An Address ON WOUND INFECTIONS;

AND ON
SOME NEW METHODS FOR THE STUDY OF THE
VARIOUS FACTORS WHICH COME INTO CON-
SIDERATION IN THEIR TREATMENT.

DELIVERED BEFORE THE ROYAL SOCIETY OF MEDICINE
ON MARCH 30TH.

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What are the Factors which Influence the Emigration of White Blood Corpuscles into the Wound?

I PASS now to consider yet another subject-matter—the emigration of leucocytes into the wound. I need not labour the point that this is a factor which may determine the issue of an infection; nor need I point out that it behoves us to acquire a control over the movement of leucocytes, and then to turn this to account, as the case may be, by activating or restraining emigration.

Broad foundations for our work have, as you know, already been laid by the brilliant researches of Metchnikoff. But it was with Metchnikoff always a question of experiments *in vivo*—that is, of experiments carried out under conditions which cannot be sufficiently simplified to give quite unambiguous answers. And we require for the elucidation of our problems and for all detail work connected therewith, absolutely simple crucial experiments, such as can only be made *in vitro*.

The line of thought which I have followed in elaborating a laboratory method for the study of the phenomena of emigration is the following: The leucocytes in extravascular blood are known to retain their emigrating power. A difficulty will, however, when we are working with extravascular blood, attach to our observations by reason of the fact that we have not at disposal such a containing membrane as the capillary wall. We are, in fact, in dealing with extravascular blood, confronted with a situation similar to that which would be encountered in observations *in vivo* if the capillary walls were to give way and we had to make observations on emigration in a portion of tissue which was flooded out by red corpuscles.

I had hoped at first to be able to circumvent this difficulty by taking advantage of the fact that when clotting occurs the red blood corpuscles become enclosed in a meshwork of fibrin, after the manner of fish in a net. But all my efforts to make the fibrin meshwork take over the office of a containing membrane were defeated. No matter how tenderly the clot was treated the meshes of the net broke, and haemorrhage from the clot interfered with the observations.

A second difficulty also presented itself. When in the living body white corpuscles emigrate into connective tissue it is possible to register their travel because they move forward through a retaining meshwork. It would not be possible to do so if they merely passed out into fluid, to be afterwards carried hither and thither by every chance convection current. Exactly the same applies to the extravascular blood. The emigrating leucocyte must be provided with some sort of scantling to move forward upon, and come to rest in.

After a time I alighted on a method which satisfies the two aforementioned experimental requirements, and which, as I think, provides all that is required for a quantitative estimation of emigration. Let me first tell you the general lines upon which the method proceeds, and then set out the details of the technique.

Principle of the Method Employed for Making Observations on Emigration.

The principle of the method is as follows: We fill in a capillary tube with blood from a prick in the finger, immediately place the capillary tube in the centrifuge, and centrifugalize until we have carried down all the corpuscles. We have now in the upper half of the tube a

plasma which has been completely freed from all formed elements; and in the lower half of the tube, at the bottom, the red blood corpuscles intermixed with a certain number of polynuclear white blood corpuscles; and above this a layer made up predominantly of white blood corpuscles—these last in the front ranks consisting almost exclusively of small and large mononuclears. The blood now clots. And this gives in the upper half of the tube a clot consisting of fibrin without any formed elements—let us call this the *white clot*—and in the lower half of the tube a clot—let us call this the *red clot*—which holds all the corpuscles in its meshes. When a chemiotactic stimulus now comes into application from above the white blood corpuscles will come out from the red clot and will travel upwards through the meshes of the white clot—afterwards maintaining their positions so as to allow of our making measurements and enumerations. We will now pass to the details of the technique.

Details relating to Apparatus and Procedure.

With regard to apparatus, all that is required is a supply of flat capillary tubes. By using flattened capillary tubes we obtain a thin clot, which can more easily be examined under the microscope.

We make these tubes—and they may conveniently be called emigration or chemiotactic tubes—either out of a small test tube, or out of a length of fairly wide-bored glass tubing. We

Fig. 5.—Method of making emigration tubes. A, Method of giving the flattened conformation to the capillary stem. B, Method of bending the tube round to form an elbow.

heat in the blowpipe flame until the glass becomes very plastic; then, making a sharp outward turn with the right wrist, bend the tube round through a right angle, giving it the proper flattened conformation; and then draw out into a long flat capillary stem (Fig. 5, A). We then cut this through at the point where it begins to lose its flattened conformation; and so leave attached to the next segment a sufficient length of tube to take hold of when we go to work upon it. When a sufficient number of lengths of flattened stem have been provided, we cut these up into segments of about 8 cm. in length; arrange them side by side after the manner of a palisade; and then with our glass writing pencil rule two lines across the face of our tubes. The first of these lines ought to fall somewhere in the middle. It is to serve as a fiducial mark for filling in the blood from the finger. The second line, which may conveniently fall at a point to about half or three-quarters of a centimetre to the end of the tube, is to serve as a fiducial mark in filling in the chemical agent whose effect we are to study.

The tube may now be used just as it is; or we may before using it furnish it with a siphon curve by bending it round at the level of our second fiducial mark. We do this by taking up each tube separately, holding it horizontally, and then passing it rapidly to and fro through a small by-pass flame. The action of gravity will then, as soon as the glass softens, bend round the tube for us (Fig. 5, B).

Method of Using the Emigration Tubes, and Bringing the Chemiotactic Agent into Application.

The emigration tube is first filled in up to the midway point with blood drawn from a puncture in the finger. In the case where we employ a bent tube this is done by letting the blood flow in through the siphon curve.

The chemiotactic agent can now be brought into application in three different ways.

1. It may simply be superimposed upon the clotted blood. This is done by using a *filiform pipette*, made by heating the stem of an ordinary capillary pipette in a small by-pass flame and drawing out, while we with a rubber teat apply internal pressure to prevent the walls of the hair-fine tube collapsing. Considered as a method for bringing a chemiotactic agent into immediate application, this

method falls short in the respect that the chemical has to diffuse down through the whole white clot before it comes into operation. In view of this, the method does not lend itself to the institution of any comparisons between normal blood and anaemic blood. For the experimental conditions are not comparable when our chemiotactic agent has, in the one case, to diffuse through a length corresponding to half the column of blood; and, in the other case, through a length which may amount to nine-tenths of that column.

2. The *second* method of bringing the chemiotactic agent into application is that of *superimposing the chemiotactic agent upon the unclotted blood* (Fig. 6, B and C).

Fig. 6.—Method of adding chemiotactic fluid to blood. A, Curved emigration tube, empty. B, Tube filled in with a column of blood, a dividing air bubble, and then, as far as the elbow, with a bacterial suspension. C, Tube after centrifugalization; the bottom of the tube is occupied by the red clot, and the intermediate portion by the white clot and implanted bacteria; at the top is the watery menstruum in which the bacteria were suspended. D, Tube filled in for traversing; in the lower portion of the capillary stem is the chemiotactic agent; in the upper part the column of blood. E, Tube after centrifugalization; the bottom of the tube is occupied by the red clot, and the intermediate portion by the white clot, containing traces of the chemiotactic agent; at the top the watery menstruum containing the bulk of this agent.

Having filled in a curved emigration tube up to mid-point mark with blood, we tilt the tube so as to take in a dividing bubble of air; then fill it up as far as the elbow with the chemiotactic agent; and then, after sealing up the distal end of the tube, proceed to centrifuge. This method is applicable, in particular, when we are working with bacterial suspensions; for while the watery suspending fluid remains, by reason of its light specific gravity, on the top, the microbes are, by virtue of their higher specific gravity, carried down into the plasma to be embedded in the white clot all the way down to the leucocytic layer.

3. The *third* method is the method of *traversing* (Fig. 6, D and E). In employing this we may use either a straight or a bent emigration tube. We fill in, first, with our column of blood; follow on with the dividing bubble of air; and then, making use of the forerunning column of blood to serve as a brake, fill in up to the fiducial point with the chemiotactic agent. Finally, we seal up that end of the tube which has served as an inlet, and place the tube in the centrifuge.

Our chemiotactic agent will now traverse the column of blood and take up a position at the top, leaving behind it in the plasma traces of whatever chemical it holds in solution. That this is what actually happens, can be shown by employing, in place of a colourless chemiotactic agent, a solution of methylene blue, or simple water. This last, when it follows on after a column of blood and dissolves the red blood corpuscles which this leaves in its wake, will come out at the top coloured with haemoglobin.

The traversing procedure will be applicable in the case where we want to bring into operation chemical agents,

and especially applicable where we want to bring such agents into instant application.

When we are working with a series of tubes, as will practically always be the case, it will be well to place each, as soon as it is filled, into iced water, or ordinary cold water. The buckets of the centrifuge will serve as convenient receptacles. After centrifugalization the emigration tubes are placed in the incubator, according to circumstances, for from three to twelve hours or more. While in the incubator the tubes may conveniently be placed upright—that is, with the white clot uppermost—in plasticine. They may also be laid on their sides, tilted a little upwards. The inverted position is to be avoided. For when we invert our tubes we bring down into the white clot a shower of red corpuscles which block emigration and also obscure the view.

Method of Bringing the Emigration Effect into View and taking Cognizance of the Results.

When all we want is to get a general idea of what is going on in the tubes, we can obtain this by introducing the unopened tube into an observation cell. A very simple form of observation cell can be made by placing small pellets of plasticine upon each of the four corners of a microscopic slide, covering in with another slide—allowing a little overlap—and then filling into the interspace either water or some more highly refractive fluid, such as glycerine or oil. By observing in such a cell we bring in view not only the leucocytes which have emigrated into the white clot, but also those which have escaped into the interspace which may develop between the clot and the walls of the capillary tube.†

For all purposes of quantitative observation we blow out our clots into water, wash carefully so as to remove leucocytes adhering to their exterior, and then mount them on a slide. We then, after cutting off the surplussage of red clot, fix by drying and stain for a few minutes in Kühne's

Fig. 7.—Clots from emigration tubes blown out and mounted. A, No emigration; B, compact emigration; C, dispersed emigration; D, method of enumerating leucocytes which have emigrated; E, bacterial colonies coming up only in portion of clot which has not been reached by the emigrating leucocytes.

methylene blue freely diluted. The specimens are examined, first, dry or in water, under a low-power objective, and afterwards in oil under an immersion.

† Attention may, in connexion with this, be called to facts which have an importance and a useful application in medicine, which happen to have also an importance and a useful application in connexion with the emigration method here under consideration. The facts I have in view are as follows: The blood of a person suffering from chilblains, or any other manifestation of a lowered blood coagulability, will, on centrifugalization, generally fail to give the kind of white clot we require for our emigration experiments—that is, one that is firm and non-contractile. This condition of things can be remedied by the exhibition of calcium salts, or, as the case may be, by appropriate additions of these salts made to the blood when filling our capillary tubes. I would in connexion with this emphasize that we have here brought into clear view what is really the material factor in connexion with the effect exerted by calcium salts in the blood.

And I may perhaps be permitted to point out, in connexion with my own work on calcium as an agent for promoting and citric acid as an agent for diminishing coagulability (BRITISH MEDICAL

* In practice the procedure of traversing is to be carried out exactly as here described. In the illustration Fig. 6, D, in order to convey the idea of traversing to the eye, the chemiotactic agent precedes instead of following the column of blood.

General Considerations relating to the Movements of White Blood Corpuscles.

Before passing to consider the question how it will be possible to arrive at a quantitative expression for the leucocytic movements induced by a chemiotactic agent, it will be well to take a general survey of the things that present themselves to view in every emigration tube.

We have to take into account in connexion with white blood corpuscles two kinds of movements. We have, on the one hand, a process of wandering at large; and, on the other hand, a directed movement—that is, a movement along some particular axis—undertaken under the direction of a chemical stimulus. We may call the first kind of movement an *eleutherotropic* movement. The second is usually known as a *chemiotactic*—I prefer to call it a *chemiotropic*—movement. It is, of course, the latter, not the former, kind of movement which we are here primarily concerned to study. For clearly it is the chemiotactically directed movements of the leucocytes towards the bacterial focus, and not their wanderings at large, which come into consideration in any conflict against infection.

None the less, a word may be said about eleutherotropic movements. One finds in every specimen of blood which has been simply centrifugalized and placed in an incubator, always a certain wandering at large of the leucocytes—in particular, the mononuclear white blood corpuscles, which have been tightly packed together by the action of the centrifuge, and are ranged at the top of the red clot, leave their ranks and wander out into the adjacent regions of the white clot. The polynuclear leucocytes also are affected by eleutherotropic wandering. They come out from the hinder ranks of the leucocytic layer, and also from deeper down in the red clot, and wander free. In our observations we leave out of account all those leucocytes that have wandered outside the white clot. We regard them as having run to waste.

A further point which claims attention in connexion with emigration is the nature of the emigrating leucocytes.

Ordinary *eleutherotropic* emigration is predominantly mononuclear, this being probably accounted for by the fact that the white blood corpuscles which are ranged up along the line which divides the red from the white clot are almost all mononuclear. In *chemiotactic* emigration we have either a differential emigration of polynuclear white corpuscles, or a mixed mononuclear and polynuclear emigration in which either the one or the other of these varieties of leucocytes may predominate. In all such mixed emigrations the polynuclear, presumably because they are faster of foot, overtake the mononuclear leucocytes and pass on and occupy the more distal portion of the field of emigration.

Method of Arriving at a Quantitative Expression respectively for "Compact" and "Dispersed" Emigration.

A quantitative expression for the emigration movement which takes place in a capillary tube can be arrived at in two ways. When we are dealing with a *compact* emigration (Fig. 7, b)—that is, where the field of emigration is quite closely packed with leucocytes—we have simply to measure the area of that field, or (and this answers the purpose perfectly well) the length of clot occupied by the emigration; and we may conveniently measure it in terms of microscopic fields. In such measurements we take off from the base line furnished by the distal border of the cone of leucocytes which occupies the base of the white clot. This represents the original leucocytic layer converted, by the contraction of the fibrin, into a cap covering the upper end of the red clot.

When, as will happen when a longer time has been allowed for the wandering out and dispersion of the leucocytes, we have before us a *dispersed* emigration (Fig. 7, c) it will no longer suffice simply to measure the distance the leucocytes have travelled into the clot. It now becomes

necessary to enumerate the emigrating cells. Employing first a low-power lens, we bring the upper edge of the cap of leucocytes to the extreme edge of our field of view. Then turning to our oil immersion, we bring this down upon the central portion of the low-power field, and make a count of all the leucocytes which lie within its purview, when necessary, helping ourselves out in our count, by introducing into the diaphragm of our eyepiece a cover-glass divided up by light rulings, made with a glass writing pencil. Having obtained in this way a value for the emigration in an arbitrarily selected portion of the first microscopic field, we go back to our low-power objective and move our specimen along until the objects on the extreme distal margin have been carried right across the field, and now lie just out of view on the other side. The further steps in the procedure are now simple repetition.

What are obtained by this method of enumeration are, of course, only arbitrary figures, and it will be realized that the comparative values arrived at will be strictly accurate only where we are employing clots of similar thickness.

Survey of the Data which the Method has Already Given.

We may now pass on to consider some of the data that the method has already given. White blood corpuscles will move out in any direction towards a chemiotactic substance. They will, however, emigrate more freely downwards, than horizontally; and more freely horizontally, than upwards.

Anaerobic conditions are more favourable to emigration than aerobic conditions. Leucocytes will travel out further in the direction of a chemiotactic substance when we absorb the oxygen in the tube with caustic alkali and pyrogallol acid and seal, than when we leave the end of the tube open to the air.

Leucocytes emigrate more abundantly in tubes standing at a temperature of 40° C. than in tubes standing at 37° C. They do not emigrate at temperatures of 10° to 15°—the temperatures which prevailed on our laboratory bench. After exposure to temperatures of 0° C. for periods of half to one hour they emigrate apparently as freely as before.

Emigration apparently goes on unaffected in the presence of ether. It is abolished or suspended in an atmosphere of chloroform.

Physiological salt solution, brought into application either by traversing or by superimposing it upon the clotted or unclotted blood, induces a very vigorous emigration of polynuclear white blood corpuscles. Weaker salt solutions induce a less vigorous emigration, and water again a less vigorous. Strong salt solutions—for example 5 per cent. salt solutions—suppress emigration.

It will be appreciated in connexion with these and all findings obtained by this method that they do not tell us the effect of reagents acting in the specified dilutions directly upon leucocytes, but only the effect of these reagents operating from a distance. In other words, our experiments do not furnish information as to what would be the effect of bringing the chemical agents in the specified concentrations directly in relation with the capillary wall.

Bacterial suspensions which have been sterilized by heating evoke, according to the dilution in which they come into application, quite different effects. The general rule applying to bacterial suspension would seem to be as follows: Concentrated suspensions usually completely suppress emigration. Ten or hundredfold diluted, these same suspensions evoke vigorous emigration. When we employ progressively higher dilutions we arrive in time at a point when the effect of the bacterial suspensions is exactly the same as those of the particular fluid which we are employing as a diluent.

Normal bloods tested with one and the same series of bacterial suspensions exhibit quite different degrees of chemiotropic sensibility. Chemiotropic sensibility, not alone to bacterial suspensions but also to physiological salt solution, is very strikingly modified in the case of patients suffering from bacterial infections. The same applies to persons inoculated with streptococcal vaccines. In five out of six men, inoculated with such a vaccine and examined both before and afterwards, the emigrating response to streptococcus was subsequently to inoculation very strikingly increased. In the case of one man it was diminished.

Results somewhat similar to those obtained with dead cultures are obtained with suspensions of living microbes (streptococci and gas phlegmon bacilli), but here the prolonging of the incubation period may strikingly alter the situation.

JOURNAL, July 29th, 1893, and July 1894; *Lancet*, January 18th, 1896, and January 3 th, 1897, that while what I have said with reference to the clinical effects exerted has been, I believe, universally confirmed and accepted; what I have said with respect to the effects of the coagulability of blood *in vitro* has been traversed. This stands, as I believe, in relation to the fact that the laboratory workers who have repeated my work have employed methods which took into account rapidity, but left out of account firmness, of coagulation. It will now, I hope, by adopting the method of centrifugalizing, and watching the effect exerted upon plasma which has been disembarassed of corpuscles, be possible to arrive at unanimity in these matters. Finally, I may direct attention to the fact that what comes into view in centrifuged, is seen also in uncentrifuged, blood. When we make to this appropriate additions of calcium salts, we obtain, as I long ago pointed out, a firmer and non-contractile clot.

What generally happens may be summed up as follows: When, by superimposing and centrifuging, a heavy sowing of microbes has been implanted into the unclotted blood, the colonies come up all along the white clot, and emigration into this is completely checked. Where only a moderate implantation of microbes has been made we have in different parts of the clot different results: Bacterial colonies develop freely in the distal area of the clot which is not invaded by emigration. In the intermediate region—that is, in the region where the microbes can grow out before the leucocytes arrive—one sees with the low-power objective areas specially crowded with leucocytes—these are in point of fact colonies which are being broken up and dispersed by invading leucocytes (Fig. 8)—and with the oil immersion one sees that every leucocyte in these crowded masses is taking up microbes, and that there is also in all this region of the clot plentiful phagocytosis of scattered microbes (Fig. 9). In the base of the white clot—that is, in the area where emigration has occurred earliest and most vigorously—one finds absolutely no trace of microbial growth.

The appearances which have just been described correspond, of course, to a period of conflict. This conflict is generally at its most interesting phase in tubes which have been incubated from three to six hours. When we come

Fig. 8.—Extreme limit of emigration, showing phagocytes attacking streptococcus colony.

later—for instance, after twelve or more hours—the conflict is over. We then find either that the white corpuscles are masters of the field and the microbes have disappeared; or else that the microbes have invaded the whole clot, and that this crumbles away as soon as it is blown out into water. There can be little doubt that the crumbling away of the clot, and the overrunning of the blood with microbes, are due to the digestion of the fibrin, and to the corruption of the blood fluids by trypsin set free from the disintegrated leucocytes.

Experiments such as these just outlined, in which living microbes are brought into application on blood, provide, in point of fact, a valuable test method. They tell us the resultant of the *chemiotropic sensibility* of the leucocytes, the *opsonic power* of the blood fluids, the *digestive capacity* of those phagocytes which come into action, and of the *antitryptic* and, where such comes into account, the *bactericidal power* of the blood fluids. We are, in fact, furnished with something like a complete evaluation of the antibacterial powers of the blood.*

There is still one element left out of account. The method here in question does not tell us anything as to whether or no there are bacterial poisons in solution in the blood fluids of the infected man. But this is a question which can be separately investigated. I have tried to find the answer by traversing normal bloods with serums derived from patients suffering from septicæmic infections. And I have by this procedure obtained in a series of cases what appeared to be a definitely chemiotropic emigration.

All that has been recounted above is, of course, only a very small beginning. But I think we may be

* It will be observed in connexion with tests thus conducted with centrifuged blood that, if we leave out of regard the centrifugation, they are in all essentials the same as the phagocytic-bactericidal tests with freshly drawn uncentrifuged blood which I have already described (*Vaccines and Drugs in Pneumonia*, Constable, 1914).

confident that the method for the study of emigration which has here been proposed will resolve important problems in connexion with infection generally, and also—and this is what specially concerns us here—some of the problems in connexion with wound infections which are now urgently pressing for a solution.

In the matter of general problems of infection it would, I think, be possible, by implanting bacteria into blood in combination with chemical agents which would respectively promote and hinder emigration, to resolve for each particular bacterial infection the question as to whether it is the leucocytes or the blood fluids which come most into consideration as destructive agencies. That problem once resolved, we should know whether we ought, in the infection in question, to direct our chief efforts to increasing the efficacy of the blood fluids or to modifying the chemiotropic sensibility of the leucocytes and encouraging emigration.

Again it looks as if it might be possible by very simple experiments to resolve the problem as to why in gonorrhoea, and also in other surface infections, the purulent discharge is suddenly arrested when the microbes succeed in invading the blood stream, or establishing themselves in an articulation or elsewhere in the interior of the body. It would seem possible (for something of this sort would seem to occur in septicæmias supervening on wound

Fig. 9.—Phagocytosis of streptococci towards extreme limit of emigration.

infections) that we may be dealing here with a paralysis of the emigrating powers of the leucocytes. Or, again, it is possible that in these cases emigration may be simply suspended by a redistribution of the chemiotactic forces. In other words, the cessation of the external discharge may simply mean that there is now in the blood a bacterial poison; and that this chemiotactic element, acting upon the leucocytes as a *vis a tergo*, counterbalances the *vis a fronte* of the chemiotactic substances produced by the bacteria on the external surfaces.

In connexion with the particular problems of wound infections we may hope at no distant date to come into possession of information which will enable us to activate or restrain, according as the one or the other may approve itself the better policy, the emigration of leucocytes into the wound. And we may hope also to determine in connexion with every antiseptic or other solution which is brought into application in a wound, whether it promotes or hinders emigration.

Finally, let me point out—for everything that concerns bacterial vaccines concerns the treatment of wound infections—that experiments on emigration will almost certainly resolve a number of important outstanding questions in connexion with vaccines. They ought very easily to resolve the question as to what is the best excipient for a vaccine—whether a menstruum which would restrain emigration, or one like physiological salt solution which would call forth a vigorous emigrating response at the point of injection. And lastly—and this would be one of the most important applications of observations on emigration—we ought to be able to determine what is in the case of each bacterial vaccine the dose which will induce the earliest possible and the most effective determination of phagocytes to the focus or foci of infection.

(To be continued.)