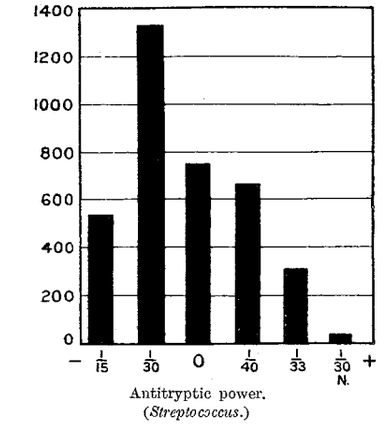


FIG. 111.—Effect of reducing the antitryptic power on the growth of bacteria in serum.

In the wound which has only been inflicted a few days, and in which there are sloughs present, the discharges are found to be either tryptic or to have a very greatly reduced antitryptic content, and in these wounds the flora is a very mixed one, anaerobic bacilli, aerobic bacilli, and pyogenic cocci being usually present. In the later stages, however, when the wound becomes clean and when the antitryptic power of the discharges is fairly high, there are usually to be found only streptococci, staphylococci, and diphtheroid bacilli, all of which are serophytes. Usually the last microbe to disappear from a wound is the streptococcus, and this is the one which grows best in the unaltered serum or lymph. *Fig.* 111 illustrates the effect of the antitryptic power on the growth in serum of *B. Welchii*, *B. sporogenes*, and streptococcus. Graduated additions of a solution of trypsin were here made to the serum, so that some of the tubes have a reduced antitryptic power, while others were actually tryptic. It will be seen that no growth has occurred in the unaltered serum with *B. Welchii* or *B. sporogenes*, although good growth and gas-formation took place when the antitryptic power was neutralized. With streptococcus a certain amount of growth was observed in the unaltered serum, but even with this organism the growth was very greatly enhanced when a small reduction in the antitryptic content was made.

We can well see that to rid a wound of the serosaprophytic organisms, it is very necessary that the antitryptic power of the discharges should be kept at a high level, and that these discharges should be kept free from corruption. The blood in all these cases is possessed of a high antitryptic content, and if the discharges have a low antitryptic power, it means that the lymph has become corrupted by broken-down leucocytes in or around the wound.

A necessary preliminary to the prevention of this corruption is the removal of all sloughs from the wound. These, where they cannot be removed by surgical means, must be got rid of by tryptic digestion, and here we find ourselves in a dilemna. On the one hand we want the antitryptic power maintained to prevent the growth of organisms, and on the other it is necessary to rid the wound of sloughs by tryptic digestion. In practice it is found to be impossible, while sloughs are present, to maintain a high antitryptic content in the discharges, so that we must, in the early days of the wound, attempt to avoid disaster by means of free drainage of the cavity of the wound, and concentrate on removal of the sloughs. This is done most rapidly by means of an intermittent application of hypertonic salt solution. This solution immediately breaks up the leucocytes with which it comes in contact, and liberates trypsin. (It has no destructive effect on trypsin itself.) The salt solution soon becomes diminished in strength (*Fig.* 129), so that all inhibitory action on tryptic digestion is lost and the liberated trypsin can act without hindrance on the sloughs. Meantime, as soon as the strength of the salt solution approaches normal, fresh leucocytes emigrate into the cavity of the wound. These are destroyed with the next application of hypertonic salt, and so the process goes on until the wound is clear of sloughs.

While this is happening in the cavity of the wound, we must not lose sight of the events which are occurring in the infected walls, for it is here that the really serious infection exists. As a result of bacterial action, leucocytes are here also breaking down and corrupting the lymph, and it is very necessary that this corrupted lymph should be removed and be replaced with fresh fluid possessing its full antitryptic and antibacterial power. Drainage of the tissues around the wound is just as important as the mere mechanical drainage of the cavity of the wound, and it can most easily be effected by the application of hypertonic salt solution, which causes a prompt outflow of lymph from the tissues into the cavity of the wound (*Fig.* 133).

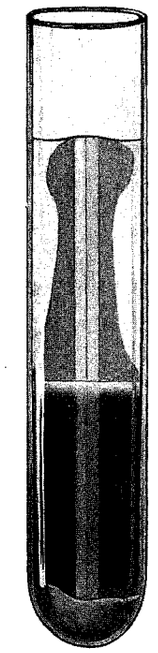


FIG. 112.– Glass laths in centrifuged blood, showing the red clot below, above this the leucocytic layer, and above this the white clot contracted round the laths.

We shall see later that the benefit derived from some of the so-called antiseptics is, in all probability, due to their exercising an action in the wound with results similar to that obtained with hypertonic salt solution.

2. Leucocytes.—

It has long been known that the chief agents in the destruction of bacteria in a wound are the leucocytes. Recently, however, Sir Almroth Wright has devised methods which have shown their remarkable potency in this connection. We can show this first with leucocytes obtained from the blood. Blood in itself contains too few leucocytes and too much fluid for the bactericidal action to be manifest; but we can obtain an aggregation of the leucocytes from the blood in various ways, and so bring them to bear on the microbe in large numbers, more or less comparable with the condition of things in a wound.

Blood is drawn from the finger into a narrow tube (*Fig.* 112) containing a small glass slip (which we have called a 'lath'), and the tube is centrifuged before the blood has time to clot.

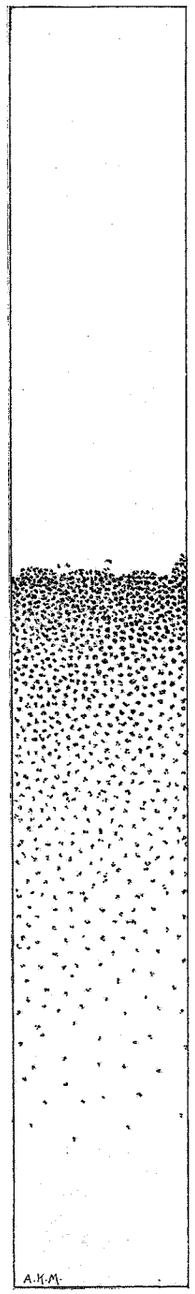


FIG. 113.—Emigrated leucocytes on a glass lath incubated in centrifuged blood for one hour.

We then have the condition represented in *Fig.* 112, i.e., the upper half of the tube is filled with plasma and the lower with corpuscles. The leucocytes, being of slightly less specific gravity than the red corpuscles, are to a certain extent aggregated in the upper layers of the corpuscular portion. If we now place the tube in the incubator for an hour, the leucocytes emigrate on to the glass lath and adhere to it, and if this lath is taken out, washed free from red corpuscles, and stained, it will be found that in the upper half, corresponding to the portion of the lath surrounded with plasma, there are no leucocytes. Towards the centre of the lath, corresponding with the upper corpuscular layer, there are an enormous number of leucocytes adhering to the lath, and these gradually diminish in numbers towards the bottom. If such a lath with the adherent leucocytes be placed on an infected agar plate, it will be found that

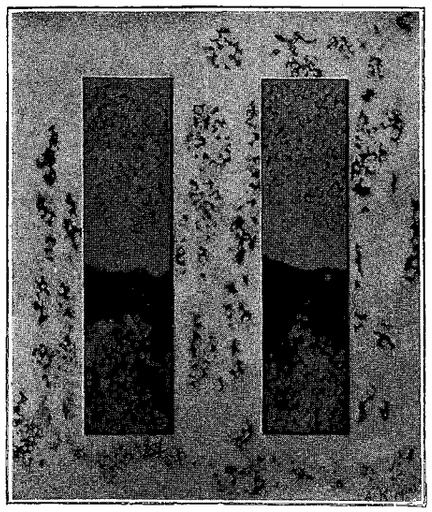


FIG. 114.—Glass laths, such as are shown in *Fig.* 113, imposed on agar surfaces implanted with staphylococcus. In *A* the leucocytes were brought into operation in serum; in *B* in normal salt solution.

the microbes are completely destroyed in the region where there are many leucocytes, while there is no inhibition of growth under the upper half of the lath on which there are no leucocytes. The distribution of the leucocytes on the lath, and the bactericidal effect of these leucocytes, are shown in *Figs.* 113, 114,

It was found also that the leucocytes had the power of destroying the microbes even after they had been washed quite free from serum. In such a case no phagocytosis took place, yet there was a very definite direct bactericidal power exercised by the leucocytes (*Fig.* 114*B*).

We see, then, that leucocytes collected from the blood are powerful bactericidal agents, and it now remains for us to see whether the leucocytes obtained from an infected wound behave in the same way.

Very soon after the infliction of a wound, leucocytes commence to collect in the walls and emigrate into the cavity of the wound, ultimately forming the cellular elements of pus. It used to be taught that pus was a collection of dead leucocytes; but this only approaches accuracy when the pus is obtained from a collection which has stagnated and in which the leucocytes have been in contact with the bacteria for a considerable time. *Fig.* 115 illustrates the bactericidal power of such a pus, in which most of the leucocytes are dead. This pus came from an unopened furuncle. Some of the pus was placed on an agar plate and covered with a cover-slip, when the pus spread out in a thin film between the cover-slip and the agar. The plate was then incubated, and innumerable colonies developed throughout the pus, thus showing that the leucocytes had lost their bactericidal action.

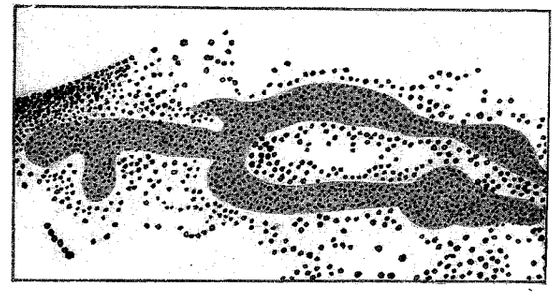


FIG. 115.—Bio-pyo culture. Pus from a furuncle spread out on agar under a cover-glass and incubated. Innumerable staphylococcus colonies have developed in the pus and in the surrounding liquor puris.

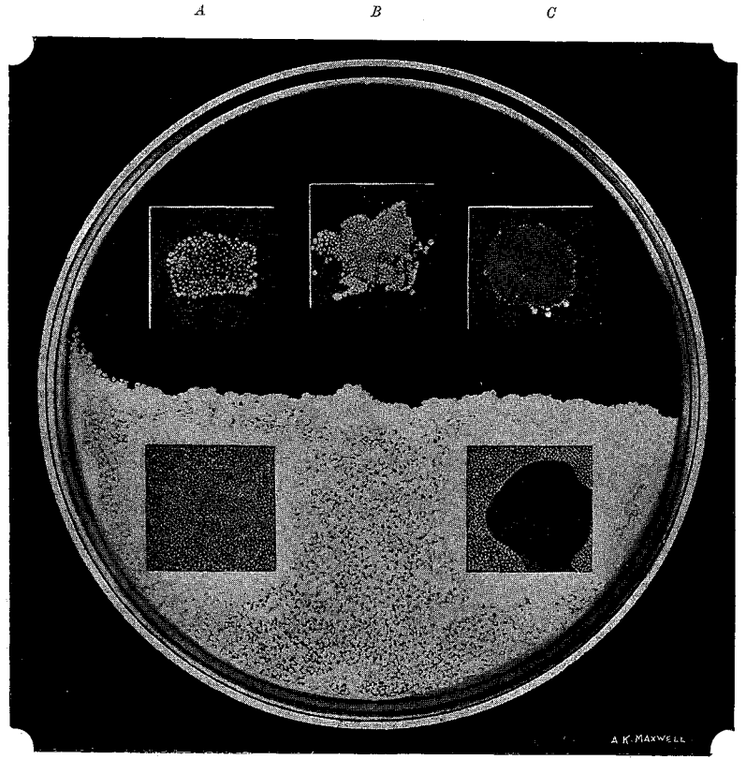


FIG. 116.—Drawing made after 24 hours' incubation of pus from a clean wound imposed under cover-glasses on sterile agar (*A*, *B*, *C*), and on agar implanted with staphylococcus (*D*, *E*). *A*, Pus heated to 48° C. *B*, Dried pus. *C*, Living pus. *D*, Pus heated to 48° C. *E*, Living pus.

This result should be contrasted with that illustrated in *Fig.* 116*C*, which represents fresh pus from an open wound treated in the same way. Here it will be seen that no colonies develop in the pus, but only a few colonies around the margin where some of the microbes have been squeezed out into the pus fluid and away from the reach of the pus-cells by the weight of the cover-slip. It will also be seen from *Fig.* 116*B* and *A* that when the leucocytes are killed by heating them to 48° C., or by drying, many colonies develop throughout the pus, giving a picture similar to that shown in *Fig.* 115 where stale pus was used.

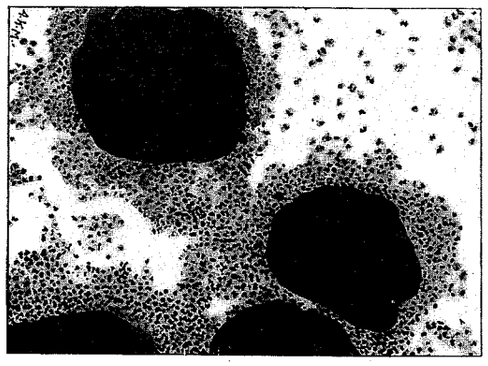


FIG. 117.—Leucocytes of pus aggregating themselves in dense rings round colonies of staphylococcus.

We have shown (*Fig.* 116*C*) that the cells of fresh pus can destroy the microbes contained in that pus, but they can do much more than this. If, instead of using a sterile agar plate, the pus is placed on an agar plate which has been heavily infected with staphylococci or streptococci, covered, and incubated, it will be found that the pus has completely destroyed the added cocci in addition to destroying its own microbes. This is shown in *Fig.* 116*E*, from which it will be seen that there is no growth where there is pus, but a very copious growth where the pus is absent. We see also from *Fig.* 116*D*

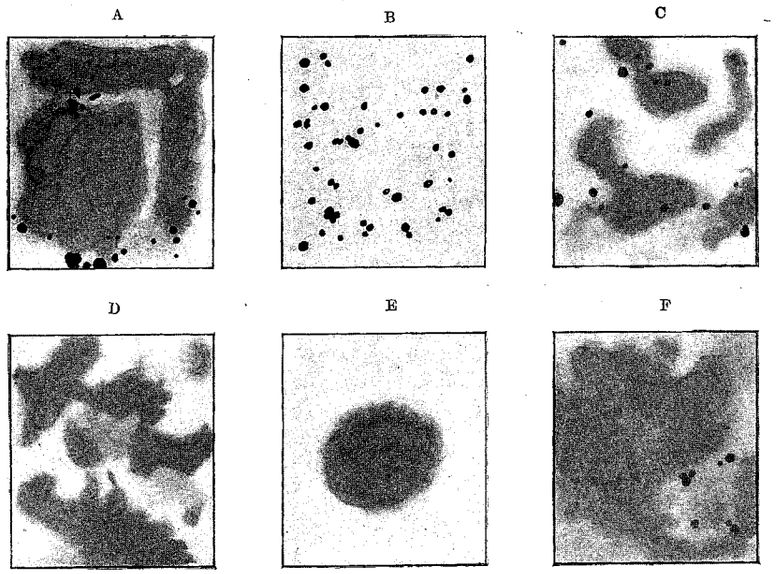


FIG. 118.—Impression cultures of a wound; A, Before washing with normal salt solution; B, Immediately after; C, 1½ hours after; D, 4 hours after; E, 8 hours after; F, 24 hours after.

that if the pus-cells are killed by heating to 47° C. there is no inhibition of the growth of these microbes.

In some cases it will be found that, even with fresh pus, one or two colonies will appear in the substance of the pus. *Fig.* 117 illustrates the microscopical appearance of

the stained cover-glass from such a specimen. It will be seen that there is an aggregation of leucocytes around the colonies, showing that there had been an attempt, although unsuccessful, on the part of the leucocytes to deal with the microbes.

If, instead of making a bio-pyo culture in the way described above, namely, by placing a small quantity of the pus on an agar plate and covering it with a cover-glass, the culture is made by dropping a sterile cover-slip on the wound and immediately transferring it to an agar plate, the result is the same. Few or no colonies develop where pus was adhering to the cover-slip, but they develop in the spaces between the islands of pus. This type of culture I have called an impression culture, and I have used it largely in studying the effect of washing a wound with various fluids, to which experiments reference will be made later. If impression cultures are made in a clean wound before washing with normal saline solution, and again at various times after (the wound meanwhile being covered with protective, so that the whole of the exudate may be retained), results are obtained such as are illustrated in *Fig.* 118. Before the dressing a number of colonies develop between the pus islands. Immediately after the dressing there are many more colonies, as most of the pus cells have been washed away by the saline, but many microbes remain and can grow unhindered. A specimen taken an hour and a half after dressing still shows a few colonies, but the four-hour and eight-hour specimens remain sterile, as here the freshly emigrated leucocytes are capable of dealing with the infection. (That living microbes were

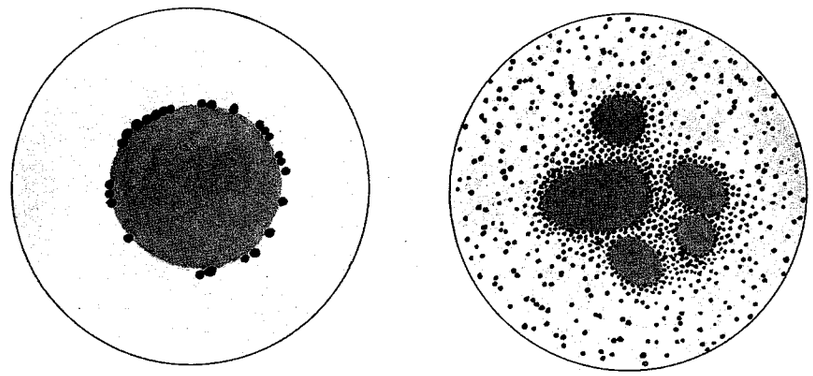


FIG. 119.—Bio-pyo cultures from clean wound. *A*, Pus unaltered; *B*, Same pus mixed with serum.

still present in these specimens when removed from the wound was shown by heating the pus to 47° C. and then placing it under a cover-slip on an agar plate, when many colonies developed throughout the pus.) The specimen taken twenty-four hours after dressing shows practically the same condition as the first specimen. This change from the eight-hour to the twenty-four-hour specimen is probably due partly to the degeneration of some of the cells and partly to the collection of fluid under the protective. The leucocyte does not swim freely in fluid, but apparently only shows active movement when it has some point of attachment, so that in any fluid collection it only exercises its functions fully on the walls of the cavity. A consideration of this is of the greatest value in wound treatment, as it explains why it is necessary to bring the surfaces together in suturing these wounds to obtain healing without suppuration, and why it is that in cavities which normally contain fluid, or into which fluid is rapidly poured as a result of an infection of pyogenic cocci, such an infection tends to have very grave results. The body fluids have normally no bactericidal action on these cocci, and the leucocytes do not have a fair chance of combating the invasion in the presence of much fluid. The effect of excess of fluid in diminishing the effective bactericidal power of the leucocytes can be shown in two ways. Bio-pyo cultures are made with pus as it is removed from the wound, and with the same

quantity of pus to which has been added an equal quantity of sterile serum. It will be found that, in the case where the pus has been diluted with serum, a large number of colonies develop, as many of the microbes find their way into the fluid out of the reach of the leucocytes. This is illustrated in *Fig.* 119.

Fig. 120 shows the result of impression cultures made from the same wound on successive days. On the first and third days (A and C) the wound had been covered for twenty-four hours with impermeable protective which retained the discharges on the surface of the wound. Here it will be seen that a fair number of colonies have developed, especially between the islands of pus. On the second day (B) the wound was covered with a thick layer of absorbent gauze, which immediately soaked up the fluid portion of the discharge and provided a framework for the leucocytes so that they could function without disability. Here we see that the impression culture was almost sterile. On the fourth day this was repeated, with the same result. We see, therefore, that for a flat granulating surface a covering of absorbent material is of great advantage, and it has also been found by experience that if it is desired to suture a wound, close apposition of the surfaces is essential to success.

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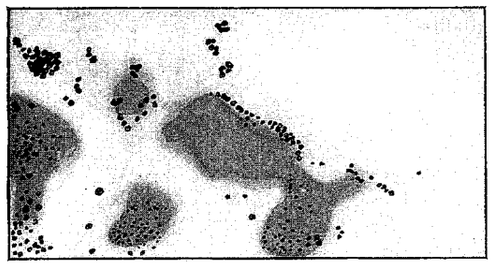
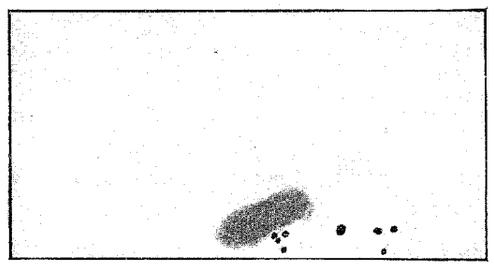


B



C

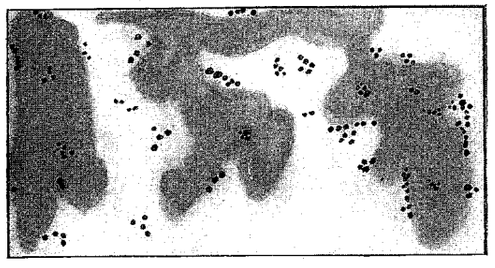


FIG. 120.—The description will be found in the text.

So far I have confined myself almost entirely to local conditions. We have seen that in the early stage it is of prime importance to clean the wound of sloughs and to drain the tissues, in addition to draining the cavity of the wound. In a later stage we have seen that we must make such conditions that the leucocytes can exert their maximum effect on the bacteria. We can, however, by the administration of vaccines in appropriate doses, assist the local defences by raising the antibacterial content of the blood fluids.

The organism which is responsible for almost all the serious infections of war wounds after the first few days is the *Streptococcus pyogenes*, just as it is of wounds in civil practice. For the last twelve or thirteen years streptococcus vaccines have been used for all manner of streptococcal infections with considerable success. One of the most striking series of cases was that published by Western[2]. This consisted of 56 cases of puerperal septicæmia, in all of which the streptococcus was obtained from the blood. By the use of vaccines he obtained 45 per cent of recoveries, as against about 10 per cent in the control cases of a similar nature not treated with vaccine.

In patients with septic wounds it is very common after an injection of streptococcus vaccine to see a drop in the temperature for one or two days, while the patient has a

general feeling of well-being. After a very considerable experience of vaccines in these cases, I feel strongly that no harm would result, but much good would follow, from the routine weekly administration of stock streptococcus vaccine in doses of from 1 to 5 million to all men with septic wounds. This streptococcus vaccine might be combined with vaccines of staphylococcus, *B. proteus*, or *B. pyocyaneus*, if they were found to be present in the wound. All vaccine treatment must, however, be secondary to proper local treatment of the wound.

ANTISEPTIC TREATMENT.

The teaching of the 'antiseptic' school is that one should aim at the sterilization of a wound with a single application of the antiseptic; failing this, it is held that the antiseptic, even if it does not completely sterilize the wound, will kill a large number of the microbes present, so that it will at least diminish the amount of the infection, and will leave a medium in the cavity of the wound in which the microbes will not flourish. It is further held that, provided the antiseptic is used in dilute solution, no damage will be done. We shall see how these teachings bear the light of experiment.

We must first, however, consider the action of these chemical antiseptics on bacteria and on leucocytes outside the body (see *Tables I, II, III*). I shall have nothing to say here regarding the action of these antiseptics on bacteria in a watery medium, as this has no bearing on their bactericidal action in an infected wound. Speaking generally, it has been found that antiseptic solutions show their maximum bactericidal action when they are allowed to act on the microbes in a watery medium; their action is more feeble when the medium is of a serous character; it is still less in blood; it is further reduced when the medium is of a purulent nature; while least of all will an antiseptic act on bacteria embedded in a piece of tissue.

Table I.—INHIBITORY POWER OF ANTISEPTICS ON THE GROWTH OF BACTERIA IN SERUM.

ANTISEPTIC	CONCENTRATION WHICH COMPLETELY INHIBITS GROWTH OF					
	B. Welchii	B. sporogenes	B. pseudotetanus	B. proteus	B. coli	Staphylococcus
Carbolic acid ..	1–200	1–200	1–200	1–200	1–200	1–200
Mercuric chloride ..	1–2000	1–2000	—	1–8000	1–16,000	1–8000
Iodine	1–500	1–500	1–500	1–500	1–500	1–500
Eusol	1–4	+ 1–2	+ 1–2	1–8	1–4	1–8
Dakin's solution ..	+ 1–2	+ 1–2	+ 1–2	+ 1–2	—	—
Chloramine-T. ..	1–60	1–50	1–80	1–50	—	—
Flavine	1–16,000	—	—	1–1000	1–500	1–16,000* / 1–60,000†
Brilliant-green ..	+ 1–1000	—	—	—	1–1000	—
Malachite-green ..	1–200	+ 1–200	+ 1–200	+ 1–200	+ 1–200	—

* If the infection is a heavy one. † If the infection is a light one.
\+ = Greater than.

Table II.—BACTERICIDAL ACTION OF ANTISEPTICS IN PUS.

ANTISEPTIC	LETHAL CONCENTRATION
Carbolic acid ..	1–50
Lysol	1–50
Iodine	1–400
Boric acid	+ 1–50
Hydrogen peroxide ..	0
Flavine	1–500
Eusol	3–1
Dakin's fluid ..	3–1

\+ = Greater than.

Table III.—Effect of Antiseptics on Leucocytes.

Antiseptic	Greatest Concentration which allows Emigration	Concentration which inhibits Phagocytosis by 50 per cent after Exposure for			
		20 minutes	2 hours	5 hours	24 hours
Carbolic acid ..	1–1600	1–500	1–1600	1–1,600	1–2000
Mercuric chloride ..	1–16,000	1–7000	1–16,000	1–16,000	—
Iodine	1–1200	—	1–500	1–1,000	1–1000
Eusol	1–8	—	1–4	1–4	1–8
Dakin's solution ..	1–16	—	1–16	1–16	1–16
Flavine	1–4000	1–500	1–1000	1–500,000	1–2,000,000
Brilliant-green ..	1–16,000	1–7000	—	1,–20000	1–160,000
Malachite-green ..	1–16,000	1–7000	1–6000	1–12,000	1–100,000
Crystal-violet ..	1–16,000	1–7000	—	—	—
Picric acid	1–800	—	—	1–400	1–800

In *Table I* are set forth the results I have obtained in testing the inhibitory power of the common antiseptics on the growth of bacteria in serum, and in *Table II* the concentrations which are necessary to kill the bacteria in pus, while *Table III* shows the strengths of antiseptic which inhibit leucocytic emigration and destroy the phagocytic power of the leucocytes.

These tables must be considered together; but before drawing any conclusions as to the value of this or that antiseptic in the treatment of a septic wound, certain very important factors, which do not appear in the tables, must be taken into account. One of the most important of these is the rate at which the antiseptic acts on the bacteria and on the leucocytes. The antiseptics which act rapidly on the bacteria will also act rapidly on the leucocytes, and any comparison of the value of antiseptics based on their action on bacteria and leucocytes will be quite fallacious unless the chemical is allowed to act on both bacteria and leucocytes for the same time, and unless such a time is chosen as is to some extent comparable with the time during which the chemical will remain active in the wound. The rate at which the antiseptic is destroyed in the wound is also of prime importance, and if a slow-acting antiseptic is rapidly destroyed in the wound, it is unlikely that it will exercise a marked bactericidal effect. The hypochlorites act very rapidly, but they are quenched almost immediately. Flavine, on the other hand, acts very slowly, but much of it is rapidly used up in dyeing the walls of the wound and the dressing.

Can a wound be sterilized by a single application of an antiseptic?

Fig. 121 represents diagrammatically the relation of the bacteria to the tissues soon after the infliction of the wound, and at a later stage when the infection has become established. In the recently inflicted wound, before the infection has grown out, the microbes are scattered throughout the wound in small numbers, either free or enclosed in pieces of earth or clothing. They are confined to those portions of the wound where they have been deposited by the missile. In the later stage, however, where the infection has grown out, the microbes are spread throughout the whole cavity and they have penetrated into the walls of the wound for a considerable depth.

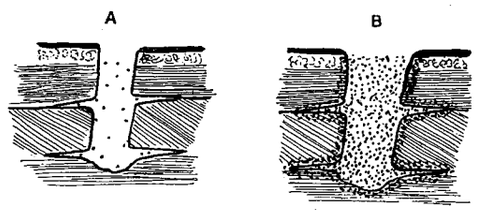


Fig. 121.—Diagram of a wound. A. Recently inflicted. B, After infection has become established. The dots in the cavity in A and in and around the cavity in B represent microbes.

It was very early found that no antiseptic was capable of sterilizing by a single application a wound in which the infection had become established. It will be seen from *Tables I, II,* and *III* that the leucocytes are more sensitive to the action of chemical antiseptics

than are the bacteria, and, in view of this, it is unlikely that any of these antiseptics have the power of penetrating into the tissues and destroying the bacteria without first killing the tissues themselves.

I have attempted to imitate a wound in which the infection is limited to the cavity, by means of a tube such as is depicted in *Fig.* 122A, which has several small processes drawn out to represent the diverticula which exist in all serious recently-inflicted gunshot wounds. Into such a tube is placed some serum which has been implanted with fæces, and which makes a fair imitation of the primary infection of a wound. After this had been incubated so that the infection might spread throughout the whole tube, I tried to sterilize it with some of the antiseptics in common use. The procedure adopted was to pour out the contents and wash the tube three or four times with the antiseptic solution by filling the tube with it and emptying it out again. Then the tube was filled with the antiseptic solution and left for twenty minutes, after which the antiseptic was poured out and replaced by serum. The tube was then incubated for twenty-four hours. The result was that there was no change in the flora of the tube on the second day. The next day I 'dressed' it in the same way, but left the antiseptic in the tube for one hour. This had no effect. The next day the antiseptic was left in the tube for three hours, but it likewise had no effect. Then the antiseptic was allowed to remain in the tube for twenty-four hours, after which it was emptied out, replaced by serum, and the tube incubated as before. Films were made of the contents of the tubes, and the microscopic appearance of these films is shown in *Fig.* 122, B to F, from which it will be seen that there is practically no difference between the tube dressed with normal saline and those in which antiseptic solutions were used. This would seem to indicate clearly that it is impossible to sterilize a wound with an antiseptic, even if it were possible to keep the antiseptic solution in the wound for a long time without dilution, and even if the walls of the wound were not infected. I have carried out the same experiment with brilliant-green and flavine, and found both equally unsuccessful.

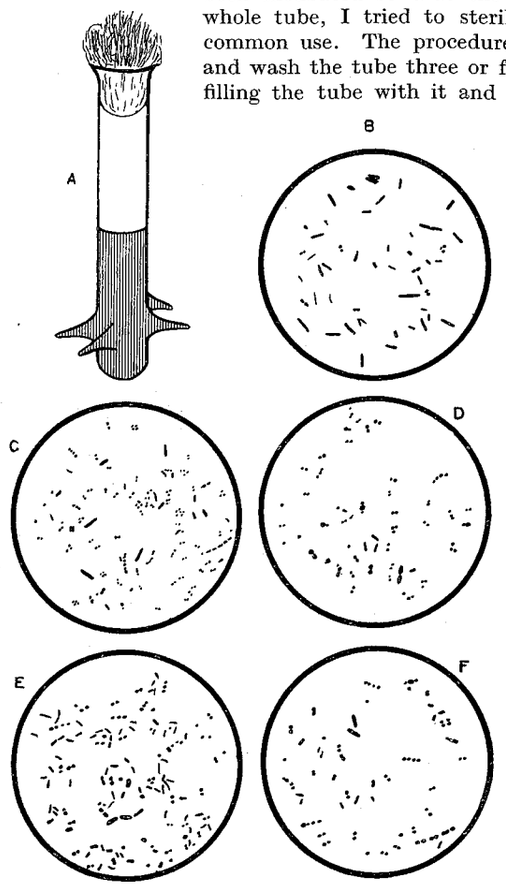


Fig. 122.—A. Artificial wound; B to F. Microscopical appearances of the serum in the artificial wound after being 'dressed' for several days with (B) normal salt solution, (C) 5% salt solution, (D) eusol, (E) Dakin's solution, (F) carbolic acid 1–40.

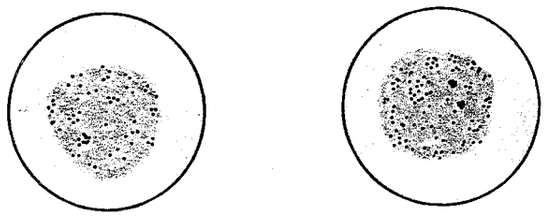
I tried to imitate the infected walls of the wound by placing a ball of infected asbestos-wool, about the size of a pea, in a test-tube, and I then attempted to sterilize it by filling the tube with Dakin's solution, which was changed at intervals during twenty-four hours. Then the Dakin's fluid was emptied out, and the tube filled with serum and incubated. The tube which had been treated with Dakin's solution gave as good a growth as the control tube which had been treated with normal salt solution, and, on examining the growth under the microscope, there was found to be no change in the original flora.

Even if it does not completely sterilize a wound, does the antiseptic at least kill some of the microbes and so reduce the active infection in the wound?

There can be no reasonable doubt that antiseptic applications kill some of the microbes in the cavity of a wound, but it does not follow from this that the active residual infection should be any less. As we have seen, the problem in an infected wound is not a simple one. In addition to any chemical antiseptic we employ, there is a very powerful antiseptic effect being constantly exercised by the protective agencies of the body. The most powerful of these is the leucocyte, which is to be found both in the cavity and the walls of the wound. We have already seen, in the consideration of the experiment illustrated in *Fig.* 116, something of the power of this natural antiseptic. Now if the chemical antiseptic solution employed, in addition to killing some of the microbes in the cavity of the wound, also destroys the leucocytes (and a consideration of *Tables I* and *III* will show that the leucocytes are more susceptible to the action of these chemicals than are the bacteria), then the result on balance may well be that there are actually more microbes that can grow and flourish after the application than there were before.

Observations were made on a wound of medium severity which was exuding much pus and which was being treated with two-hourly irrigations of eusol through Carrel's tubes. Just before one of the irrigations a pipette was introduced along the side of the Carrel tube, and some of the pus was withdrawn. The wound was then irrigated with eusol by the surgeon in the usual way, and five minutes afterwards a pipette was again introduced along the wall of the tube as nearly as possible in the same situation as before, and some of the purulent discharge was withdrawn. The same quantity of each of these two samples of pus were placed on an agar plate and covered with cover-glasses, after which the plate was incubated.

Fig. 123 represents the resulting growth of microbes from pus before and after irrigation, and it will be seen that, so far from there being any diminution in the number of colonies, there is rather an increase in the latter.



Before. 5 minutes after.

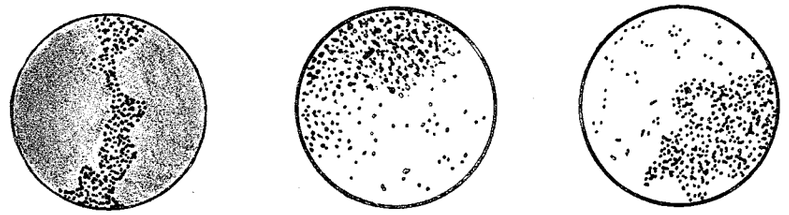
FIG. 123.—Bio-pyo cultures made before and after irrigation of a wound with eusol.

Again, if a clean flat granulating wound be taken and impression cultures be made before and after the application of any of the common antiseptic solutions, it will be found that the amount of growth obtained after the application is greater than it was before. This is shown in *Fig.* 124.

A clean, flat, granulating wound such as this, without pockets or macroscopic sloughs, should be absolutely the most favourable for the observation of any antiseptic action which a fluid may possess. Yet we see that, so far from sterilizing the wound, the growth obtained, even from the surface, is much greater after than it was before the application of the antiseptic. The natural antiseptic powers of the pus are done away with, but the microbes are not completely destroyed, and those which are left are allowed to grow unhindered until such time as fresh pus-cells can emigrate to keep them in check.

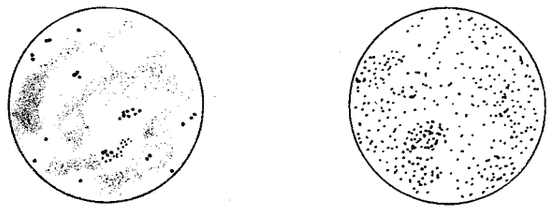
A consideration of the leucocidal property of antiseptics will show us that certain antiseptics are suitable for the washing of a wound, while others are bad. We can take it as certain that these antiseptic solutions will kill the leucocytes in greater dilution than they will the microbes. If we desire, therefore, an antiseptic solution with which to wash

out a wound, we should choose one which loses its antileucocytic power rapidly and which exercises its antiseptic action very quickly. We then have the washing effect of the fluid without doing much damage to the wound. If, however, we use an antiseptic which will



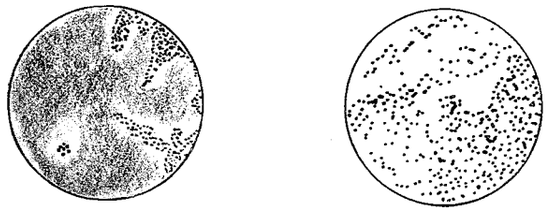
A *B* *C*

A, Before washing. *B*, After washing with carbolic acid (1–40). *C*, After eusol following carbolic acid (1–40).



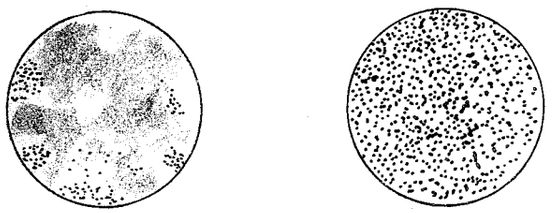
Before After

Eusol.



Before After

0·05 Perchloride of mercury.



Before After

Peroxide of hydrogen.

FIG. 124.—Impression cultures from granulating wounds before and after irrigation with antiseptics.

only gradually disappear, we shall have a considerable interval during which the fluid has no lethal effect on the microbes, while it will completely inhibit the leucocytes within its sphere of action. There is also the question of the stimulation of the growth of microbes by weak solutions of these chemical antiseptics, which will be considered in the next section.

There is only one way in which I have been able to sterilize the surface of a flat granulating wound by means of a chemical antiseptic. Such a wound was taken and divided into two halves. One half was washed with normal saline and the other with eusol; impression cultures were then made, and it was found that there was no very great difference between the two halves (*Fig.* 125, B, C). The whole wound was then washed with eusol, and cultures were again made from the two halves, when it was found that the part which had been originally washed with saline was sterile (E), while the half which had been washed twice with eusol still gave a growth (D).

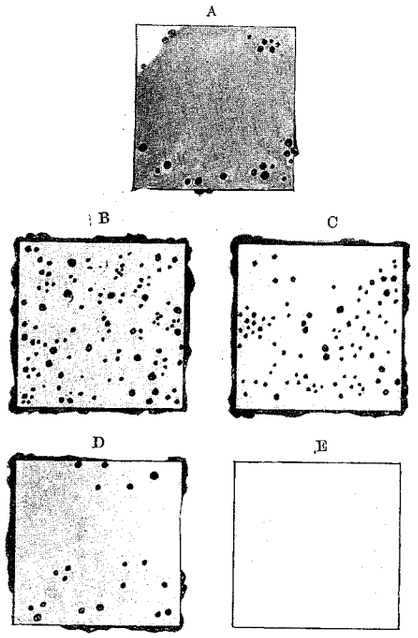


FIG. 125.—B-iopyo-culture from clean wound, and cultures made from it after washing with eusol and physiological salt solution. Description in text.

A preliminary wash with carbolic acid before the eusol did not enable the latter to sterilize the surface (*Fig.* 124). It would seem that the saline solution removed the albuminous material from the surface of the wound, and then the eusol was able to act on the bacteria in practically a watery medium, in which it is very active; this would seem to be the best way of ridding the surface of a wound of bacteria.

One great advantage of eusol and Dakin's solution is that they disappear as active

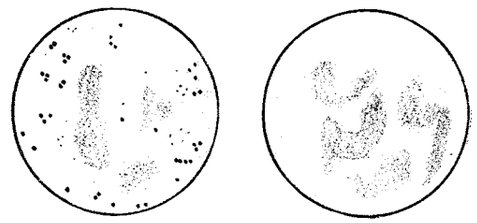


FIG. 126.—Impression cultures of a wound.

chemical agents in a few minutes and do not have any lasting deleterious effect on the leucocytes. This is shown in *Fig.* 126, which shows the results of impression cultures from a wound at intervals after washing with eusol. It will be seen that four hours after dressing, the culture remained sterile, showing that, during this interval, fresh leucocytes had emigrated in sufficient numbers to deal with the microbes on the surface of the wound.

Will the antiseptic application leave in the wound a medium which will inhibit the growth of microbes?

We have seen above that the antiseptics have potentialities for harm, in that they destroy leucocytes in a less concentration than that in which they will kill microbes. They may have yet another action which must be considered—the stimulation of the growth of microbes in serum by these chemicals. In experiments on this subject, we have used as a medium serum to which has been added trypsin sufficient to neutralize the antitryptic power of the serum or actually to render it tryptic. This is an imitation of the condition of things found in pus fluids. The serum was implanted with microbes, and then the antiseptic was added in various dilutions. Usually we have taken microbes which produced gas from serum, as this rendered the experiment easier in that the amount of gas produced gave an indication of the amount of growth of the microbe. The experiments were carried out in small test-tubes, and, as soon as the mixtures were made, melted

vaseline was poured on the top of the fluid in the tube and allowed to set. The gas, as it was produced by the growth of the microbe, pushed up the vaseline plug so that the total amount of gas produced could readily be measured. *Fig.* 127 illustrates this method, which was introduced by Beattie.[3] This figure also shows the effect of carbolic acid on the growth in serum of *B. Welchii*. It will be seen that growth is completely inhibited by 0·5 per cent of carbolic acid, but there is a regular rise in the amount of gas-formation

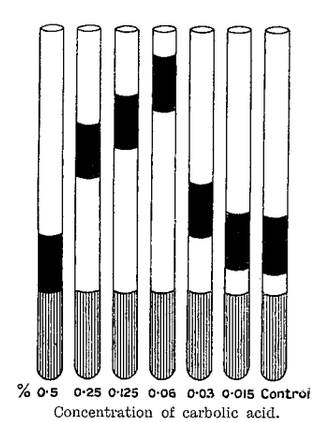


FIG. 127.—Influence of carbolic acid on the growth of *B. Welchii* in serum.

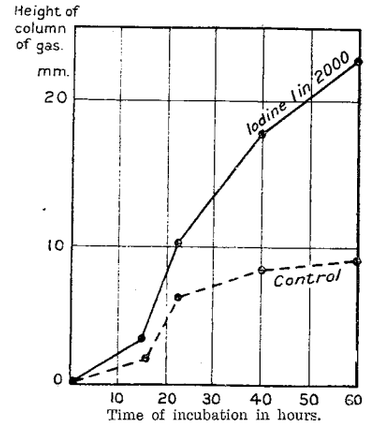


FIG. 128.—Curves of gas formation by *B. Welchii* in serum and in serum containing iodine 1-2000.

up to a maximum in the tube containing 0·06 per cent carbolic acid, after which the amount of gas formed is less; but even in the tube containing 0·015 per cent there is rather more gas-formation than in the control tube which contained no carbolic acid.

The results of this method can also be registered in the form of a chart by recording the amount of gas-formation after varying times of incubation. Such a chart is shown in *Fig.* 128, which depicts the rate of gas-formation by *B. Welchii* in serum and in serum containing iodine 1–2000.

It is not to be assumed, however, that every antiseptic will aid the growth of every microbe. The diagrams (*Figs.* 129A to H) show the effect of antiseptics on the growth of bacteria in serum. The height of the columns indicates the amount of gas

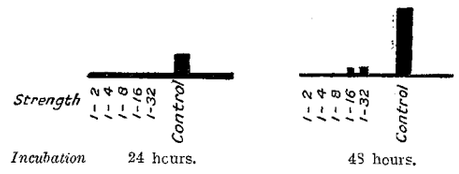


FIG. 129A.—Effect of eusol on the growth of *B. Welchii* in serum.

produced by the organism growing in serum containing the concentration of antiseptic indicated. In every case there was no antiseptic in the control tube, this being replaced by normal salt solution. Dakin's solution and eusol will be seen to act only as inhibitants for *B. Welchii*, whereas both of these solutions strongly favour the growth of *B. sporogenes*. In the experiment illustrated where *B. sporogenes* was grown in eusol and serum, the amount of the bacillus was small, so that it was some time before growth proceeded far enough to manifest itself by gas-formation. In this experiment growth was first noticed in the tube containing equal parts of eusol and serum on the third day,

whereas in the control tube without eusol, gas-production only started on the eighth day. During the prolonged latent period in this experiment some change in the serum was probably taking place which was necessary for the growth of *B. sporogenes*, and it would appear that the eusol had hastened this change.

This experiment is, in some ways, comparable with what happens in a wound in the early stages, where there is only a small implantation of bacilli into a medium consisting

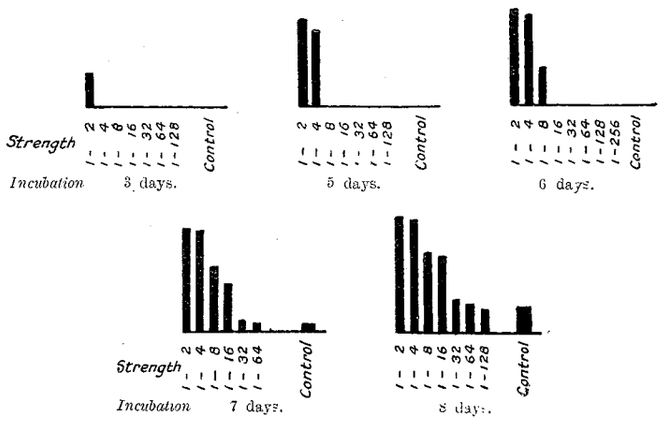


FIG. 129B.—Effect of eusol on the growth of *B. sporogenes* in serum.

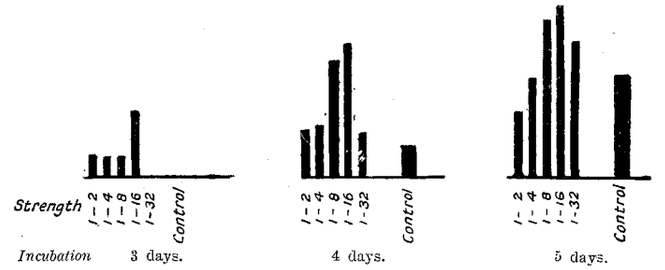


FIG. 129C.—Effect of eusol on the growth of pseudo-tetanus bacilli in serum.

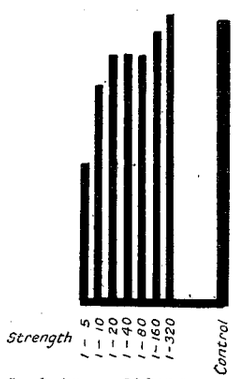


FIG. 129D.—Effect of Dakin's solution on the growth of *B. Welchii* in serum.

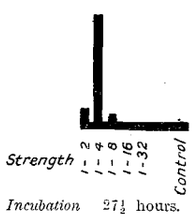


FIG. 129E.—Effect of Dakin's solution on the growth of *B. sporogenes* in serum.

largely of blood and serum, and it seems possible that the introduction of eusol into such a wound would hasten the growth of *B. sporogenes*, which is an almost universal primary infection.

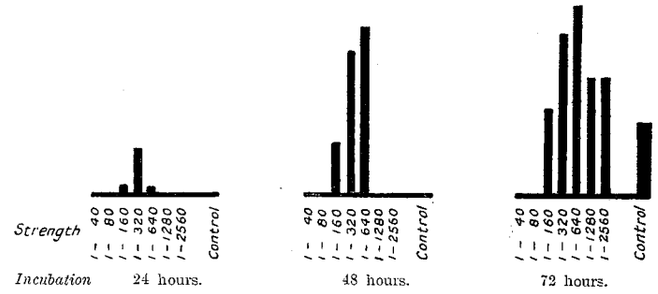


FIG. 129F.—Effect of chloramine-T on the growth of *B. sporogenes* in serum.

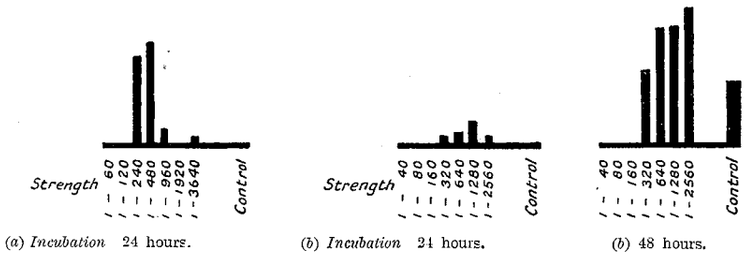


FIG. 129G.—Effect of chloramine-T, (*a*) on the growth of *B. Welchii* in serum; (*b*) on the growth of *B. proteus* in serum.

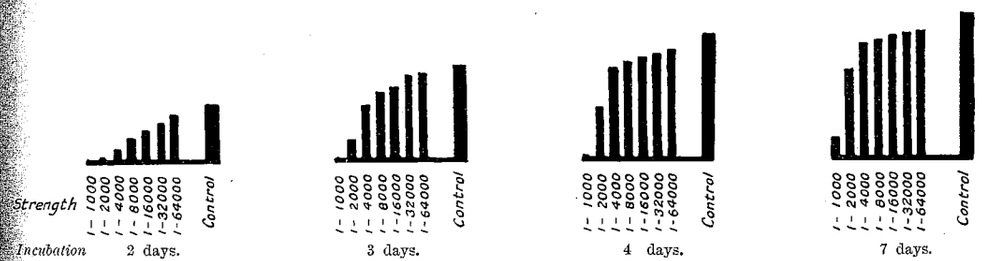


FIG. 129H.—Effect of iodine on the growth of *B. sporogenes* in serum.

From the foregoing experiments we can draw the following conclusions :—

1. It is not possible to sterilize a septic wound with a single application of any of the commonly used antiseptics.
2. Although, doubtless, the antiseptic kills some of the microbes in the cavity of the wound, it also inhibits the leucocytes, so that the active residual infection may actually be increased.
3. The antiseptic does not, of necessity, leave in the wound a medium which is inhibitory to the growth of microbes; on the contrary, it may have a stimulating effect on their growth.

PHYSIOLOGICAL ACTION OF ANTISEPTIC SOLUTIONS IN A WOUND.

In the study of antiseptics for wound treatment the action of the antiseptic on bacteria has been largely followed; but comparatively little attention has been paid to the physiological action of these solutions. I have attempted to make some observations on these lines by using cup-shaped wounds into which I could place a known

volume of fluid, and from the cavity of which I could remove the *whole* of the fluid. In this way I was enabled to ascertain something of the fate of the antiseptic solutions when introduced into a wound, the nature of the physiological reaction of the tissues to such fluids, and the bactericidal effect of the solutions *in vivo*.

Wounds suitable for such experimentation were rare, but I was able to make a series of observations on two wounds of very different character. Both were of old standing; one had a floor of necrosed bone, and from this wound there was a very copious purulent exudate; whereas the other was completely lined with granulation tissue, and there was only a moderate exudate of a serous character. The first wound also was heavily infected with a very mixed flora, while the second was only lightly infected with pyogenic cocci.

The procedure adopted was, in the morning, to empty the cavity completely of the exudate which had collected over-night, and then allow it to collect for a given time (usually about one hour), after which the cavity was completely emptied and the amount of exudate measured. This gave a base line of the amount of natural exudate on the particular day, and also gave information as to the nature of the exudate. The wound was then thoroughly cleansed with normal salt solution until the washings were quite clear, after which a known quantity of the solution on which observations were being made was introduced and left for five minutes. Then the cavity was completely emptied with a pipette, and a fresh volume of the fluid was introduced and removed after ten minutes. This procedure was repeated for longer and longer intervals, the portions of fluid being carefully collected and measured. During the time the fluid was in the wound it was not disturbed, so that really the changes observed were less than they would be in a wound where the fluid is spread in a thin layer over a large surface.

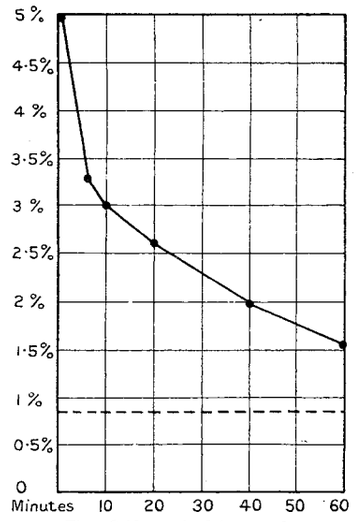


FIG. 130.—Diminution in concentration of 5 per cent saline solution in a wound.

Rate of Disappearance of the Active Agent.—

Physiological Salt Solution.—The salt content of this fluid remained unchanged.

Hypertonic Salt Solution.—The rate of diminution in the concentration of the salt is shown in *Fig.* 130, from which it will be seen that there is a gradual reduction in the strength, so that after one hour it has become reduced from 5 per cent to 2 per cent.

Eusol.—The amount of hypochlorite present in the solution was titrated with potassium iodide and sodium thiosulphate, and the results obtained are shown in *Fig.* 131. The original titre was 0·4 per cent, but this was reduced in five minutes to less than 0·1 per cent. A reference to *Tables I* and *II* will show that this concentration is almost without antiseptic value in serum or pus. In another experiment, in which the eusol was kept agitated during the whole time it was in the wound, the strength had diminished from 0·4 per cent to 0·16 per cent in one minute and to 0·04 per cent in five minutes. (This experiment is recorded in *Fig.* 131 as a broken line.)

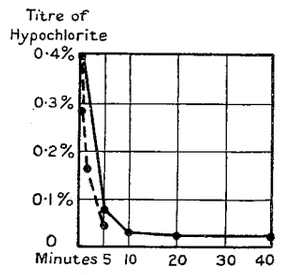


FIG. 131.—Rate of destruction of eusol in a wound.

Dakin's Solution.—This was titrated in the same way as eusol. The results are shown in *Fig.* 132, from which it will be seen that after five minutes the strength had fallen from 0·5 per cent (the original strength) to 0·13 per cent, and after ten minutes to

0·11 per cent. Reference to *Tables I* and *II* will show that after five minutes this solution possesses little, if any, bactericidal power in serum or pus. It is to be noted also, in connection with *Fig.* 132, that after a few seconds (less than half a minute) the strength had fallen from 0·5 per cent to 0·22 per cent of hypochlorite.

Chloramine-T.—This was tested by a method which proved unsatisfactory. It showed, however, that after ten minutes there was an enormous reduction in the strength of the chloramine-T solution.

Flavine.—The strength of this substance was tested by matching the colour of the fluid removed from the wound with solutions of flavine of a known strength. The original strength was 1–1000, and this was reduced after a few seconds to 1–1500, after thirty minutes to 1–2000, after sixty minutes to 1–3000, after two hours to 1–3500, and after fifteen hours and a half to 1–16,000.

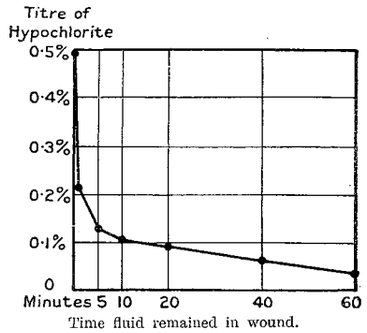


FIG. 132.—Rate of destruction of Dakin's solution in a wound.

Amount of the Exudate.—*Fig.* 133 shows the rate of exudation per hour from the wound before the application of the fluid, during the time the fluid is in the wound, and again for various times after the fluid had been removed. It will be seen from this that there is a very great increase of the exudate into hypertonic salt solution, and for a short time (less than thirty minutes) after the wound has been emptied of this solution. The increased exudate after the removal of the salt solution is due to the presence of salt in hypertonic solution in the walls of the wound, as the exudate removed thirty minutes after withdrawal of the salt solution was distinctly hypertonic.

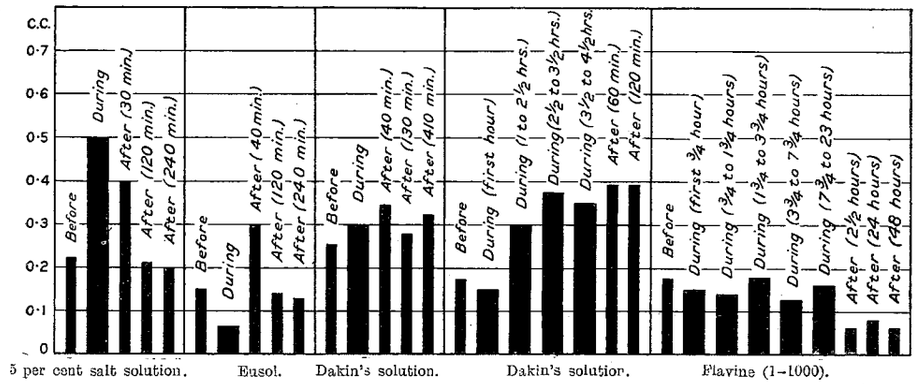


FIG. 133.—Transudation of fluid per hour into a wound cavity before, during, and after the application of salt solution, eusol, Dakin's solution, and flavine.

With eusol there is some diminution of the exudate while the eusol is in the wound (for one hour), but this is followed by a very considerable increase for a time (less than forty minutes) after. When Dakin's solution was placed in the wound for one hour there was a slight increase in the rate of exudation into the Dakin's solution, and this was followed by a further increase for forty minutes after. When, however, Dakin's solution was applied in four changes extending over four and a half hours on the following day, there was a marked increase in the last three and a half hours during which the solution was in the wound, and the rate of exudation was about doubled for at least two hours after the Dakin solution was removed. With flavine up to the time of its removal after

twenty-three hours there was a tendency to a diminution of the amount of exudate. Then, the yellow scum which is characteristic of flavine had formed, and after the flavine was removed, the exudate for at least two days was reduced to something less than half of what it had originally been.

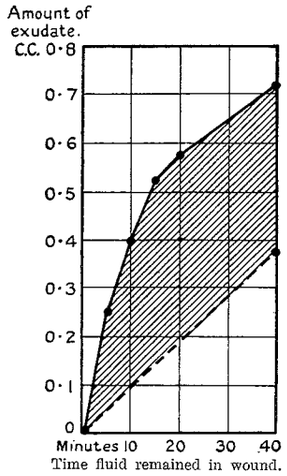


FIG. 134.—Rate of exudation of fluid into a wound cavity filled with 5 per cent salt solution. The continuous line represents the amount of exudate. The dotted line represents the amount of exudate into the empty cavity of the wound on the same day. The shaded area indicates the amount of exudate which is actually due to the salt solution.

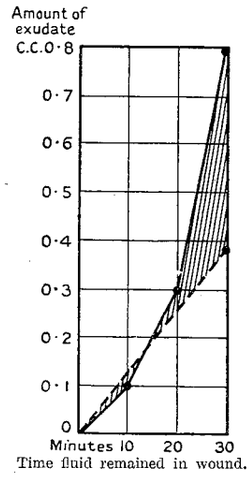


FIG. 135.—Rate of exudation into a wound filled with chloramine-T (4 per cent). Continuous line represents amount of exudate into cavity filled with chloramine-T. Dotted line represents amount of exudate into empty cavity on the same day. Shaded area indicates amount of exudate due to chloramine-T.

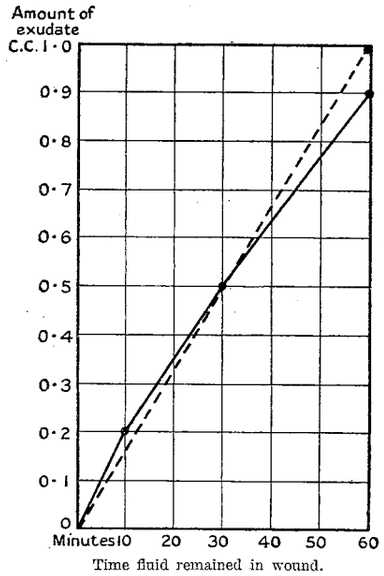


FIG. 136.—Rate of exudation into a wound filled with chloramine-T (1 per cent). Continuous line = exudate into cavity filled with chloramine-T. Dotted line = exudate into empty cavity on same day.

The rate of transudation into hypertonic salt solution is shown in *Fig.* 134. This should be read in conjunction with *Fig.* 130, and it will be seen that there is a very rapid outflow of fluid at first, but this falls off just as the strength of the salt solution diminishes. The shape of this exudation curve shows that the increase in the amount of fluid is directly due to the 'drawing' action of the salt, and not to an irritation of the walls of the wound, as there is no latent period, but when the salt is strongest the amount of exudate is greatest, and immediately the strength of the salt falls off the rate of flow diminishes.

This chart should be contrasted with *Fig.* 135, which shows the rate of exudation into 4 per cent chloramine-T. This concentration of chloramine-T was too strong for wound treatment, and when put into a wound it caused sufficient irritation to produce some capillary oozing after some minutes. Here it will be seen that there is no increase in the amount of exudate for twenty minutes, and then there is a very rapid outflow of fluid which is a reflection of the irritant action of the solution.

Chloramine-T in a 1 per cent solution had practically no influence on the exudation from the wound (*Fig.* 136).

Eusol, like chloramine-T in strong solution, at first shows a slight diminution in the amount of exudate, but this is followed by a very considerable increase (*Fig.* 137).

A consideration of *Fig.* 133 will show that Dakin's solution also has a mild irritant effect on the wound. This solution was only used in connection with the second of the wounds experimented on, from which there was normally only a small amount of exudate ; but the rate of exudation into the fluid, and also into the empty cavity of the wound before and after the application of the fluid, is set forth in *Fig.* 138. It will be seen that at first the rate of exudation into the Dakin solution followed the same line as the exudation before the application ; but it leaves this line, and in forty minutes has almost reached the line depicting the rate of exudation after the application. This is exactly what would be expected if the fluid exercised a mild irritant action.

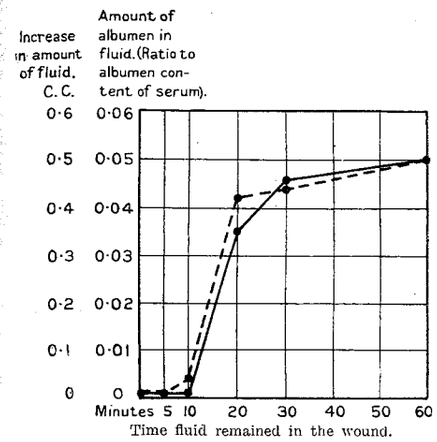


FIG. 137.—Rate of exudation of fluid (continuous line) and of albumin (dotted line) into a wound containing eusol.

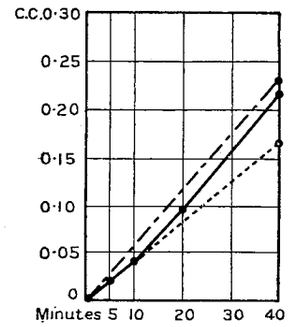


FIG. 138.—Rate of exudation of fluid into a wound cavity containing Dakin's solution, shown by the black line. The dotted line represents the exudate before application, and the thin interrupted line the exudate after application.

Amount of Albumin in the Exudate.—It has been asserted frequently that hypertonic salt solution, while it could by osmotic action attract water from the tissues, could not possibly attract albumins, as these are little subject to osmotic influences. I have measured the amount of albumin which was attracted into 5 per cent salt solution in a wound, and have compared this with the amount which passed out into normal salt solution. The results are seen in *Fig.* 139, from which it is clear that hypertonic salt solution draws an albuminous fluid from the tissues. This Sir Almroth Wright has always maintained, and has supported his contention by many *in vitro* experiments. Here his conclusions are absolutely confirmed by *in vivo* observations.

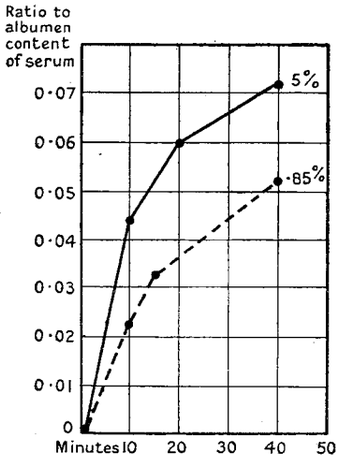


FIG. 139.—Amount of albumin in the exudate from a wound filled with normal and hypertonic salt solutions.

The amount of albumin put out into eusol is shown in *Fig.* 137, which shows that at first there is no albumin in the fluid ; but after some twenty minutes there is a very rapid increase in the amount, and the curve follows very closely the curve of the amount of exudate. This is what would be expected if the increased flow is due to an irritation effect, as such an exudate would correspond merely to the ordinary inflammatory exudate. The same is true of chloramine-T.

Leucocytes in the Exudate.—The number of leucocytes varied very much in the two wounds on which observations were made, and we will deal chiefly with the findings in the first wound, which normally had a large flow of fluid containing many leucocytes (about one million per cm.).

Table IV.

Time during which Fluid remained in the Wound	Normal Salt	5 per cent Salt	Eusol	Chloramine-T 1 per cent
5 mins.	36,000	16,400	? *	? *
10 ,, ...	70,000	36,400	? *	? *
20 ,, ...	346,000	62,000	350,000	91,200
30 ,, ...	434,000	125,000	—	—
60 ,, ...	593,000	234,000	480,000	742,000

* Unable to count. Leucocytes broken up by chemical.

It will be seen from this table that all the non-physiological fluids introduced into the wound had a marked restraining effect on the emigration of the leucocytes. In the fluids, and particularly the hypochlorites, which were left in the wound for only five or ten minutes, accurate counts of the leucocytes could not be made, as the fluid had not lost its leucolytic power during the short time it had been in the wound. With 4 per cent chloramine-T no counts could be made, as the chemical had acted on the albumins with the formation of a glutinous mass. In connection with flavine (only tested in the second wound) the number of leucocytes fell from 4350 per c.mm. in the specimen of flavine which had been left in the wound for one hour to 850 per c.mm. in the specimen left in the wound for four hours. In the specimen left in the wound for fifteen and a half hours, it was impossible to count the leucocytes with even approximate accuracy, as by this time the wound had become covered with the characteristic membrane, portions of which came away with the fluid, and which, on microscopical examination, seemed to be composed chiefly of leucocytes in various stages of degeneration. In the exudate into the cavity for two days afterwards there were almost no leucocytes, apart from those contained in the membranous covering, which persisted for this time.

It will be seen that with the hypochlorite solutions tested (eusol and chloramine-T), the power of inhibition of the leucocytic emigration ceased in about twenty minutes, and after one hour the number of leucocytes in the exudate was almost as great with eusol as, and greater with chloramine-T than, it was with physiological salt solution. This is what would be expected when the rate is considered at which the active agent of these fluids is destroyed in the wound.

Bactericidal Action of Antiseptics tested in Vivo.—With normal and hypertonic salt solutions no bactericidal action was to be expected and no observations were made.

Chloramine-T 4 per cent.—To estimate the number of living microbes in the fluid removed from the wound after various intervals, 10 c.mm. of the fluid was plated in agar immediately after removal. The results obtained in this way with 4 per cent chloramine-T were as follows :—

Table V.

Time Chloramine-T remained in the Wound	No. of Colonies developing from 10 c.mm.
5 minutes	4
10 ,, ...	5
20 ,, ...	30 (surface overgrown with *B. proteus*)
30 ,, ...	79 ,, ,, ,, ,,
50 ,, ...	764 ,, ,, ,, ,,
Preliminary wash with normal salt	872 ,, ,, ,, ,,
Wash with normal salt immediately after the chloramine-T.	800 ,, ,, ,, ,,

We see from this table that even the specimen of chloramine-T which was removed after being in the wound for only five minutes (when the chloramine-T. was exercising its maximum bactericidal power) was not sterile. That the antiseptic had no real effect on the bacterial flora is shown by the fact that the wash with normal salt solution immediately after the application of the antiseptic gave, within the limits of experimental error, the same number of colonies as the wash with salt solution before the experiment started. It is to be remembered that here we are dealing with a solution of chloramine-T which is stronger than that used in the treatment of wounds, and which we have shown to be markedly irritant in the wound.

The microscopical appearance of the chloramine-T removed from the wound after remaining there for fifty minutes is shown in *Fig.* 140, from which it will be seen that it is very similar to ordinary pus, and a consideration of *Table V* will show that the microbes present in the pus were for the most part alive.

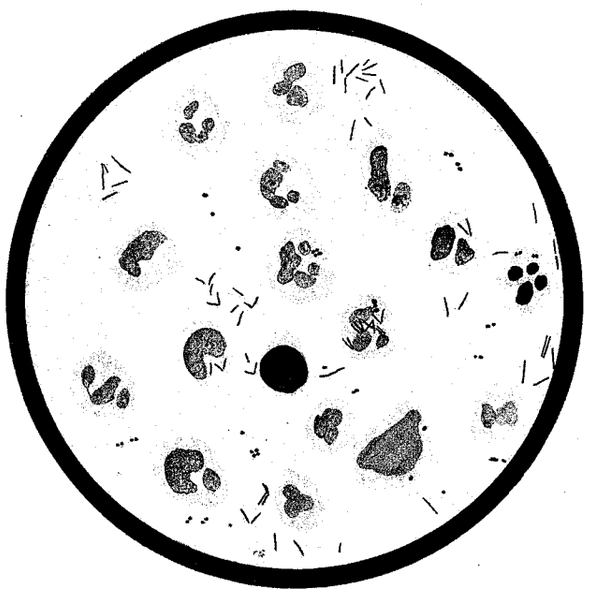


Fig. 140.—Microscopical appearance of fluid removed from a wound 60 minutes after being filled with chloramine-T (4 per cent).

Dakin's Solution. — Ten c.mm. of the exudate before the application of Dakin's solution, and the same quantity of the exudate for one hour after the application of the fluid, were planted out in agar, and showed in the exudate 91 colonies before the experiment and 95 colonies after. In this experiment the Dakin's fluid was renewed four times during four and a half hours. This shows that the application of Dakin's solution to a clean wound without sloughs has no action in diminishing the number of microbes in the wound. If this solution really exercised a bactericidal effect in the wound, one would have expected some evidence of this in the above experiment, and if there is no bactericidal effect manifest when the fluid (renewed four times) has acted for four and a half hours, it is unlikely that there would be any bactericidal effect when the fluid is changed every two hours over several days, as in the Carrel method of treatment. Carrel has claimed that he has been able to sterilize wounds by means of repeated instillations of Dakin's solution, and he attributes to this solution great powers as an antiseptic. To accomplish this sterilization the solution is introduced into the wound every two hours for several days, so that in an average case there may be 60 to 144 instillations. We have seen that after five—or at a generous estimate ten—minutes, the strength of the Dakin solution falls below the point at which it is bactericidal in serum or pus, so that for one hour and fifty minutes out of every two hours there is no antiseptic in the wound. We have seen also that after, roughly, one irrigation with Dakin's solution per hour, the amount of the residual infection is unaltered. It seems inconceivable, therefore, that the Dakin fluid used after the method of Carrel can have any appreciable action as an antiseptic, and it seems reasonable, therefore, to assume that the benefit which Carrel derived from this solution was to be attributed, not to its bactericidal action, but to some other function

which it performed in the wound, and not the least of its functions is its power of rapidly combining with the albumins in the wound and so disappearing as an active chemical agent.

Eusol.—Ten c.mm. volumes of eusol which had been allowed to remain in the wound for varying intervals were, as soon as they were removed from the wound, diluted 1000 times, and of this dilution 10 c.mm. volumes were planted in agar. The result was as follows :—

Table VI.

Time Fluid remained in the Wound	No. of Colonies Developing	Equal to No. of Colonies per c.c. of Original Fluid.
5 mins.	2	200,000
20 ,, ...	50	5,000,000
30 ,, ...	40	4,000,000
60 ,, ...	25	2,500,000

It will be seen that eusol was not capable of sterilizing the cavity of the wound, and that after a few minutes it apparently lost all its bactericidal power.

It is to be noted, also, that the maximum count of bacteria was obtained in the sample of eusol which had remained in the cavity of the wound for twenty minutes. It may be that this is due to the fact that although in the first few minutes the eusol may destroy some of the microbes, the leucocidal effect of the solution will persist for a longer time, so that in the twenty-minute sample the leucocytes have been put out of action for practically the whole period, while the bactericidal action of the solution was only effective for a few minutes. In the samples which had remained in the wound for longer periods, the diminution in the bacterial count might well be due to the activities of the leucocytes, no longer restrained by the action of the eusol, this having been completely dissipated.

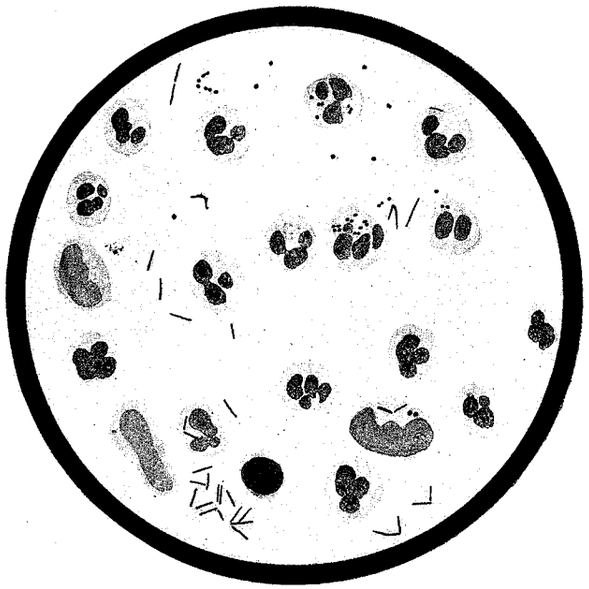


Fig. 141.—Microscopical appearance of fluid removed from a wound 20 minutes after being filled with eusol.

The microscopical appearance of the eusol removed from the wound after twenty minutes is shown in *Fig.* 141.

Flavine.—The number of living microbes in the wound after the application of flavine (1–1000) for a few hours could not be even approximately estimated, owing to the yellow membrane with which the wound had become covered by that time. Portions of this membrane came away in the fluid, and a satisfactory emulsion could not be made. The colonies, therefore, came up in clumps, and the number of colonies in each clump could not be estimated. It appeared, however, that the number of living microbes in the fluid

increased the longer the flavine had remained in the wound, up to fifteen and a half hours, which was the longest time during which observations were made.

Effect of Fluids introduced into the Wound on the Antitryptic Power of the Exudate.—The results obtained in this connection are set forth in *Fig.* 142. It will be seen that hypertonic salt solution reduces the antitryptic power of the exudate slightly for a short time after its application. With eusol the reduction is very marked, but after about forty minutes the exudate had returned to normal. With the Dakin solution applied for four and a half hours the reduction was very marked, and persisted for at least two hours. Flavine had no effect on the antitryptic power.

The effect of this reduction of the antitryptic power is twofold. In the first place, as has been shown by Sir Almroth Wright, this reduction will favour the growth of all the microbes in the wound (*Fig.* 111); and in the second place it will remove the chief obstacle to the tryptic digestion of sloughs. The former would seem, on the face of it, to be a bad effect, whereas the second can only be favourable. A balance must be struck between these two actions, and in different stages of the wound they will have different relative importance. In the early stage, when there are many sloughs on the surface, there is practically always a mixed flora, and the discharge is usually either tryptic or possesses very little antitryptic power. In such a case, therefore, little harm is going to result in a further reduction of the antitryptic power, and the all-important thing in such a condition is to get rid of the sloughs. We see, then, that the hypochlorite solutions, eusol and Dakin's solution, by reducing the antitryptic power of the discharges will favour the tryptic digestion of the sloughs; but another thing to be remembered is that these solutions have some destructive action on the tryptic ferments. We are confronted, therefore, in connection with these solutions with a destructive effect on trypsin and also a very great reduction in the antitryptic content of the discharges. The effect on trypsin, however, is not a very marked one, and it is very possible that the reputation which Dakin's solution has acquired for cleansing a wound of sloughs is due chiefly to its powerful action in the reduction of the antitryptic power of the exudate.

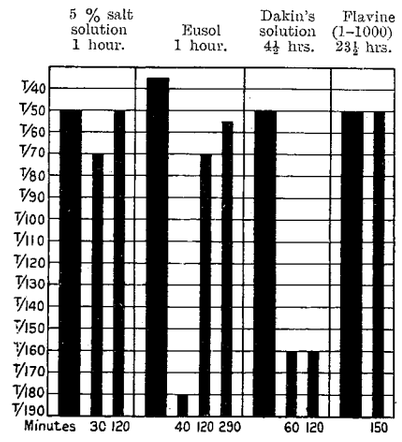


Fig. 142.—Antitryptic power of the exudate into a wound cavity before and after the application of salt solution, eusol, Dakin's solution, and flavine.

Thick columns = antitryptic power of exudate before application.

Thin columns = antitryptic power of exudate after application.

The times recorded show the interval which had elapsed between the removal of the test fluid and the withdrawal of the exudate.

It is true that Dakin's solution has a very marked effect in dissolving cells; for instance, if an emulsion of liver cells be put into Dakin's solution, they are completely dissolved in a few minutes; but this solvent effect is not so evident when connective tissue is placed in the solution. Sloughs are adherent in the wound by virtue of connective tissue, so the direct solvent action of Dakin's solution would have little effect in the separation of these, and it is much more likely that they are separated by a process of autodigestion. So far as I have seen, there is no application which removes sloughs from a wound so quickly as hypertonic salt solution, and we can see that this may well be so, as this solution has no destructive effect on the tryptic ferments, as has Dakin's solution.

Following from the experiments cited above, we see that none of the so-called antiseptics which are commonly used in wounds are capable of exercising any appreciable bactericidal action in an infected wound. We have seen that they are incapable of sterilizing a glass tube with one or two processes much smaller than the diverticula to be found

in a gunshot wound. Much less would they have any sterilizing action on a wound with infected walls (as all wounds have when the infection has become established). We have seen also that when put into a comparatively clean wound they have no sterilizing action. Also, that when a perfectly clean flat granulating wound without diverticula is washed with these antiseptics and impression cultures are made before and after the application, there is no reduction in the number of microbes which develop in the cultures, but, on the other hand, there is usually an increase in the number of colonies owing to the destruction or removal of the chief natural means of defence, namely, the leucocytes.

It is very noteworthy that the antiseptic which has the most marked inhibitory power on the growth of microbes in a serous fluid should have been found unsuitable for the continued treatment of a wound. Of all the antiseptics in common use there can be no doubt that flavine exercises the most powerful inhibitory action on the growth of microbes in serous fluids ; yet it has been generally found in France that this fluid was not suited for the continued treatment of an infected wound. This is, in all probability, due to its very strong and continued action in destroying the leucocytes in the wound. It was introduced partly because it was supposed to be innocuous or almost innocuous to leucocytes ; but I have shown elsewhere that it is a slow-acting but extremely powerful leucocidal

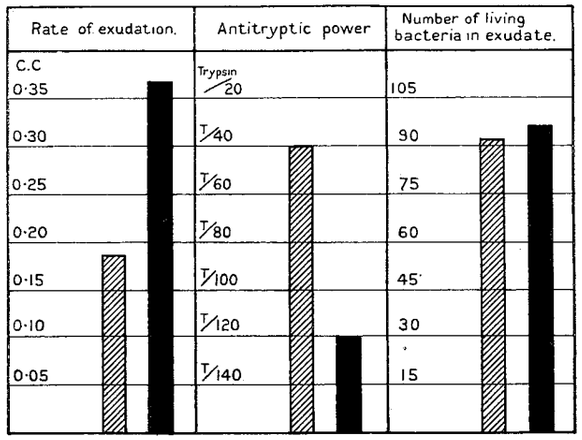


FIG. 143.—Effect of Dakin's solution applied to a wound for 4½ hours in four changes. Shaded column = exudate before application. Black column = exudate after application.

agent, destroying them in twenty-four hours in as great a dilution as 1–2,000,000. The result of this seems to have been that all reparative processes in the wound come to a standstill.

Eusol and Dakin's solution, on the other hand, have found very many supporters in the treatment of wounds in which the infection has become established. We have seen that when these are put into a wound the active agent disappears in a few minutes, after which the natural defensive mechanism can proceed unhindered. These solutions also cause a mild irritation in the wound, which results in a greatly increased flow of lymph from the tissues, thus imitating the tissue-draining character of hypertonic salt solution. The exudate, after the application of these fluids, also has a very low antitryptic content, thus favouring the tryptic digestion of the sloughs. The bactericidal action of these solutions in a septic wound appears to be negligible. *Fig.* 143 illustrates graphically the results of the application of Dakin's solution to a wound, so far as they could be tested. It will be seen from this that whilst as regards the amount and the antitryptic power of the exudation there were marked changes, yet as regards the number of bacteria in the exudate before and after the application of the fluid there was no change. In view of

this, it is not unreasonable to assume that the benefit which the wound derived from the application of Dakin's solution was due, not to its bactericidal action, but to its stimulation of the physiological processes occurring in the wound.

The only other agent which has obtained any great measure of support in the later treatment of infected wounds has been Rutherford Morison's B.I.P.P. This is not in itself a bactericidal substance, and in contact with tissues or tissue fluids we have never been able to demonstrate that it has any bactericidal action ; but just as it has no bactericidal action, so it has no toxic or repellant action on the leucocytes, and it can easily be demonstrated that these will emigrate in large numbers on to a surface to which B.I.P.P. has been applied. We have seen (*Fig.* 124) that when a wound is washed with a solution of the common antiseptics, the number of colonies which develop in an impression culture is greater after the washing than before. Rutherford Morison has always insisted that after surgical treatment of the wound and the application of B.I.P.P. the wound should not be touched for a considerable time, and it is possible that much of the success of this treatment has been due to leaving the wound alone. The actual infection would be dealt with by the leucocytes. It is possible also that the greasy nature of the application prevents the shutting up of the discharges which would take place in a wound left alone after treatment with a watery fluid. If this were so, then pastes containing substances less poisonous than bismuth and iodoform would give equally good results, and certain reports seem to show that this is the case.

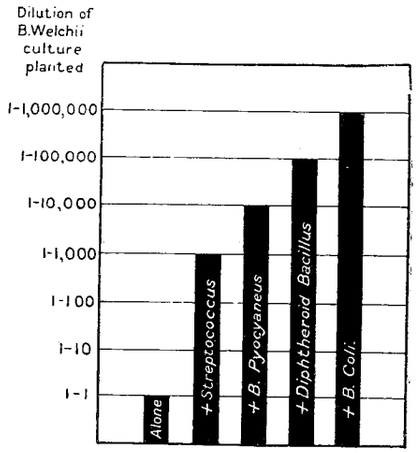


FIG. 144.—Influence of the presence of other bacteria on the growth of *B. Welchii* in serum. Columns represent the dilutions in which growth of *B. Welchii* occurred.

In view of the observations I have made, and which are quoted above, I venture to suggest *that the antiseptics at present in use will only exercise a beneficial effect in a septic wound if they possess the property of stimulating or conserving the natural defensive mechanism of the body against infection.*

If such a thesis be true, then it brings the 'antiseptic' and the 'physiological' treatments on to the same basis, and it also makes it necessary, in the estimation of the value of an antiseptic, to study its effect on the tissues more than its effect on the bacteria.

ANTISEPTICS IN PROPHYLACTIC TREATMENT OF WOUNDS.

Up to the present we have been dealing with the treatment of a wound in which the infection has become established ; but even more important is the prevention of infection in the treatment of war wounds, and it is here that antiseptics find their true application. It has often been said that these war wounds contain already so many microbes that it is little use taking extreme precautions regarding their treatment, as a few microbes more or less will make no difference. In *Fig.* 144 I have shown graphically the effect which certain microbes have in stimulating the growth of *B. Welchii*, and we have shown that almost all the microbes which constitute the primary infection of the wound, or which are put in later by the surgeon or the sister, have a mutually beneficial symbiotic action. It follows from this that even where the wound is apparently badly infected, the same care should be exercised in the dressing as would be taken if the wound were a sterile one. It has been shown[4] that *Streptococcus pyogenes*, which is responsible for most of the severe sepsis in the later stages, and causes more deaths from sepsis than all the others put

together, is present in only 15 per cent of the wounds when they arrive at the clearing stations.

I have found that this organism is present in about 20 per cent of cases when they arrive at the base when they have been sent on immediately after excision and packing of the wound. After they have been at a base hospital, however, for more than a week, *Streptococcus pyogenes* is present in almost all the wounds (I speak here only of wounds which have not been sutured but which have been repeatedly dressed). It would appear, therefore, that faulty technique in dressing the wounds was responsible for the almost universal presence of this, the most dangerous of all the microbes found in the wound. The only way to avoid this spread of infection is by the most careful technique in dressing and by the lavish use of antiseptics *outside* the wound. All these antiseptic solutions are powerful bactericidal agents in a watery medium, and outside the body the strength can be increased, as no question of damage to the tissues comes in here. In civil hospitals the equipment is such that sterilization by heat has largely displaced sterilization by antiseptics, but in military hospitals in the field the equipment is, of necessity, more primitive, and there sterility can only be obtained by the lavish use of chemical antiseptics.

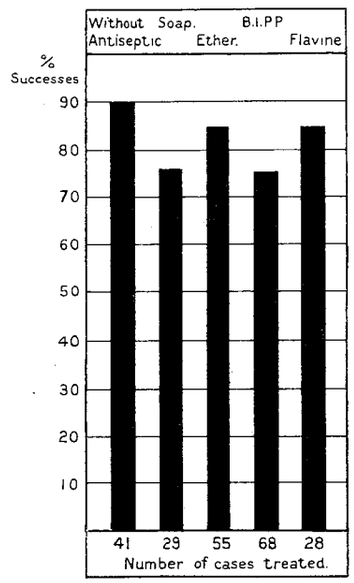


Fig. 145.—Results of wound treatment by various agents.

In connection with the prophylaxis of infection, we have the question of the early treatment of the wound before the infection has had time to grow out and while it is confined to the cavity of the wound. There is no question about the efficacy of early and thorough surgical treatment ; that has proved its value without a doubt. There has been controversy, however, regarding the value or otherwise of chemical agents as adjuncts to this surgical cleansing. An experiment was carried out about a year ago at No. 10 Casualty Clearing Station in which primary suture was done on wounds excised and treated with various agents. The results obtained are shown in graphic form in *Fig.* 145, from which it will be seen that there is no great difference between the number of successes whatever the method of treatment. It is true that the largest percentage of successes was in the group sewn up without the application of any antiseptic ; but this is explained away by saying that in most cases the surgeon only dared to sew up wounds in this way when they were not very serious. It has been found in several large series of delayed primary sutures that the results were practically identical whether the wound had been packed with gauze soaked in an antiseptic, or whether plain gauze had been used as the primary dressing after excision of the wound at the casualty clearing station.

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 packs. In all cases I found microbes present, and there seemed to be little difference between the bacterial content of the different varieties of pack. This observation, taken into consideration with the results of delayed primary suture at the base and with primary suture at the front, would seem to show that the antiseptic plays no part in the primary treatment of a wound.

If this is so, then there is a very great disadvantage in the use of an antiseptic from the surgeon's point of view. It is very difficult for the surgeon not to be deluded into the belief that he has in the antiseptic a second string to his bow, and consequently it will tend to make him less careful in his surgical treatment of the wound. If he knows that he has

nothing to fall back on, then, even with the most conscientious individuals, the surgery would improve. Because of this alone it would be well if the treatment of the *wound* with antiseptics in the early stage were abandoned and the surgeon relied on his skill alone. All the great successes of primary wound treatment have been due to efficient surgery, and it seems a pity that the surgeon should wish to share his glory with a chemical antiseptic of more than doubtful utility.

Many of the experiments quoted above were the results of 'team' work, and I do not wish to close without thanking my chief, Sir Almroth Wright, and my colleagues at St. Mary's Hospital and at No. 13 General Hospital, especially Captains Douglas and Colebrook, for permission to use work in which they took a share.

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[2] *Lancet*, 1912.
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