A Lecture ON WOUND INFECTIONS AND THEIR TREATMENT.

DELIVERED (WITH DEMONSTRATIONS) AT THE OPENING OF
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PART I.

IN undertaking to deliver an introductory lecture on such an occasion as this I feel myself under a very heavy disability. While I am impressed—very profoundly impressed—with the ingenuity and value of the mechanical devices and appliances which have been brought together within these walls, I am, as you know, quite lacking in that expert knowledge required for the proper appraisal of such things. There is, however, one—and it is, I would urge, by far the most important—aspect of wounds which does fall within my particular department of study. It is this that emboldens me to address you on this occasion.

I would, at the very outset, put it to you that the distinction between *sick* and *wounded* is, from the point of view of science, an entirely improper one. Those who are classed as wounded are as universally as, perhaps even more universally than, those classified as sick, suffering from bacterial infection.

Ever since the days when Lister demonstrated that sepsis in surgical wounds was avoidable, very little study has been devoted to the bacterial infections which here come into consideration. For the surgeon has set before himself as his goal, not the successful treatment of septic infections of wounds, but their avoidance. And who will say that he is wrong? Less excusable, as it seems to me, was it for him to think that he had in reserve—if occasion should ever require it—an effective ready-made method of treatment for septic infections of wounds. After a year of war there are on that point very few illusions left.

There has in the meanwhile, I hope, been growing up a conviction that we shall not arrive at an effective treatment of wounds without strenuous study of the infecting microbes, the conditions in the wound, and the therapeutic agents which we employ, and the defensive operations of the organism.

Let me, drawing upon the research work which my fellow workers and I have been doing in France, try to put before you, as briefly as may be, in connexion with these questions, such points as seem to me most deserving of attention.

The wounded man seen just after he has received his wound is a man seen in the *incubation period* of his infection just after the microbes have been implanted—these being, as you know, carried into the wound, upon particles of infected skin and clothing.

The problem presented by the patient when first seen accordingly shapes itself thus. What is here the chance that the defensive mechanism of the body will prove adequate for the destruction of the microbes; and may we reasonably look to see the infection aborted? And in the event of the infection not being extinguished, what kind of course will it shape?

The extinction or non-extinction of the infection will depend upon the amplitude of the microbial implantation, and the favourable or unfavourable physiological conditions in the wound.

Rifle Wounds Traversing only Soft Parts.

The first type of wound which we have to consider is the perforating rifle wound where the bullet traverses the soft parts without touching bone. Here, nearly always, the bullet will cut into the wall of blood vessels. As a result there will be haemorrhage from the wound, and when the blood coagulates the track will become plugged

with clot. Later, when the patient is moved and the wound thereby disturbed, oozing may take place from the track.

Here we have a very light microbial implantation—only small numbers of microbes having been left behind by the missile on its passage through the soft tissues. Further, the walls of the track will have been scoured by the out-flowing blood. And finally, such microbes as are here left are enveloped in clot—in other words, we have here brought to bear upon the residual microbes the full bactericidal power of the volume of blood which occupies the wound. We have, in fact, exactly the same conditions as we have in experiments conducted in capillary tubes with a very small number of microbes implanted into a unit volume of blood.

As a sequela, as indicated above, we may have oozing. It is not, as might be supposed, blood which then escapes from the mouth of the wound. It is a thinner incoagulable fluid, containing only a small proportion of red corpuscles. It is, to come to the point, serum expelled from the clot by the *vis a tergo* of blood pouring into the upper reaches of the wound. There attaches, as I think, to this oozing a therapeutic significance. It is, in point of fact, equivalent to a reinforcement of the clot in protective elements.

You have already seized the point that when oozing occurs the serum of the clot—and serum is a fluid which when implanted with microbes tends to lose its antibacterial power—is replaced by blood fluids fresh from the vessels. And you appreciate that the outflowing stream of serum will tend to wash microbes out from the clot and convey them out of the wound. But it might, perhaps, be overlooked that when oozing takes place the clot is reinforced by red, and, this is important, by white corpuscles.

What, I take it, is occurring in the oozing wound can be brought quite easily before the eye in the capillary tube.

And let me describe the experiment in detail, for I want you here at the outset to have before you a perfectly clear idea of the method of working in capillary tubes with *washes* and *mural implantations*.

Description of a Reinforcing Experiment.

We take a capillary tube pipette fitted with a teat, place upon the stem a fiducial mark, fill in with a 15 per cent. solution of citrate of soda, blow this out—leaving thus what we may call a *wash*¹ on the walls of the pipette—fill up with a unit volume of blood, and then mix by moving the blood with its wash of citrate up and down in the stem of the tube.

We now, leaving in the distal end of the stem an air space somewhat greater than that occupied by our unit volume, draw up into our pipette a suspension of microbes and blow it out again, leaving a wash of microbes on the wall. We follow on now with a unit volume of blood and incorporate into this the microbes left on the wall (this is what I mean by a *mural implantation*). Finally, we carry our volume of uncoagulable, and our volume of implanted coagulable, blood up some little distance away from the mouth of the pipette, and then seal up our tube. The implanted blood now clots, giving us an ordinary *unreinforced* clot. Such a clot is shown diagrammatically and on an enlarged scale in Fig. 1, B.

It consists of a meshwork with spaces shown as half filled in with corpuscles and half with serum—we have in the blood, it will be remembered, almost equal volumes of corpuscles and serum.

To imitate the conditions in the oozing wound we have now to feed the incoagulable blood in the upper part of our tube

¹ A series of weighings made with the form of balance used by Bang in weighing drops of blood on filter paper for sugar estimations have brought out the fact that a "wash" of watery fluid in a capillary tube corresponds to between a twentieth and a thirtieth of a unit volume.

Fig. 1.—A. In the neck of the capillary pipette we have the reinforcing unclotted blood, and in the distal end of the stem the blood clot which has been implanted with microbes and connexion has been made between the two by the introduction of a very fine glass stylet.

B. We have here a more magnified image of the clot showing the creeping down of reinforcing blood along the stylet, and below the clot, the expelled serum that previously filled in its meshes.

into the coagulum in the lower part of our tube. We do this by taking a fine filament of glass (made by drawing out a capillary tube in the by-pass flame of a Bunsen burner) and passing it down through the unclotted into the clotted blood (Fig. 1, A). Contact having in this manner been established, both the fluid and the formed elements of the uncoagulated blood creep slowly down along the capillary filament into the clot (Fig. 1, B). Arrived there, they expel the serum from the clot (Fig. 1, B)—driving it out of the tube if we have broken off the tip of the pipette—and occupy all the unfilled spaces.

In this way we not only carry out from the clot blood fluids which may be exhausted of their antibacterial elements, but we fill up the clot with red and white corpuscles, bringing to the leucocytes of the clot, which, of course, are the elements which count, a reinforcement of 100 per cent.

Going back now to our rifle-bullet wound, we have to consider what will happen if the implanted microbes are not killed off. In this case we shall presently find an infected purulent fluid coming down along the walls of the track; and very soon the blood clot will disintegrate and come away, and we shall have a surface infection all along the track. We now pass to consider other types of wounds.

Rifle Wounds where the Bullet has Comminuted Bone.

Where a projectile comes up against a bone it will shatter it and make a large exit wound by blowing out the fragments and splinters, at the same time scattering the charge of microbes, which would otherwise have been sown only on the walls of the track, far and wide through the tissues.

We have here obviously very unfavourable physiological conditions. For not only is the circulation in the wound completely disorganized, but the wound will contain bone and soft tissues cut off from their blood supply and bound to necrose and slough; and deep in the tissues we have an implantation of microbes which no outflowing blood will wash away. And even more important, the outflow of lymph from the wounded surface—an outflow which meant the continuous replacement of invalidated by potent lymph—is going to be arrested by desiccation of the external wound surfaces. The wound, in other words, will, if left to itself, become *lymph-bound*. And, finally, the wound is, by virtue of its large exposed surface, going to lie open to all manner of after-infection from without.

These same unfavourable physiological conditions are associated also with shrapnel, shell, and bomb wounds. We shall consider them further in connexion with these.

Severe Shrapnel, Shell, and Bomb Wounds.

The essential feature about this class of wound is that we have here blunt or flat missiles, and that by consequence, as compared with bullet wounds, we have less penetration and perforation, and proportionately more bruising; and at the same time larger portions of infected skin and clothing are carried in by the missile. In other words, we have in the wound much worse physiological conditions; and along with this a heavier microbial implantation.

It will be a convenient arrangement to consider, in connexion with the severer types of wound, the effects of the bruising and the cutting off of the blood supply and the implantation of microbes into devitalized tissues; and then, in connexion with the lighter types of wound, to deal with the effects of the bacterial implantation into tissues not devitalized.

The disorganization and the shutting off of blood supply which is the feature of all severe wounds is followed, of course, by mortification; and thereafter the necrotic tissues fall a prey to every type of microbe: serophytic and sero-saprophytic, aerobic and anaerobic, non-sporing and sporing. And all these influences, working in combination, will cause the tissues to turn black and putrefy and disintegrate: giving, where the wound is allowed to desiccate, a condition of *dry gangrene*. Under the black gangrenous coating there will, if the infection fails to spread to the underlying tissues, and if things are not composed otherwise by the growing out of the tetanus bacillus in the necrotic tissues, gradually be formed a line of demarcation. And finally, the gangrenous layer will be exfoliated, leaving underneath a granulating membrane and a surface infection. Essentially the same sequence of events will supervene if the wound is kept moist. Only here we shall have *moist gangrene*, and the necrotic tissues will be converted into sloughs, and there will be earlier and more profuse suppuration.

Slighter Punched-in Wounds Produced by Shrapnel and Shell.

We come now to the lighter wounds which are inflicted by blunt missiles, to the wounds which we may call *punched-in wounds*. They are comparatively superficial wounds with steep sides going down to a floor sunk below the level of the surrounding skin. Here the microbes have been carried in over an area corresponding to the superficies of the wound, and they are implanted into the walls and the floor. What is important in connexion with this implantation is, that it is made not into an open track from the walls of which the microbes might be washed off by outflowing blood, nor yet into effused blood, which is up to a point a very uncongenial culture medium for microbes; but into lymph standing in lymph spaces. Now the lymph in such spaces is only under very low pressure—a pressure as low as, or lower than, that of the capillaries—and by consequence when lymph spaces are broken into we have nothing in any way analogous to the outflow of blood from a wounded vein or artery. There will at most be a little weeping of lymph, and the conditions will be comparable to those produced if, after dealing a heavy blow with a hammer upon the bark of a tree, there followed a little exudation which afterwards dried, forming an impermeable coating. It is clear that we should then have, instead of a washing away, an embedding of microbes in the outflowing fluid, and an incorporation of these into the subjacent tissue. The conditions in the punched-in wound are, as you will now see, very unfavourable. They will be unfavourable, first, because the antibacterial power of the lymph with which the microbes are brought in contact is bound to be quickly exhausted; and, further, because with the arrest of the lymph flow there will no longer be any renewal of the lymph. Again, the conditions are unfavourable also in the respect that the leucocytes coming, as they do in such a case, only tardily into a zone which is already poisoned by microbes, cannot press home their attack. The emigrating leucocytes will therefore do nothing but choke up the tissues for a considerable area round each focus of bacterial infection.

In this way we get in the course of a very few days all round our punched-in wound a hard infiltrated edge, margined towards the healthy skin by a zone of pale pink; and in the indurated walls of the wound we have an *imprisoned infection*. That infection will now extend, and, if it finds its way outwards to the surface too solidly obstructed, will spread inwards; giving rise, according to circumstances and the character of the microbic infection, to *cellulitis* or *gas gangrene*. In the ordinary course, however, the infection will manage to break through to the surface, and then again we have an ordinary suppurating surface with an *infection of flowing discharges*.

It is important to appreciate that the processes that have just been described take place not only in the punched-in wound, but everywhere where microbes are implanted into lymph spaces, and where we have afterwards an effusion of lymph which desiccates and seals up the wound.

And precisely the same sequence of events as follows upon the original implantation of microbes will, just so long as the microbes still maintain themselves in the walls of the tissues, recur if at any moment the wound is allowed to desiccate and become lymph-bound. A setback of this kind will, for instance, almost inevitably follow when, by the transporting of the patient from hospital to hospital, the washing out of the infected walls of the wound is interrupted.

Nature of the Microbic Infection met with in Wounds.

We now pass to consider very briefly the nature of the microbes which are carried into wounds from the soiled skin and clothing of the soldier. These microbes may, as I pointed out in a previous lecture, be classified—and the classification is important for treatment as well as for the understanding of the mode of infection and of the evolution of the wound—into two main classes, a class of *serophytes* which (presumably because they find ready-made pabulum in the blood fluids) can live and multiply in serum; and a class of *sero-saprophytes* which, so far as we know, can develop in the blood fluids only after these have lost their antitryptic property—the property in question being that which inhibits those digestive processes which would be capable of converting the native

albumins of the serum into pabulum for microbes. Intermediate in character between the serophytes and serosaprophytes is a class of microbes which cannot grow in the serum when we make only a small implantation, but which, no doubt owing to the fact that they bring into operation powerful digestive ferments,² succeed in establishing themselves when we make a heavy implantation. We may call these *imperfect* or *secondary serophytes*.

To the category of *serophytes* belong the streptococcus and the staphylococcus—the latter being far inferior to the former with respect to its power of multiplying in unaltered serum. To the category of *imperfect serophytes* belong the *Bacillus aerogenes capsulatus* of Welch (*Bacillus perfringens*); the *Bacillus proteus*; its close congener, the *Bacillus pyocyaneus*; and the wisp-shaped diphtheroid bacillus commonly found in foul suppurating wounds.

To the class of *sero-saprophytes* belong the larger number of microbes found in such wounds.

It will suffice here to bring out a few of the more important points in connexion with the serophytes and imperfect serophytes which are found in wounds.

The microbe most universally present is a streptococcus. It differs in very many respects from the classical *Streptococcus pyogenes*, which is met with, though much more rarely, in wounds. In film preparations of pus the streptococcus here in question shows up nearly always as a diplococcus. As obtained from agar and broth cultures, the elements of the diplococcus are lancet-shaped, and they are bent into an angle. To follow the French description, they resemble a circumflex accent or take the form of saddle-bags (*formes en besace*). In broth cultures we have interspersed with these a few short chains. The colonies as they grow upon agar are more opaque, less sharply margined, and somewhat larger than those of the *Streptococcus pyogenes*. Instead of being as colourless as glass and severely discrete, they show up as very faintly grey-green, and, when planted closely, tend to run together. As compared with the ordinary *Streptococcus pyogenes*, growth is also much more rapid—luxuriant cultures being obtained at 37°C. on broth and agar in four or five hours. Moreover, growth is obtained, not only at 37°C., but also at the temperature of the laboratory bench.

The most remarkable characteristic of this streptococcus is, however, the freedom with which it grows out in normal serum, and also upon agar when transplanted in blood. When we implant into blood in emigration tubes, putting the tubes directly into the centrifuge, and from this into the incubator, we obtain after three to five hours with a moderate implantation a growth in the form of diplococci and short chains permeating the whole white clot; or, with very light implantation, a growth in the form of colonies clearly visible to the naked eye and consisting of typical convoluted chains made up of indefinitely numerous elements. In the case where we implant into blood and then implant the blood culture on agar, we have very opaque white convex colonies which may be as much as half, or even one, centimetre in diameter; and which, except for the fact that they are rather moister, closely resemble staphylococcus colonies. These are made up of lancet-shaped diplococci, which might easily be taken for pneumococci. The surrounding blood is not haemolyzed.

There will be no doubt in the mind of any one who has studied descriptions and illustrations of the *enterococcus* and its mode of growth on ordinary media as given in French bacteriological textbooks that the streptococcus here in question is the *enterococcus* of the French authors. Moreover, it may be taken as assured—for we have compared our cultures of streptococci from wounds with a series of cultures of streptococci obtained by Professor Dreyer and his colleagues from the stools of patients who were being searched in the ordinary way for typhoid and paratyphoid bacilli—that the streptococcus we are here considering is the ordinary streptococcus of the faeces. And assurance is made still more complete by the fact that when searching normal faeces by the *faeco-sero-*

*culture method*³ my fellow worker, Lieutenant A. C. Iuman invariably obtained from the faeces in his after-washes a pure culture of a streptococcus which was, in all the above-mentioned morphological and biological characters, indistinguishable from that which is practically invariably present in the wounds. We may therefore take it as unquestionable that the streptococcus which is commonest in wounds is of faecal derivation, and both our *faeco-* and *pyo-sero-cultures* show that if the smallest possible implantation of this microbe is made, in no matter what bacterial admixture, into serum, it will immediately grow out there.

With regard to the presence of staphylococcus in wounds, it may be pointed out that, by reason of its wide distribution in the skin and its serophytic properties, it is bound to be present in practically all wounds. We shall, however, presently, in discussing the results of our *pyo-sero-cultures*, appreciate that its growth in the wound is very quickly restricted by changes in the blood fluids (and with these there may possibly be associated also changes in the leucocytes) which are produced by the immunizing responses of the patient.

Like the staphylococcus and the *Streptococcus faecalis*, which we have just been discussing, the *bacillus* of Welch—which is also, of course, a constant inhabitant of the faeces—is implanted, one may take it, into every wound. This microbe, be it noted, is only an imperfect serophyte. In point of fact, as my fellow worker, Captain d'Este Emery, has succeeded in showing, the serum exerts upon the bacillus of Welch a very considerable bactericidal power; and it is therefore in serum cultures only the surviving microbes which grow and multiply, and in blood cultures only those which both resist the action of the serum and also elude destruction by phagocytes. All this means—and clinical experience amply bears this out—that if we can bring the blood fluids and leucocytes to bear on Welch's bacillus we have very little indeed to fear from it.

What little requires to be said about the *Bacillus pyocyaneus* and *proteus* and other members of the class of imperfect serophytes may for the moment be reserved.

Before passing on something may appropriately be said about methods of cultivation, and I may limit myself to the description of methods of obtaining cultures in serum. For it is only by implantation into serum that we learn what microbes threaten danger, and how far the body is protecting itself against these. And again it is only by the method of serum culture that we can, when dealing with a complicated mixture of microbes, choose out from among these those which we ought to employ as vaccines.

What we want for these purposes is a cultivation method which will enable us to make first a moderate implantation and then a series of smaller and smaller implantations.

The *wet wall method*, or as we may also call it when implanting microbes the *method of mural implantation*, gives us what we here want, and there are two different procedures by which we can obtain the progressively diminishing implantations we require. We may call these respectively the *wash* and *after-wash* and the *wave-wash* methods.

Wash and After-wash Method.—Here taking a capillary pipette fitted with a teat and furnished with a fiducial mark, we fill it up to that mark with such a mixed bacterial culture as is furnished by pus, sputum, or faeces, and then expel this volume, leaving behind a *unit wash* on the walls of our capillary stem. Into the tube thus primed we now aspirate a *unit volume* of serum, then an air-bubble, and then nine or more unit volumes of serum separated by air-bubbles. In this way we obtain in successive volumes of serum a series of smaller and smaller implantations. If we want, as we may often do, a series of implantations in which the number of microbes falls off more steeply, we can, after filling in the first unit volume of serum and carrying it some little distance up the stem, resect this just above the level of our mural implantation, completing after that in the ordinary way the filling in of the pipette. Or we can, leaving the stem unresected, arrive at the same result by making each unit volume, as we fill it in, pass several times up and down over the implanted portion of the tube, thus washing off the microbes more effectively.

³This method is modelled in all respects upon the *pyo-sero-culture method* presently to be described.

⁴It may be well to bring out at this point that, though one could hardly, when dealing with a solution of a chemical agent, ask for a steeper series of descending dilutions than that furnished by the *wash* and *after wash* method—for we go down here by steps of 25, 625, 15 625, 400 000, and 10 000 000 quite a different standard comes into application when diluting a microbial culture. For in the first case we are dealing with dissolved chemical agents, and imponderable quantities do not come into the account; and in the second case we are dealing with particulate matter, and quite imponderable quantities come very easily into reckoning. And, in point of fact, experiment shows that in pipettes implanted with a unit wash of pus and then filled in by the *wash* and *after-wash* method with unit volumes of serum we implant, in many cases as far as the fifteenth and in some cases as far as the twentieth *after-wash*.

²The suggestion here made wins support from the fact that the streptococcus, which we may take as the type of a true serophyte, does not, when growing in clear serum, exert any reduction in its antitryptic power, whereas both the *Bacillus proteus* and the bacillus of Welch do this. And it will be remembered in this connexion that the streptococcus does not liquefy gelatin, while the *Bacillus proteus* and the bacillus of Welch rapidly digest albuminous substances (even coagulated white of egg) and gelatin, and, as the case may be, fat, urea, and other substances.

Wave-wash Method.—Here, we place two fiducial marks on the stem of our pipette, and then, using the proximal mark for the serum and the distal for the air bubble, fill in with unit volumes of serum separated off from each other by measured instead of, as in the ordinary way, with unmeasured volumes of air. Then at the end of the series we fill in up to the proximal mark with the diluted bacterial suspension which is to serve as our implanting fluid. Retaining this accurately in place, we now place further fiducial marks on our capillary stem, marking off in this way the distal end of the last and penultimate of our volumes of serum. We now, relaxing the pressure of our fingers upon the teat, carry the whole system of volumes and intervening bubbles upwards in our capillary stem, arresting the movement as soon as the distal end of the implanting fluid comes level with the first, or, if we prefer it, the second, of the fiducial marks we have just inscribed. We may call this implanting movement a *wave wash*, and may speak of a *one-unit* or *two-unit wave* according as the implanting fluid is carried up into the segment of tube belonging to the next or the next but one volume of serum. We make a series of these wave washes (generally a series of six or twelve), making with these not only an implantation of microbes from the implanting fluid into the neighbouring volume or volumes of serum, but also at the same time implantations from the implanted into the previously unimplanted volumes of serum.

Method of making Anaerobic Cultivations in Capillary Tubes.—When we want to cultivate under anaerobic conditions we proceed as follows, employing, according as we want to cultivate only one volume of serum, or a series of volumes of serum, the first or the second of the procedures now to be described.

Method of making a Single as distinguished from a Multiple Anaerobic Culture in a Capillary Tube.—Taking a capillary pipette fitted with a teat we thrust the stem deep down into a rubber tube delivering hydrogen or ordinary coal gas, and now alternately compressing and relaxing pressure upon the teat we expel the air, and fill teat and pipette with unmixed gas. We now withdraw the end of the pipette from the delivery tube, keeping as we do so the orifice of the pipette directed downwards, and then, after expelling a little of the gas, draw up from a drop of implanted serum placed ready to hand a convenient quantity into the stem of the pipette. Keeping the serum close down to the orifice of the pipette so as to prevent any entrance of air, we now once more thrust the capillary stem deep into the gas delivery tube, and draw in sufficient gas to give us comfortable space at the end for sealing up the orifice in the by-pass of a Bunsen burner. We complete our operations by burning through the tube capillary stem just below the barrel of the pipette.

Method of making Anaerobic Cultures of a Series of Volumes of Serum Implanted by the "Wash and After-wash" or the "Wave-wash" Methods.—We begin by fitting to our gas delivery tube the barrel of a capillary pipette which has been truncated by resecting the stem just below the neck. We sterilize this by flaming, and let our hydrogen or coal gas run through it sufficiently long to expel all admixture of air. Then, directing the orifice downwards, we turn off the gas and compress the rubber tube between the forefinger and thumb of the left hand so as to expel a little of the contained gas. Then we dip the orifice of the glass end-piece into a large drop of serum placed ready at hand and draw this up, and then, by regulating the pressure with our fingers, keep the serum closely applied to the open orifice (Fig. 2). We now take into our right hand a capillary pipette furnished with a teat and a fiducial mark, and implanted, if we are using the wash and after-wash method, with a wash of a microbial suspension. The tip of the pipette is now introduced into the serum in the mouth of the glass end-piece, and we now draw up into it first, as in Fig. 2, A, a unit volume of serum, and then pushing, as in Fig. 2, B, the point out into the gas

FIG. 2.

which is trapped by the serum, a bubble of gas; and so on in series until we have filled up the stem of our capillary pipette.

Response of the Wounded Man to his Wound Infection and Blood Changes Induced in him by Auto-inoculation.

In wound infections, as everywhere where bacterial toxins are elaborated and absorbed into the blood, the machinery of immunization is after a time called into operation, and as a result the blood is put into a better condition for defence. And then begins a serious conflict between the invaded organism and the invading microbes.

It is in connexion with septicæmic invasions, such, for

instance, as typhoid fever, gradually becoming to be understood that it is by the event of this conflict that the issue is decided; and that the physician in attendance is not following, much less directing, events. But it is as yet a quite unfamiliar thesis that the wounded, like the typhoid, patient is reacting to his infection with a systemic immunizing response; and that the changes so induced in the blood exert a quite decisive influence on the course of the infection, while the surgeon who is dressing the wound and making local applications, is only in a subordinate way helping or, as the case may be, hindering the curative procedures of Nature.

It will be well before considering the surgeon's task to take cognisance of what the immunizing responses of the patient are doing in the matter of fortifying his blood.

We have, of course, in connexion with this only very imperfect knowledge, and we shall therefore have to keep very close to our experimental data. It will be convenient to marshal our facts under headings supplied by our methods of blood testing.

Data Furnished by Measurements of the Antitryptic Power of the Patient's Serum.

Reference has already been made to the antitryptic power of the blood fluids. In point of fact, the antitryptic power of the serum acts as a check upon all microbial growth.⁵ In the case of *sero-saprophytic bacteria* it completely balks their efforts to establish themselves in the serum. In the case of *imperfect serophytes* it places a very formidable obstacle in the way of growth. And in the case of *serophytes proper* it also, as a comparison between serums of low and high antitryptic index, and between serums which have, and serums which have not, received an addition of trypsin shows, determines whether the culture in serum⁶ shall be scanty or luxuriant.

It will be obvious from the above that an increase of the antitryptic power of the blood fluids would operate in restraint of any blood invasion, and that it would also, so far as the blood fluids came into application unaltered, restrain the growth of all forms of microbes, both in tissues and in the wound. In other words, an increase of antitryptic power would operate as a non-specific factor in immunization. Now we have, as I already indicated in my last lecture,⁷ in every or practically every wounded man a notable increase in the antitryptic power of his serum. Already within thirty six hours or less after the infliction of the wound the antitryptic index has risen far above the level of the normal; and reckoning the antitryptic power of the normal serum as *unity*, antitryptic indices of four and five are very common in the patients under treatment at the base.

More than that, we have found a quite similar but smaller increase in the antitryptic power of the blood after inoculating ourselves—one of us with typhoid vaccine, another of us with streptococcus vaccine, and a third with staphylococcus vaccine.

And let me here recall to you that attention has, in connexion with inoculations against plague, typhoid fever, and pneumonia, been time and again called to the probability that these vaccines give some protection against diseases other than the particular disease which the inoculation is designed to ward off. Particularly convincing in this respect are the results in the form of *diminished incidence of "other diseases"* which were obtained in South Africa by the inoculation of pneumococcus vaccine upon the Premier Mine.⁸ And I think all those who have had much experience of vaccines will have seen cases where therapeutic effects lying quite outside the range of the particular vaccine employed, and therefore as we thought not quite creditable to science, have been obtained by vaccine therapy.

⁵ The antitryptic power of the blood fluids represents, be it noted, much more than merely a power of inhibiting microbial growth. With the loss of antitryptic power go lost also the complementing, opsonic, bactericidal and coagulating powers of the blood.

⁶ Where instead of implanting streptococci into serum free from leucocytes we implant into blood containing leucocytes these are broken down, with the result that the antitryptic power is diminished and we have then, as already described in connexion with emigration tube cultures, always after a sufficient interval, luxuriant growth.

⁷ Vide Section I, Subsection of Section 3, last paragraph.

⁸ Vide Appendix II in my *Treatise on Preventive Inoculation against Pneumonia in the African Native*. Constable, London. 1914.

Data Furnished by Pyo-sero-culture.

The method of pyo-sero-culture was described in my last lecture (Section 1, Subsection 1).⁹ We may, however, substitute for the lengthier procedure there set out the wash and after-wash method (*vide supra*).

Having by this method introduced into pipettes similarly implanted with the patient's pus—into the one a series of unit volumes of the patient's blood, and into the other a series of unit volumes of a normal serum—and having incubated these for from six to twelve hours, we now follow the procedure portrayed in Fig. 3, A, *infra*. Beginning with the volume nearest the distal end of the stem which has received the smallest implantation, we blow out our successive serum cultures in separate drops on to the surface of a sterile slide; and then, with a platinum needle or glass filament, stroke out these in succession upon an agar plate, making in this way a series of linear implantations. On incubating our plates we then obtain, with the serum of a wounded man who is uninfluenced by his infection, exactly the same results as with the normal serum. In the case of

Fig. 3.—Method of Pyo-sero-culture.

A, Pipette which has been implanted by the wet-wall method and has then been filled in by the wash and after-wash procedure with unit volumes of serum. By the side of the pipette to the right are ranged a series of discs representing the series of unit volumes of serum blown out in order from the pipette, and, finally, to the right of the discs are a series of lines representing linear implantations made upon agar.

B, Results of the series of linear implantations made with the unit volumes of the patient's serum.

C, Results of the series of linear implantations made with the unit volumes of the normal serum which was used as a control.

a wounded man in the negative phase of his infection, we obtain cultures containing a variety of microbes (all of them members of the class of imperfect saprophytes) unrepresented in the cultures made with the normal serum; and, moreover, as compared with these, the cultures made with the patient's serum are more luxuriant and extend also into higher dilutions. In cultures made with the serum of a wounded man who is making satisfactory immunizing response—and the illustration reproduces the results actually obtained, by implanting the pus of such a patient into his own serum, and into that of a normal man—we have in both series of pyo-sero-cultures only serophytes, staphylococci and streptococci, but while the cultures made with the patient's serum (Fig. 3, B) furnish only a few discrete colonies, and these only the first wash and two after-washes, we have with the normal

serum (Fig. 3, C) massed colonies in the first seven plantings, and the first eight plantings furnish cultures.

The significance of such data will, perhaps, best be appraised if we compare the method of sero-pyo-culture—considered as a procedure for the appraisal of the clinical condition and prospects of the wounded man—with the method of *pyo-culture* which Delbet has quite recently proposed to use for these same purposes.

The real value of the method of *sero-pyo-culture* lies in this, that it tells us up to what point the blood fluids of the patient would, without aid received from the leucocytes, be capable of protecting themselves against an implantation of microbes, and specifically against an implantation of microbes from the wound. Now merely to tell us this, is not to tell us how the microbes are going to fare in the wound. For we are not entitled to assume of the blood fluids that they will in the wound obtain unrestricted access to the microbes; nor of the leucocytes that they will not die in the wound, converting thereby the effused lymph into a favourable culture medium for microbes. Still, evidence of satisfactory immunizing response obtained by the method of pyo-sero-culture is, so far as it goes, evidence of favourable import, always with the proviso that the wound be treated in a rational manner.

The method of *pyo-culture* aims at arriving by an idyllically simple procedure going straight to its goal, at a trustworthy forecast of the future of the wound. It is suggested that by merely incubating the pus and then examining microscopically to see whether the number of microbes has increased or remained stationary we shall be able to foretell the future, and to decide, where the clinical appearances seem to prescribe amputation or other radical surgical procedure, whether operation can be avoided. In connexion with this proposed method of investigation, the first point to note is that the pictures which are furnished by it are generally the very reverse of clear, and that we are not in any way helped by controlling, as we are invited to do, our culture in pus by cultures made by implanting pus into bouillon. In point of fact, this particular method of controlling our results could only mislead, for the common pyogenic micro-organisms, and in particular the coliform organisms, would, no matter how sparingly they might be present in pus, always grow out luxuriantly in the bouillon culture. But let us suppose for a moment the case that we have been able to satisfy ourselves that the micro-organisms in our pyo-culture have increased, or to satisfy ourselves that they have not increased; and let us then ask ourselves what prognostic value would attach to the one or the other finding. There cannot be any doubt as to the answers to these questions. A multiplication of the microbes in the pyo-culture would, of course, indicate an unsatisfactory condition in the wound; but we should not know whether to interpret this as a consequence of a defective immunizing response on the part of the patient, or as a consequence of the surgeon having suffered the wound discharges to accumulate and become corrupt in the wound. On the other hand, if the microbes definitely failed to grow out in the patient's pus, this would clearly suggest that the patient was making good immunizing response; and that, in correspondence with this, the wound would, given requisite attention, be likely to progress favourably.

Data Furnished by Measurements of the Opsonic Power of the Patient's Serum.

The changes in the serum which gradually render it a more and more uncongenial culture medium for microbial growth have their counterpart in changes in the serum which render the microbes an easier prey for phagocytes. These changes in the opsonic power of the serum are exactly similar to those which manifest themselves in all other forms of localized infection; and we find, when we look for them, in connexion with wounds, all the phenomena of autoinoculation with which we are familiar in other bacterial infections. And every day it becomes clearer that every displacement or movement of a fractured and infected limb—such displacements, for instance, as are associated with the transport of the wounded man, or the sagging of his limb when it is dressed, and also all those unguarded passive movements which the surgeon or orderly may inflict when the patient is under an anaesthetic—operate as autoinoculations, and are followed by that sequence of negative and positive phases which we are accustomed to witness after the stirring up of a focus

⁹ BRITISH MEDICAL JOURNAL, April 10th, 1915, p. 625.

of infection by active exercise, massage, or the application of Bier's bandages.¹⁰

Data Furnished by a Study of the Emigration Response of the Patient's Leucocytes.

As was already brought out in my last lecture, comparative experiments made with the method of testing emigration there described show that we have in patients who have made immunizing response to their wound infections or to the inoculation of streptococcus vaccines, an emigration response to streptococcus implantation which is strikingly greater than that of the normal man. I do not, until the method of estimation shall have been further improved, desire to be more detailed on this question. I prefer to pass on to consider the bactericidal power of the whole blood, for we have in this the resultant of all those factors in immunity which we have been separately considering.

Data Furnished by a Study of the Bactericidal Power of the Whole Blood.

The experiments on the bactericidal power of the whole blood which I undertook in connexion with work on pneumonia in South Africa¹¹ constituted a first attempt to make a complete evaluation of the bactericidal power of the blood fluids and leucocytes working in conjunction and reinforcing each other.

Let me lead up to what I have to say upon this matter by reminding you in connexion with these experiments with the pneumococcus that the blood fluids exert no bactericidal effect upon that microbe. And let me also very briefly go back over the procedure which I employed.

That procedure was to make by the wet-wall method implantations of the pneumococcus into blood drawn direct from the finger, or, as the case might be, into

washed corpuscles suspended in serum, decalcified blood, blood kept liquid upon ice, and into what I will, to distinguish it from *exvascular* blood, venture barbarously to call *excoagular* blood. I mean by that—the red and white corpuscles and serum which come out from the blood clot when it contracts. The experiments conducted with all these different varieties of blood gave, as perhaps some of you will remember, very remarkable but undeniably paradoxical results. With blood drawn directly from the finger a very striking bactericidal effect—an effect which was upon occasion equivalent to a killing off of 600,000, to as much as 1,000,000 pneumococci per cubic centimetre of blood—was achieved. But it was not in every instance achieved.

With none of the other varieties of blood was any bactericidal effect achieved; or if there was any effect it was very insignificant and very inconstant. The reason for these diverging results did not at that time appear. I made some guesses which fell wide of the mark.

This was the position of the question when I again, in connexion with the streptococcus, addressed myself to the study of the

bactericidal power of the whole blood. And the situation to be dealt with was here essentially the same as in

the case of the pneumococcus, saving only in the respect that the serum is a much better culture medium for the streptococcus than for the pneumococcus.

During many months of work only very disappointing results were obtained. The blood of normal men, the blood of wounded men who were progressing favourably, and my own blood and that of two of my colleagues after we had been inoculated with streptococcus vaccine, alike gave, when implanted with streptococcus in a graduated manner, as good as no evidence of any bactericidal power. Or at any rate only very inconstant results were obtained.

This was even a more violent paradox than that encountered with the pneumococcus, for the blood that failed to kill streptococcus was not blood which had in any way been tampered with, but blood drawn directly from the finger; and it is, in view of the favourable event of most streptococcus infections, and the striking therapeutic effects obtained by streptococcus vaccine, impossible to doubt that streptococci are killed in the blood.

At the end, a possible explanation of the contradiction between the results obtained *in vivo* and *in vitro* suggested itself. I suddenly woke up to the fact that there was a flaw in the technique I was employing.

The nature of this flaw will be understood on referring to the accompanying diagrams. In my experiments the implantation of microbes into the blood was made by what I have called the method of mural implantation. And the particular form of mural implantation employed was the *complete mural implantation* (Fig. 4, B).

Now what follows in the case where we implant into a blood which afterwards clots is brought before the eye in Fig. 5. When the clot contracts the space which was at the outset filled with unclotted blood and then with coagulum (Fig. 5, A) divides itself up into two regions (Fig. 5, B), a region occupied by clear serum and a region occupied in part by the clot and in part by the corpuscles which have fallen out from the clot and have ranged themselves round its base. The *first* of these regions is one in which streptococci—supposing they had once managed to find their way there—would be quite safe from phagocytic attack and would find conditions favourable to their growth. In the *second* region, inasmuch as the phagocytes would be crawling about and fossicking for microbes in the clot and escaped red corpuscles, there would at any rate be a chance of the leucocytes extinguishing the infection. *Mutatis mutandis*, this would apply also in experiments conducted with the different varieties of incoagulable blood. In each case the corpuscles would settle to the bottom, and there would be left above them a band of clear serum where microbes would be quite safe from phagocytic attack.

It will be obvious that these quite elementary considerations provide a very simple explanation of all the blank results obtained in our bactericidal experiments. They do more than that. They point the way to a better technique. For clearly, if instead of making a *complete mural implantation*, we now limit ourselves to what I may call a *wainscot implantation* (Fig. 4, A) we shall be planting all our microbes into a region of the blood where they will have to run the gauntlet of the white corpuscles; and there will no longer be for them, as there was with the complete mural implantation, any direct way of escape into a region of safety provided by the serum.

It will suffice to say in connexion with this improved technique that it has answered even beyond expectation, and that when we limit ourselves to a wainscot implantation, and make implantations with graduated dilutions of streptococcus, we obtain not only with *exvascular* blood but also with *excoagular* and citrated blood conclusive evidence of bactericidal power.

Let me, however, make the following point plain. Even with this improved technique, the demon of chance has

Fig. 4.—The figure shows two forms of mural implantation into blood—in A a "wainscot," in B a "complete mural" implantation. In A the distal end of the capillary stem has, as a first operation, been fitted on as far as the upper fiducial mark with blood. An air bubble was then drawn in; after this the tip of the tube was dipped into a microbial suspension, and then was allowed to run in as far as the distal fiducial mark. After this the microbial suspension was expelled and the blood was then, to receive its "wainscot implantation," driven down to the orifice of the tube. In B, where the blood receives a "complete mural implantation," an essentially similar procedure was followed.

¹⁰ Vide in this connexion the autoinoculation curves set out in the author's *Studies on Immunization*. Constable, London.

¹¹ *Lancet*, January 3rd, 1914, p. 1.

Fig. 5.

not been exorcised from our experiments. And since chance will always, in our experiments, play a part in the ordering and disposition of leucocytes, it will be impossible in the case where the bactericidal power of the blood is centred upon these, and so operates only where gravity permits, to get regular quantitatively accurate results, such as are attainable when the bactericidal power is centred in the serum, and so operates uniformly through the medium.

I would ask you to note this point. It is necessary to emphasize it here where we are considering the blood changes produced by immunizing response to autoinoculation, and it will be necessary to emphasize it again when I come to speak of vaccines and their employment in connexion with wound infections.

But we have not, in the matter of the leucocytes and the factors which determine the bactericidal effect they exert, got quite to the end of our story. You will find the following experiment very full of instruction, and it has, as we shall presently see, very important applications in connexion with the treatment of wounds.

We take a couple of looped pipettes, such as are shown in Fig. 7, and we proceed as follows. In a first operation

Fig. 6.

Fig. 7.

Fig. 6.—The figure shows a magnified view of the distal ends of the capillary pipette represented in Fig. 7, filled in with citrated blood, which is disposed, with respect to microbes positioned at +, in A in the *agathotropic*, and in B in the *kakotropic* arrangement. In A the pipette has been placed upright, in B in the inverted position, and in each case the blood has settled down in three layers, the red corpuscles below, a layer of white corpuscles above them, and at the top clear plasma.

Fig. 7.—In A and B the elements of the blood had before implantation been allowed to settle down respectively into the "agathotropic" and "kakotropic" arrangement, and we have represented here the final result, the nutrient medium remaining in the former case sterile, while it shows in the latter a microbial growth.

we fill into the cultivation chamber in the barrel of the pipette a nutrient medium which will reveal by a colour change any growth of streptococcus (we have such a medium in cane-sugar litmus-broth). We then fill up to a fiducial mark on the stem with a 15 per cent. solution of citrate of soda, blow this out again, and then fill in with a unit volume of blood from the sterile finger. We now, after carrying the blood several times up and down the capillary stem so as to mix thoroughly with the citrate, seal up the end of the tube. This done we place our pipettes in a test-tube rack—the one in the upright position, the other with the tip turned upwards—and we let the blood sediment. The red corpuscles will now, in the upright pipette, be brought to the distal, and in the inverted pipette to the proximal, end of the volume of decalcified blood. (Fig. 6, A and B.) And in each case there will be on the top of the red a layer of white corpuscles, and above this a layer of clear plasma. We now, taking, of course,

the pipettes in hand one after the other, make into each a minimal implantation of streptococcus. We do this by just dipping the tip of the tube into a streptococcus suspension and then very cautiously bringing the corpuscles, or, as the case may be, the plasma, into contact with the infected inner surface of the tip of the tube. We now incubate the pipettes for three or more hours—the one in the upright, the other in the inverted position. And, finally, using the procedure as described in my textbook of technique,¹² we carry up the implanted blood into the nutrient fluid provided in the incubation chamber.

We obtain in experiments of this sort always one invariable result. In the case of the pipette which is kept upright—that is, in the case where the implanted streptococci have to run the gauntlet of the white corpuscles—the microbes are all killed off, and in consequence our nutrient medium undergoes no change. In the case where the pipette is inverted and we implant directly into the plasma the streptococci survive, and our nutrient fluid changes from blue to red.

And we can vary our experiment in all sorts of ways, and still each time get the same result. For instance, we can, by very simple devices, cut the red corpuscles out of our experiment and work, as in the wound, only with leucocytes and plasma. Or we can at the outset invert our pipettes, and then, after implanting into the plasma, dispose them upright, thus raining down our leucocytes upon the microbes and aborting the infection.

All this shows, as I think, that we have arrived at a generalization which must be important for practice. Let me formulate it for you thus.

Where the leucocytes are in the front rank and the blood fluids behind, the elements of the blood are marshalled in that order which is most favourable for combating streptococcal infections. I will venture to call that arrangement the *agathotropic* arrangement; but I should have no quarrel with any lover of superlatives who might feel moved to call it the *aristotropic* arrangement; nor yet with any grammatical purist who might prefer to call it the *eutropic* arrangement; I would only insist that where we set out to extinguish a streptococcal infection we ought to place the leucocytes in the forefront of the battle.

When we have realized what is the favourable arrangement of the elements of the blood for the combating of streptococcus infections, there will have come home to us also that there is an unfavourable arrangement. And we have seen that the unfavourable arrangement is that in which the blood fluids are ranged up in front and the leucocytes are behind. I would ask you to let me call that the *kakotropic*, but there is no reason why you should not call it the *kakistotropic* or the *dystropic* arrangement.

With this I bring to a conclusion the preliminary portion of our subject matter, and let me say that of the lessons that can be drawn from it many will only appear later.

(To be continued.)

¹² *Technique of the Teat and Capillary Glass Tube*. Constable, London.

A COMMISSION has been appointed by the Governor of Indiana to investigate the causes and means of prevention of mental deficiency in that State. We learn from the *New York Medical Record* that a report recently published by the American Eugenic Society recommends the sterilization of an increasing number of defectives each year until the number reaches about half a million. It is estimated that if this recommendation were carried out the propagation of the mentally unfit in the United States would be practically at an end.

In the September number of the *Cronica Medico-Quirurgica* of Havana some particulars are given of the Java School of Medicine, founded in the city of Batavia in 1865. It has 116 students, and degrees are conferred on some ten or twelve candidates every year. As the population of the Dutch Indies is 52 millions, these figures show that the medical profession is not overcrowded there. The course of study extends over nine years, of which three are spent in an arts school and six in the School of Medicine. The lectures are given in Dutch. Forty bodies are dissected each year, and the clinical and bacteriological teaching is said to be sound. Admission to the classes is free; the students are lodged and boarded without charge and receive an allowance of 6 to 10 dollars a month from the Government. A new school, with accommodation for 400 students, is now being built at the cost of the State.