THE INFLUENCE OF ANTISEPTICS ON THE ACTIVITIES OF LEUCOCYTES AND ON THE HEALING OF WOUNDS.

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PART II.

IN the BRITISH MEDICAL JOURNAL of June 3rd, 1916 (Part I), I described a method (the use of pigment particles) for the study of the activities of leucocytes in closed aseptic and in open granulating wounds. By the application of indigo, carmine, or carbon particles to different kinds of wounds under different conditions of treatment we were enabled to follow the three chief phases of phagocytosis—namely, emigration of leucocytes, ingestion of foreign substances, and return immigration of loaded cells. We also found that return immigration of loaded phagocytes was probably intimately associated with the phenomena of latent infection and recrudescence sepsis in wounds.

In the present communication I propose to describe some observations which throw further light on this important problem of return immigration. Here, as on the former occasion, we must distinguish carefully between wounds in which the pigment particles have been introduced under aseptic conditions during operation and infected wounds in which pathogenic organisms and their toxins are also present.

The Transportation of Pigment Particles in the Tissues.

It will be necessary first to describe some experiments on animals undertaken with the object of tracing the movement of the pigment particles in the tissues, as it is only under exceptional circumstances that this phenomenon can be observed in the human subject.

EXPERIMENT 1.—Aseptic Thread in Muscle.

A carmine thread was introduced under aseptic conditions into the thigh muscles of an anaesthetized guinea-pig. The

FIG. 1.—Drawing from section through thigh in neighbourhood of carmine thread. Guinea-pig 7. Shows carmine particles A in leucocytes entering capillary and in B fibroblasts. $\frac{1}{12}$ in. obj., A oc.

animal was killed seven days later, and the limb hardened in formalin. Suitably stained embedded sections of the muscular tissues in the neighbourhood of the thread showed collections of pigment-loaded leucocytes along the intermuscular connective tissue strands, especially around the blood vessels. In some cases pigment-carrying cells could be traced in the walls and in the lumen of the capillaries. (See Fig. 1.)

EXPERIMENT 2.—Granulating Wound.

In the case of the open granulating wound in guinea-pig 9 (described in Part I) which had been dressed with indigo gauze, a further fact was also noted. Although the pigment had been applied uniformly over the whole surface of the wound it was not carried away uniformly by the phagocytes over the whole area. The wound on section was of the shape shown in the

diagram, and the return stream of pigment-loaded cells was most marked round the periphery, in the angles formed by the

apposition of the roof and floor of the wound in the situations marked by the crosses. (See Fig. 2.)

The stream of pigment-loaded cells was greatest in fact where the vascular and connective tissue structures favoured movements of phagocytes. These favourable conditions were not present equally all over the floor of the wound, but chiefly in the angles where the floor and the roof meet. These are also the situations where the spread of infection takes place in wounds. Other examples of unequal pigment flow will be described in connexion with wounds which involve bone tissue, but the close

FIG. 2.—Drawing from section through granulating wound dressed with indigo. Guinea-pig 9. Shows indigo carried by leucocytes, A, from surface of wound into deeper tissues. $\frac{1}{2}$ in. obj., A oc.

association which exists between the transportation of pigment by wandering cells, and the spread of infection in wounds, supports the suggestion made in the earlier paper that the spread of infection in a wound depends not only on the active growth of the infecting organisms, but on a transportation of the same organisms by wandering cells. I shall, however, return to this subject later; at present I wish to emphasize the fact that transportation of pigment particles and spread of infection both tend to take place by way of the connective tissues rather than along the muscles in the limbs, or the parenchymatous tissues in the internal organs. This does not apply to acute cases of gas gangrene, in which the infection spreads also along the muscle fibres.

EXPERIMENT 3.—Aseptic Thread in Testis.

A section was taken from the testis of a guinea-pig at some distance from the site of the insertion of an indigo thread introduced a week previously. The pigment-bearing cells occupy the strands of areolar tissue which connect the seminiferous tubules; they are not found in the epithelium lining the tubules. The indigo chiefly occupies the fusiform fibroblast cells. (See Fig. 3.)

The pigment is undoubtedly taken up by the polymorph leucocytes which collect among the fibres of the indigo thread. In an open granulating wound it is taken up by the leucocytes which emigrate on to the surface of the pigment-coated granulation tissues. Having ingested the pigment these loaded leucocytes (provided they are not thrown off as dead pus cells) can be traced in return-immigration through the granulation tissue to the underlying fibrous and muscular tissue. The paths followed lie chiefly around the blood vessels and the strands of areolar tissue which connect together the skin, muscles, and other structures, or, as in this case, the seminiferous tubules. The loaded leucocytes enter the general circulation, whence they eventually reach the liver and spleen, or they are carried by the lymph channels to the nearest lymph glands, where the pigment granules can be found in considerable

numbers, generally in the peripheral rather than the central portion of the gland.

At this point we reach a break in the chain of direct observation. We have already seen that the pigment is

FIG. 3.—Drawing from section of testis after insertion of indigo thread. Guinea-pig 7. Shows indigo grains, A, in the connective tissue cells between the tubules. 1/8 in. obj., A oc.

taken up by the fixed tissue cells. Inasmuch, however, as these pigment particles neither multiply nor have power of independent movement, inasmuch also as there are no fibroblasts on the surface of the granulating wound or in the interstices of the pigment thread in the closed wound, it is evident that the pigment must be carried to the fixed tissue cells by the polymorph phagocytes. The question arises how this transference is effected. We can trace the loaded leucocytes entering the blood vessels and the tissue spaces; we next find the pigment granules inside the fixed tissue cells. Do the fixed tissue cells and the endothelial cells in their turn ingest the loaded leucocytes, or do the leucocytes disengage the pigment granules after they have reached the tissue spaces, and are the granules so liberated taken up by the connective tissue cells? Close observation of the behaviour of pigment-carrying leucocytes does not suggest that these cells are ingested *en masse* by the fibroblasts.

The Movements of Pigment Particles in Bone Tissues.

It so happens that certain amputation stumps in wounded soldiers which necessitate the removal later of extruded bone afford a favourable opportunity for studying this problem in the human subject. (See Fig. 4.) In several such cases I have had the granulation tissue which forms a cap on the end of the extruded bone dressed for some days or weeks before re-amputation with sterilized indigo or carbon, or latterly with sterilized rice starch. After re-amputation, films from the marrow from the shaft of the removed bone at a distance of one or more inches from the dressed end, have in some instances shown pigment granules in the marrow cells. (See Fig. 5.) Pigment can be seen inside the leucocytes which pass in more or less continuous streams from the surface of the stump to the somewhat abrupt line where the granulation tissue joins the marrow tissue proper. Here there is a break in the continuity of flow; a few loaded cells can be seen in the spaces amongst the marrow cells, but for the most part the pigment in the deeper cells is in the form of minute granules, unlike the coarser masses in the leucocytes; this is especially noticeable in the case of starch granules, and I have not come across any evidence of the ingestion of loaded leucocytes *en masse* by tissue cells. These facts, as far as they go, suggest a disengagement of pigment by the wandering cells and

FIG. 4.—Drawing of necrotic bone; re-amputation of thigh stump. A, Granulations in medullary canal; B, granulations covering soft tissues; C, shell of necrotic bone. Pigment dusted on A recovered from marrow cells at X.

granules, and I have not come across any evidence of the ingestion of loaded leucocytes *en masse* by tissue cells. These facts, as far as they go, suggest a disengagement of pigment by the wandering cells and

a reingestion of the liberated particles by the tissue cells. Pigment-bearing leucocytes pass from the surface to the deeper layers in isolated vertical streams. There is no equal downward flow over the whole of the

FIG. 5.—Drawing from film of marrow cells from shaft of femur two inches above granulating surface. A, Shows indigo grains in myelocytes, 1/12 in. oil im., A oc.

surface of the granulating area. Depressions on the raw surface appear to act as reservoirs, in which phagocytes collect, and from which they pass directly down to the deeper layers. The furrows between the little knobs of granulation tissue and the groove which forms between the protuberant granulations and the edge of the necrotic bone act as foci for these pigment streams. This affords further evidence of the close analogy which exists between the movements of pigment-loaded leucocytes and the spread of infection in wounds. (See Figs. 6, 7, 8.)

It is anaerobically infected compound fractures and sinuses leading down to necrotic bone which are most liable to recrudescence sepsis. Recent experience in the transplantation of bone has shown that if a bone graft is composed of both compact and endosteal tissue, if it is firmly contacted with living host bone, and if it is early provided with an efficient new blood supply, such a bone graft will prove very resistant to infection. How can we reconcile these apparently contradictory characters in bone tissues: Liability to infection and necrobiosis with a marked tendency to retain infecting organisms and to exhibit recrudescence sepsis under certain kinds of traumatism on the one hand, with a marked capacity to resist infection, when used as a bone graft, on the other? We have

FIG. 6.—Drawing from section through granulation tissue covering re-amputated stump (leg) after application of sterilized rice starch. 2/3 in. obj., A oc. Shows starch grains (coloured blue with iodine) A passing from the surface of the wound into the deeper tissues (the lines from A intersect the surface of the wound on the left).

seen that the marrow cells in the human subject have a considerable capacity for ingesting pigment particles and possibly (but this requires further investigation) of digesting micro-organisms, in which respect they resemble their near relations the polymorph leucocytes. It would seem, therefore, that the problem is bound up with the phagocytic capacity of the marrow cells. In healthy

conditions of the wound and with a good blood supply the cells in the cancellous tissues can deal with a moderate infection. If, however, the injury to the tissues is severe and the infection virulent, as in gunshot wounds, the

FIG. 7.—Drawing from area included in circle in Fig. 6; higher magnification. $\frac{1}{12}$ in. water im., A oc. Shows starch grains in connective tissue cells, A.

organisms can entrench themselves in the bone and become inaccessible to the phagocytes. Or if after ingesting the organisms the marrow cells themselves lose their vitality, then the imprisoned organisms may be liberated and recrudescence sepsis may arise. Microscopic sections from the granulation tissue from these stumps which have been dressed with some easily recognized pigment (such as sterilized rice starch) before re-ampputation, show an outward stream of empty cells emigrating on to the surface and a return or immigrating stream of loaded cells passing from the surface to the deeper layers of the granulation tissue, and in some cases to the marrow tissue. Beyond this point it is difficult to follow the ultimate destination of the phagocytes. In experimental wounds in animals pigment carrying cells are found in the nearest lymph glands. Some after entering the general circulation reach the liver and spleen. The presence of minute particles of pigment in the tissue cells at a distance from the end of the bone suggests that an unloading

FIG. 8.—Drawing from section through granulation tissue covering re-amputated stump (femur). Previous application of starch for ten days. Shows starch grains in leucocytes and fibroblasts, A. $\frac{1}{12}$ in. obj., B oc.

process may take place by which pigment is discharged by the leucocytes and taken up by the tissue cells in a disintegrated form.

Phagocytosis (especially Return-immigration) and Immunity.

A complete visualization of the whole process of the leucocytic circulation in wounds would enable us to realize the extent to which the process of immunization is bound up with efficient phagocytosis, especially in its return-immigration phase. The production of antibodies depends on the introduction into the blood, lymph, or tissues of the

host of either living or dead organisms or their toxins. The microbial products which stimulate the defensive reaction are carried into the body by the blood or lymph, or they are transported thither by wandering cells. We have already seen that the return-immigration of leucocytes which constantly goes on in open as in closed wounds provides a mechanism by which pigment particles are carried into the tissues, and there are reasons for thinking that organisms and their products are transported in the same way. It depends on the efficiency with which the returning phagocytes digest the interned organisms whether this process of natural vaccination shall take place by dosage with living or with dead organisms. While all the phases of phagocytic activity—namely, emigration, ingestion, digestion, and return-immigration—are important, the factor of return-immigration of germ-laden cells may prove to be of greater significance in the immunizing process than has hitherto been realized. It is possible that return-immigration may provide the mechanism by which dosage is regulated under normal conditions in a wound. It is upon dosage, the amount and the frequency with which toxins are introduced, that the success of natural as of artificial immunization largely depends. Any interference, therefore, which may derange this delicate mechanism is liable to do more harm than good.

Summary.

We shall now attempt to bring the facts derived from a study of the movements of pigment particles when introduced experimentally into wounds into relation with the known facts about the spread of infection in the tissues and the behaviour of phagocytes towards ingested organisms.

First, as regards the phagocytes, Peyton Rous and F. S. Jones¹ showed that certain kinds of pathogenic organisms, when ingested by leucocytes, were thereby protected from the bactericidal and bacteriolytic action of antibodies present in the serum. On the death of the phagocytes, the organisms so liberated were capable of renewed growth. The same observers also found that potassium cyanide in N/150 concentration killed typhoid bacilli while free in a suspension of guinea-pig's corpuscles without killing those cells, but that the bacilli which had been ingested by the corpuscles were protected from the action of the potassium cyanide and grew when the emulsion was plated out. Kenneth Goadby,² from a bacteriological examination of sinuses and from a consideration of the action of autogenous vaccines in preventing the reinfection which so frequently occurs in sinus cases, concludes that reinfection is closely associated with phagocytic action.

Dr. Crosswell Shearer and Dr. Warren Crowe³ have recently shown that the meningococcus may be ingested by human leucocytes in large numbers without undergoing digestion, and they have recovered living germs from these leucocytes in a number of cases. They also bring forward evidence in favour of the view that under certain conditions the leucocytes may act as the carriers of the infection from the nasal passages to the central nervous system. The recovery of pigment-loaded leucocytes from the cerebro spinal fluid after spraying the nasal mucous surfaces with some innocuous pigment would go far to settle this problem.

My own observations suggest that return-immigration of leucocytes is intimately associated with the spread of infection and recrudescence sepsis in wounds.

In the second place, the part played by the fixed tissue cells in infection is a very important one. The observations of Kyes show the important part played by the haemophagocytic cells of the liver and spleen in destroying pneumococci injected into the general circulation of the pigeon, and Bull, at the Rockefeller Institute, has shown that intravascularly agglutinated bacilli ingested by polymorph leucocytes are carried to the liver and spleen and ingested by the cells of those organs. In this paper I have described the process of ingestion by the fixed tissue cells of pigment granules carried by leucocytes and the ingestion of pigment granules brought by phagocytes to the liver and spleen cells. The exact method by which the transference of pigment is effected, whether by internment of the leucocytes *en masse* or by a process of disengagement and reingestion, requires further elucidation. It is, however, clear that a close analogy exists between the

transportation of pigment by wandering cells and the spread of infection in wounds. The spread of infection is due to a breakdown in one or more of the factors on which phagocytosis depends. Either the wandering phagocytes do not emigrate into the invaded area in sufficient numbers, or, having emigrated, they do not ingest the organisms, or the breakdown of the defensive mechanism may occur at a later stage. The phagocytes may emigrate and successfully intern the invading organisms, but they may fail to digest them, or things may go wrong during return-immigration. The organisms may be liberated by the leucocytes while still alive, just as the pigment granules may be liberated, and they may retain sufficient virulence to originate a recrudescence of infection in a new area. It is quite likely that some part of the autointoxication which attends the too free movement of infected limbs may depend on a liberation of ingested organisms brought about by injury to germ-containing leucocytes rather than to a mechanical pumping of the organisms or their toxins into the tissues.

Practical Conclusions.

If it be true that some part (how large a part is at present unknown) of the spread of infection in wounds is due to a breakdown in the defensive mechanism of phagocytosis, of which return-immigration is only one phase; if, in other words, a certain number of pus cells in infected wounds re-enter the circulation or the tissues carrying ingested but still living organisms with them, and if a certain proportion of the latent infection in sinus cases and the recrudescence of sepsis which breaks out in healed wounds is due to this same cause, then any method of wound treatment to be successful must deal with the problem of the return-immigration of germ-laden phagocytes. The subject is one, however, of great delicacy and difficulty. If antiseptic solutions when applied to wounds are sufficiently strong to kill the phagocytes and their cargoes, and so prevent their re-entrance into the tissues they will also inhibit emigration and phagocytosis. The irrigation of a wound with normal saline favours emigration and ingestion, but it does not prevent return-immigration. The application of hypertonic saline, on the other hand, may prevent return-immigration by killing the leucocytes, but it also checks emigration and phagocytosis.

A few experiments have been carried out to test the effect of various antiseptic agents on the capacity to ingest pigment or starch by the leucocytes and pus cells exposed to the action of such reagents when applied to wounds. The pus from a case of compound fracture of the tibia in which a cup-shaped granulating wound was dressed with the mixture of iodoform, bismuth subnitrate, and liquid paraffin known as "Bipp," to which also some sterilized rice starch had been added, showed that the percentage of leucocytes containing starch grains was reduced, while the proportion of starch grains free in the discharge was increased, although a considerable proportion of the pus cells from the same wound retained their vitality.

It is interesting to note that the starch grains in the pus from wounds which have been treated with the iodoform, bismuth, and paraffin paste are colourless. The grains in the interior of the phagocytes and the free grains only show the blue colour characteristic of the presence of free iodine after that reagent has been added. This bears on the question of the liberation of iodine in wounds dressed with iodoform paste.

Samples of pus from wounds previously dressed with gauze soaked in Dakin's solution (Dautresne's formula) with the addition of a little rice starch show that the inhibitory effect on the ingestive capacity of the leucocytes is less marked than in pus from wounds dressed with the iodoform paste. In one superficial granulating and healing wound, dressed twice daily with soaks of Dakin's fluid, nearly all the starch grains were ingested by the leucocytes in the fibres of the gauze dressing.

Certain facts stand out prominently in this world-wide and still unsolved problem of the routine conversion of infected into sterile wounds in war. An experiment in immunization is now being carried out in Europe on a colossal scale. The control of the virulent infection set up in the wounded tissues is far and away the biggest factor in the problem of the treatment of wounds in war. One is struck by the rapidity and efficiency of the immunizing process as carried out by Nature in some cases

and by the failure of it in others. The difficulty is that we cannot distinguish beforehand those cases in which it will succeed from those in which it will partly or completely fail.

REFERENCES.

¹ *Journ. Exper. Med.*, vol. xxv. ² *Lancet*, July 15th, 1916. ³ *Proc. Roy. Soc. of Med.*, November 9th; *BRITISH MEDICAL JOURNAL*, November 25th, 1916, p. 725.

OBSERVATIONS UPON DYSENTERY CARRIERS.

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DURING the winter of 1915 several hundred typhoidal dysentery cases, in varying stages of convalescence, came under our observation for the purpose of eliminating, by bacteriological examination, those carrying infection. The methods we adopted in the routine examinations were the same in all cases no matter what was the original diagnosis accompanying the patient from overseas. We were led to adopt this uniformity of procedure as many of the cases had been diagnosed at the outset on clinical grounds only, and cases labelled "typhoid" or "paratyphoid" we found on examination to be dysentery.

The urine and faeces of all cases were examined and the procedure was as follows: The morning specimen of urine was obtained, the patient being instructed that while micturating he was to collect the latter portion in a sterile bottle. This was then centrifugized and the deposit planted out upon plates of MacConkey's bile salt neutral red lactose agar. For faeces a portion was emulsified in ordinary broth, and, after standing, the emulsion was plated upon MacConkey's medium. A small quantity of a second portion of the faeces was emulsified in double-strength Gram's iodine and then a drop of the emulsion was examined microscopically for protozoa. Sometimes an emulsion in saline was examined in addition. After forty-eight hours' incubation the plates were examined and likely colonies subcultured into broth, agar, and various sugar media. Finally, probable cultures were submitted to macroscopic agglutination tests with known serums.

All the cases were examined many times over so as to ensure that no carrier would slip through the net. This involved a large amount of work, as during the winter six months we made about 5,000 examinations altogether, but when the work is organized it can easily be overtaken.

BACILLARY CARRIERS.

We have only met with six cases carrying the *Bacillus dysenteriae*. Three were carriers of *B. dysenteriae* "Y" and three of *B. dysenteriae* Shiga. One of these latter was a combined infection with *Entamoeba histolytica*.

Of the three "Y" infections two of the patients contracted the disease in Gallipoli, and only one gave a history of typical dysenteric symptoms with blood and mucus in the faeces. His illness began in August, 1915, at Gallipoli; he had a relapse in the following October, and we recovered the "Y" bacillus from his faeces on January 28th, 1916.

The second case of "Y" infection contracted in Gallipoli had its onset about the beginning of October, 1915, with general malaise and fever; a week later the bowels became loose for four or five days only, during which period the patient was, at times, only partly conscious, but there was never any blood or mucus at any time, and the diarrhoea never recurred. On January 25th, 1916, we recovered the "Y" bacillus from the faeces, and on February 22nd and March 17th we again isolated the same organism from the faeces. Four examinations later, in April, gave negative results. The third case of "Y" infection is unusually interesting, for the man apparently contracted his infection in hospital. He was attached to a hospital in Lemnos as an orderly and was frequently on duty in the dysentery ward. He had not been anywhere else, and about the middle of October, 1915, after three months' duty in hospital, he was taken ill. For two to three weeks he was fevered and felt ill, but the symptoms were very indefinite and there was nothing to suggest that he was suffering from dysentery. The motions were normal and the bowels tended to be constipated, requiring enemata every second day during the first three weeks of his illness. Five weeks after the onset of his illness he was transferred home, and on January 25th, 1916, we