CHEMOTHERAPY: YESTERDAY, TODAY AND TOMORROW

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The title which I gave for this lecture was too ambitious. It would require a whole series of lectures to do it justice. I must limit it to chemotherapy of bacterial infections, but even that subject is too large for one lecture, so I propose to restrict myself for the most part to chemotherapeutic happenings of which I have first-hand knowledge.

Bacterial infections have existed since time immemorial, and physicians, in all ages, have tried to deal effectively with them. It has been our good fortune to have lived in an era when many of these infections have, for the first time, been brought under control, and there is promise of more advances in the near future.

Until the middle of last century there was practically no knowledge of the bacterial nature of the infections, so before that time everyone was working in the dark, and many and curious were the prescriptions used in the combat against bacterial infections; but we shall get no profit by discussing these.

In the consideration of almost every branch of bacteriology we go back to Pasteur and the latter half of the nineteenth century. Pasteur proved that certain fermentations were due to the action of microbes and that microbes were living objects which did not arise *de novo* from the putrescible material but were descendants of previously existing organisms. Pasteur himself did not do any serious work on chemotherapy, but his earlier bacteriological work stimulated Lister, who had putrefaction of wounds very much at heart, to engage himself on the subject.

There are some who use the word chemotherapy in a very limited sense to cover those methods in which the chemical is administered in such a way that it gets into the blood and attacks the infecting microbes through the circulation in concentrations sufficient to destroy them or modify their growth. This is too narrow a definition, and I shall use chemotherapy to cover any treatment in which a chemical is administered in a manner directly

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injurious to the microbes infecting the body. In this latter sense antiseptic treatment comes under chemotherapy – call it local chemotherapy if you like. The same general laws govern the treatment whether it is local or systemic but there are certain particulars in which one has to draw a distinction. There are many chemicals used locally which are so poisonous to the human organism as a whole that they cannot be used for systemic treatment, but large numbers of these, although they had considerable vogue in the past when perhaps there was nothing better, are practically useless as chemotherapeutic agents except in the prophylactic sense. On the other hand, there are some chemicals, e.g. the sulphonamides, which are powerful agents for systemic treatment but which are frequently of little use when locally applied to a suppurating area, as their action is neutralized by substances occurring in the pus.

If a chemical is to be effective in the treatment of established infection it is necessary that, in addition to killing or inhibiting the growth of the microbes on the surface, it should be able to diffuse into the tissues to reach the microbes there. In a septic wound there are, of course, microbes in the cavity of the wound, but far more important are those which have invaded the walls.

Lister, like all other surgeons of 90 years ago, was struggling with the problem of septic wounds. To him, Pasteur's work came as a ray of light in the darkness. Putrefaction was due to living microbes which were introduced from outside. He set to work to prevent them being introduced. He cleansed his hands and his instruments, and treated them with chemicals, and he used a carbolic spray to kill bacteria in the air and prevent them reaching his operation wounds. In this way he revolutionized surgery.

That was prophylactic chemotherapy. Lister himself recognized that carbolic acid, which was his standby as an antiseptic, was very poisonous to the tissues. There are times, however, when if it is possible to kill all the bacteria in an infected area with a chemical it may be worth while to sacrifice certain tissues locally, but the usefulness of such toxic chemicals is very strictly limited.

As a result of the success of Lister's antiseptic treatment, a large variety of chemicals were introduced as local chemotherapeutic agents for the treatment of localized infections. In time, bacteriology was put on a sound basis, and the antibacterial effect of these chemicals could be tested in the laboratory. In the early days of the laboratory investigation of these antiseptics (or local chemotherapeutic agents), little attention was paid to anything but their action on bacteria in a watery medium. This resulted in very high values being given to substances like mercuric chloride, which could be diluted about half a million times before it lost its power of inhibiting the growth of bacteria when tested in watery medium, but which was largely 'quenched' in the presence of serum or blood.

But none of these chemicals had much effect in destroying bacteria once

they had invaded the tissues. I commenced medicine in the early years of this century. Then Lister's methods were rather discredited – asepsis had taken the place of antiseptics for prophylactic chemotherapy, but for the treatment of infections which were already established, a great variety of chemicals were used. Carbolic acid, boric acid, mercuric chloride, silver salts, iodine, etc., were used extensively on septic conditions, but there did not in most cases appear to be any striking benefit except perhaps in some superficial infections. These chemicals were used quite empirically and were, I suppose, a relic of the antiseptic days when they had proved valuable in prophylaxis. They failed in treatment. They were either non-diffusible, or if they were diffusible they poisoned the tissues more than the bacteria. One of the first things I learnt in the casualty room was not to put a carbolic compress on a septic finger or carbolic gangrene was likely to result.

Then came the war of 1914–18. The aseptic surgeons were suddenly presented with masses of wounds, all of which became infected. The primary infection was from the soil and the soldiers' clothing and was largely anaerobic, but after a week or more in hospital this was replaced by the usual septic infection of civil life – mainly staphylococci, streptococci and coliform and diphtheria bacilli.

Into these wounds all manner of chemicals were poured in an attempt to destroy the infecting microbes. It was not so difficult sometimes to get rid of the majority of the microbes in the cavity of the wound – they could for the most part be washed out by simple irrigation with normal saline – but none of the chemicals had much action on the bacteria in the infected wound walls.

I might here show you a simple experiment which illustrated the inability of chemicals to sterilize even the cavity of a wound. From a test-tube some small processes were drawn to imitate the irregular processes in the cavity of a war wound. The tube was now filled with serum and infected with the usual bacteria which were found in wounds. Here we had an irregular infected cavity, but there was no possibility of the microbes invading the walls (Fig. 1). The 'artificial wound' was 'dressed' by inverting the tube and allowing the fluid to escape. This was replaced with an antiseptic which was allowed to remain in the tube for various times up to 24 hours, after which it was poured out and replaced by serum. The tube was then incubated, and next day there was a copious growth of bacteria with all the chemical antiseptics tested. The antiseptic had been unable to diffuse into the processes and kill the bacteria there, so as soon as the antiseptic was removed they grew out again and contaminated the whole tube.

Another observation made in the 1914–18 war is of some importance in local chemotherapy. In 1917 probably the most favoured method of treatment of a septic wound was the Carrell Dakin treatment. Dakin's fluid (sodium hypochlorite) was instilled into a wound every 2 hours. I had an opportunity of studying the length of time that Dakin's fluid remained active in a wound; I found a cup-shaped wound into which I could put a fluid and

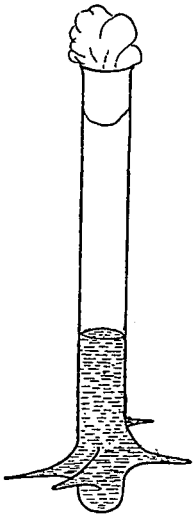

Fig. 1. Artificial wound.

withdraw the whole of it after any interval. When Dakin's fluid was left in such a wound for 10 minutes its potency had diminished below the limit at which it was antiseptic in serum. It followed from this that, for 1 hour and 50 minutes out of every 2 hours, there was no effective chemical antiseptic in the wound. But Dakin's fluid had another quite unexpected action. After it had been applied, it caused a marked increase in the transudation of fluid from the walls of the wound which persisted for some time after fluid was removed.

Fig. 2 illustrates this increased transudation. Incidentally the number of living bacteria was not reduced in the exudate after the application of Dakin's fluid for 4½ hours.

I suggest that the chief virtue of Dakin's fluid was not direct antiseptic action, but that it lay in this power of stimulating the exudation of fluid from the infected walls of the wound, thus draining the oedematous tissues just as did hypertonic saline solution, which was another favourite dressing for a septic wound. But Dakin's fluid *in vitro* in the absence of serum or pus was a powerful antibacterial agent, so all its benefits in treatment were ascribed to its direct antiseptic action.

It is desirable in the investigation of the action of these chemicals to see how long they remain active in the body, and before we class them as chemotherapeutic agents we should see, if possible, whether the apparently beneficial effect is due to a direct antibacterial action.

I have said that Lister recognized the local toxic action of his favourite antiseptic, carbolic acid, but the toxic action of many of its successors was not so obvious and was sometimes forgotten.

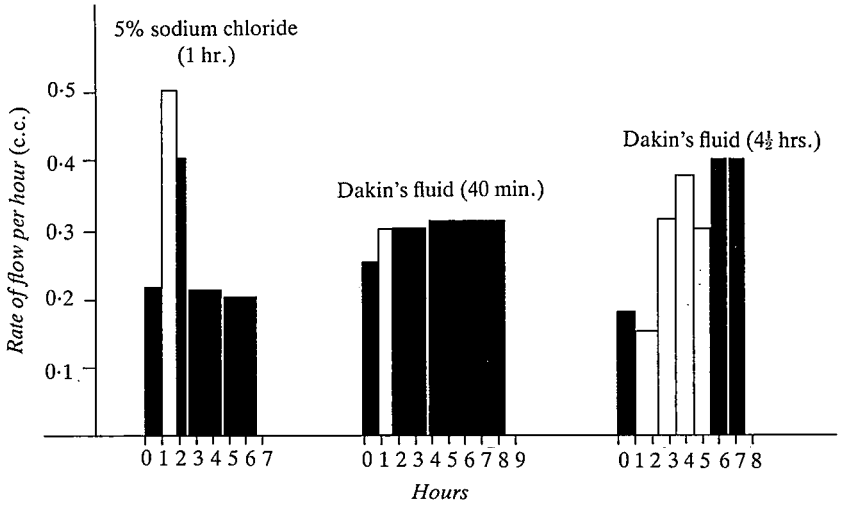

Fig. 2. Increased transudation. Dakin's fluid. Black columns = before and after. White columns = during application.

Later, and especially in the war of 1914–18, some notice was taken of the action of these chemicals on cells and especially on leucocytes, as it was not difficult to test the effect of a chemical on leucocytic function. Most usually it was the phagocytic power of the leucocytes which was tested, but the methods adopted did not always give a true picture. The effect of the chemical on bacteria was tested by its power to inhibit growth and its effect on leucocytes by its power to inhibit phagocytosis. This at first sight seems a perfectly good method, but actually the chemical acts on the bacteria during a period of hours, whereas in the phagocytic experiments the maximum time of action was 15 minutes. When blood is mixed with bacteria phagocytosis takes place very rapidly, and even in 5 minutes the cells will take up large numbers of microbes. That being so, if a chemical is added to the mixture which does not have a *rapid* lethal action on the leucocyte the latter continues to phagocyte the bacteria, and if the observation is ended after 15 minutes quite a false idea is obtained as to the destructive action of the chemical on the leucocyte. Acriflavine is a good example of this. It is a slow-acting bactericidal and leucocidal agent. If its antileucocytic power is tested only for 15 minutes it has been found that it requires a dilution of 1 in 500 to reduce the amount of phagocytosis by 50%. If, however, the chemical is allowed to remain in contact with the blood for 5 hours before the phagocytic test is made, it is found that a 1 in 500 000 dilution will cause a 50% reduction in the amount of phagocytosis. As it takes a 1 in 200 000 dilution to inhibit the growth of bacteria the 'Therapeutic index' calculated after 15 minutes' exposure of leucocytes is 400, whereas if the time of exposure had been 5

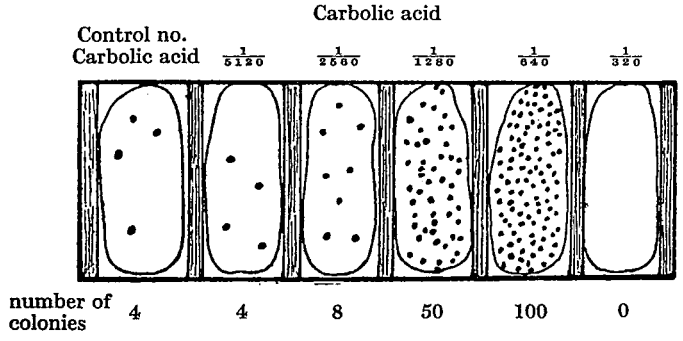

Fig. 3. Effect of carbolic acid on bacteria and leucocytes in human blood.

hours (a more reasonable time) it would have been 0.4 – a considerable difference.

In 1924 I adapted Wright's slide-cell method to show in one experiment the action of chemicals on bacteria and on leucocytes. Dilutions of the chemical were made in normal saline, and to these was added an equal volume of human blood suitably infected with the test bacteria (staphylococci or streptococci). These mixtures were run into slide cells, sealed and incubated.[1] Normal human blood kills off about 95% of the bacteria, but if the leucocytes are removed the bactericidal power disappears. In the case of all the chemical antiseptics in use there was a range of concentration where the chemical destroyed the leucocytes without interfering with the growth of the bacteria. This destruction of the leucocytes removed the natural antibacterial power of the blood, and resulted in an increased growth of the bacteria. This I regard as the most important series of experiments I have ever done, and it had a certain bearing on more recent advances of which I shall speak later.

Fig. 3 shows the result obtained with carbolic acid. There is an antileucocytic zone of concentration resulting in increased growth of the bacteria, and at a concentration of 1 in 640 the bactericidal power of the blood is completely destroyed and every microbe grows.

Let us now come to chemotherapy in the narrower sense, i.e. the attack on the infective microbe through the circulation.

There was the mercury treatment of syphilis. Mercury was swallowed, injected or rubbed into the skin. There were no tests proving that it ever reached the circulation in concentrations inimical to the spirochaete, but from the clinical results we may presume that something happened after a strenuous course of mercury which influenced the disease. With no more

[1]With slow-acting antiseptics the chemical was mixed with the blood and allowed to stand for a suitable interval, after which the bacteria were added and the mixture incubated.

evidence we might assume that potassium iodide had some direct action on the infective agent of syphilis, actinomycosis and other diseases.

In the early days of antiseptics it was shown that formalin inhibited the growth of the tubercle bacillus in quite high dilution. An eminent physician in the days of my youth recommended that formalin should be injected intravenously for the treatment of tubercle. I should like to show what happens when formalin is added to blood infected with *Staphylococcus*, an organism at least as susceptible to its action as is the tubercle bacillus.

The experiment is similar to the one I have already described with carbolic acid and the result is much the same; there is a range of concentration which encourages growth by destroying the leucocytes. Actually the amount administered was very much less than that necessary to influence the growth of the bacteria in any way. It was fortunate, perhaps, that sufficient of the chemical could not be injected to destroy the leucocytes (Fig. 4A).

Exactly the same may be said of quinine, which was recommended as an injection for the treatment of streptococcal septicaemia (Fig. 4B).

Eusol, too, was recommended in 1915 as an intravenous injection for *Streptococcus* septicaemia. I show you the result of mixing Eusol with infected human blood (Fig. 4C). If several litres could have been injected, the leucocytes would have suffered, but fortunately that was not possible.

Mercuric chloride has been recommended as an intravenous injection for streptococcal septicaemia. This is more interesting. When it is tested with *Staphylococcus* it gives a result similar to those quoted above, but when *Streptococcus pyogenes* is used as the test organism an extraordinary result is obtained (Fig. 5). Here 1/20 000 destroys leucocytic action and allows the streptococci to grow, but weaker dilutions completely stop growth of the streptococci – but only if the leucocytes are present. The leucocytes cannot do this by themselves (vide control) nor can the mercuric chloride (vide 1/20 000 cell), but in some way the combined effect of the two can completely inhibit growth. If conditions are carefully adjusted, this inhibitory effect can be seen with a dilution of almost 1 in half a million – a quantity which can almost be reached by a therapeutic dose of the drug.

Scientific chemotherapy dates from Ehrlich and scientific chemotherapy of a bacterial disease from Ehrlich's Salvarsan, which in 1910 revolutionized the treatment of syphilis. The story of Salvarsan has often been told, and I need not go further into it except to say that it was the first real success in the chemotherapeutic treatment of a bacterial disease. Ehrlich originally aimed at 'Therapia magna sterilisans', which can be explained as a blitz sufficient to destroy at once all the infecting microbes. This idea was not quite realized, and now the treatment of syphilis with arsenical preparations is a long-drawn-out affair. But it was extraordinarily successful treatment, and stimulated work on further chemotherapeutic drugs. While they had success in some parasitic diseases the ordinary bacteria which infect us were still unaffected.

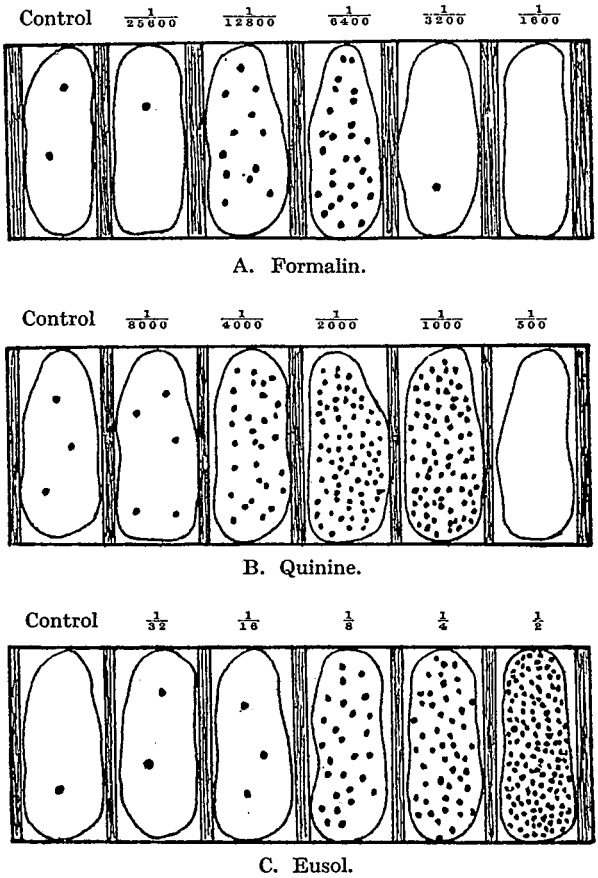

Fig. 4. Effect of chemicals on the bactericidal power of human blood. Black spots represent bacterial colonies.

Some aniline dyes were shown by Churchman many years ago to have remarkable selective properties as antibacterial agents, and they became prominent as antiseptics in septic wounds after Browning in 1917 described the action of acriflavine. This substance has been recommended as a chemotherapeutic agent for intravenous injection, but it proved to have some toxicity for the liver. When it is injected, it very rapidly disappears from the blood and all the tissues are stained except the nervous system. The following figures give the antibacterial power of the blood before and at intervals after an intravenous injection of acriflavine, and these are con-

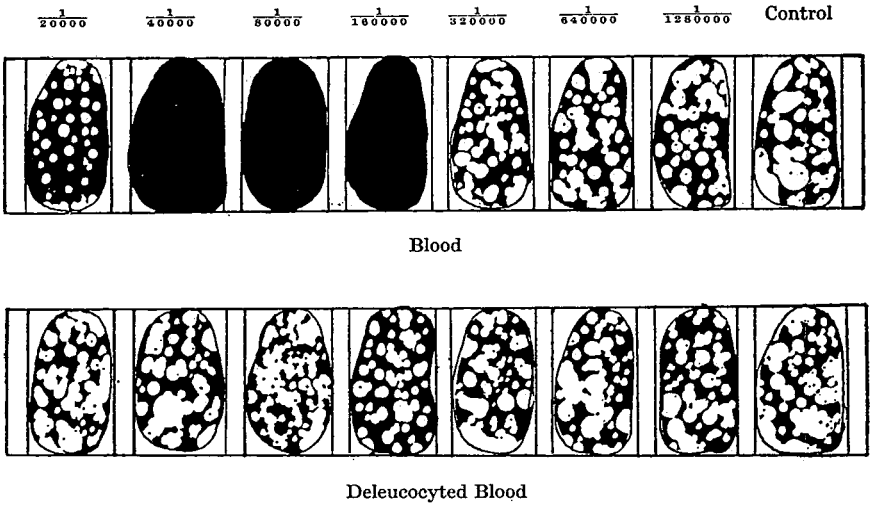

Fig. 5. Action of mercuric chloride on haemolytic streptococci in blood.

trasted with the result obtained after an intravenous injection of hypertonic sodium chloride:

Bactericidal power of blood after intravenous injection (in rabbit)

A. Acriflavine 20 cc of 1/1000	Number of colonies	B. NaCl 3 cc of 10%	Number of colonies
Before injection	87	Before	25
1 min after injection	62	2 min after	28
3 min after injection	87	30 min after	8
45 min after injection	92	2 h after	0
		6 h after	0

Whereas the increased antibacterial power after acriflavine was slight and evanescent, there was a very considerable increase after sodium chloride which lasted for hours. This could not be due directly to the sodium chloride, which in itself was not antibacterial, and the only change that could be discovered was some rise in the opsonic power of the serum. This could not be true chemotherapy, but it illustrates another of the factors which have to be borne in mind in the investigation of the action of these drugs.

Then Sanocrysin was introduced as a chemotherapeutic agent against the tubercle bacillus, and it was said that after administration so many tubercle bacilli were destroyed that an antitubercular serum had to be given to prevent poisoning with the toxins of the dead bacilli. This was another

failure; Fry showed that Sanocrysin in the concentrations used had no action on the growth of the tubercle bacillus, but it is still used for the treatment of tuberculosis, although it is not with the idea of direct chemotherapeutic action.

Long before this it had been noticed that some microbes were antagonistic to others – Pasteur himself was the first to show this – and some microbic substances or antibiotics had been used for local treatment for their direct effect on the infection. Notable among these was pycocyanase – a product of *B. pyocyaneus* – which was introduced early in the century. It was not very successful and fell into disuse.

I have said something of what happened in the past – let us say up to 10 years ago. That was yesterday.

Now we have to go on to a consideration of the chemotherapeutic happenings of today, and by today I mean the last decade. Things have moved indeed, and it is safe to say that in the last ten years more advances have been made in the chemotherapy of bacterial infections than in the whole history of medicine.

TODAY

It was in 1932 that a sulphonamide of the dye chrysoidine was prepared, and in 1935 Domagk showed that this compound (Prontosil) had a curative action on mice infected with streptococci. It was only in 1936, however, that its extraordinary clinical action in streptococcal septicaemia in man was brought out. Thus just 10 years ago and 26 years after Ehrlich had made history by producing Salvarsan, the medical world woke up to find another drug which controlled a bacterial disease. Not a venereal disease this time, but a common septic infection which unfortunately not infrequently supervened in one of the necessary events of life – childbirth.

Before the announcement of the merits of the drug, Prontosil, the industrialists concerned had perfected their preparations and patents. Fortunately for the world, however, Tréfouel and his colleagues in Paris soon showed that Prontosil acted by being broken up in the body with the liberation of sulphanilamide, and this simple drug, on which there were no patents, would do all that Prontosil could do. Sulphanilamide affected streptococcal, gonococcal and meningococcal infections as well as *B. coli* infections in the urinary tract, but it was too weak to deal with infections due to organisms like pneumococci and staphylococci.

Two years later Ewins produced sulphapyridine – another drug of the same series – and Whitby showed that this was powerful enough to deal with pneumococcal infections. This again created a great stir, for pneumonia is a condition which may come to every home.

The hunt was now on and chemists everywhere were preparing new sulphonamides – sulphathiazole appeared, which was still more powerful on