root pains or root symptoms for some time, weeks or months, before the onset of the paraplegia would be in favour of compression by growth and against functional disease. After a time in other cases I have found that though sensation to touch, pain, and temperature have been felt quite well the vibrating sensation has been lost, and if, in spite of suggestions to the contrary, the patient persists in these statements, we have a valuable indication against the diagnosis of functional disease. Later a definite Babinski reflex may be obtained and incontinence of urine may occur; these would be symptoms indicating organic disease.

In a number of cases where the symptoms later have indicated combined postero-lateral degeneration or sclerosis, at the earliest stage the symptoms may be only slight weakness or heaviness in the legs and indefinite and slight unsteadiness in walking, and functional disease has to be considered. In such cases I have found the vibrating sensation lost whilst other forms of sensation are felt. At this stage also only an indefinite and occasional Babinski reflex may be obtained. From the loss of the vibrating sensation without loss of other forms of sensation in these cases I have excluded functional affections and later definite Babinski reflexes, ankle clonus, paresis, ataxia, and the symptoms of postero-lateral degeneration have developed.

Difficulty in differential diagnosis occasionally occurs in cases of paralysis or paresis of the muscles below the knee, of the muscles which dorsiflex the foot (anterior tibial muscles), and of those which plantar-flex the foot. The symptom may be bilateral or unilateral. Various lesions of the cord or peripheral nerves may produce this form of paralysis. Sensation and the bladder and rectum may be unaffected, or sensation may be only slightly affected. The knee-jerks may be obtained; ankle clonus is not obtained, and the plantar reflexes are lost or feeble or indefinite. But usually the tendo Achillis reflex is of diagnostic service. If the tendo Achillis reflex is *lost* the disease is organic. If the disease is functional the tendo Achillis reflex will always be obtained (it might also be obtained in very localized organic lesions). If the anterior tibial muscles only are affected the tendo Achillis reflex may be obtained in organic cases, but in these cases the patient would be able to stand on his toes (with his heels raised from the ground) but not on the heels with toes raised off the ground.

We meet with many cases of functional paresis among soldiers sent home from active service, presenting three pronounced symptoms—rigidity, paresis, and tremor of the legs. Sensation is normal, the reflexes are normal or merely increased, and the bladder and rectum are unaffected. In some of the cases the symptoms have followed an injury and the cases are often incorrectly diagnosed as "spastic paraplegia." Injury and bullet wounds of the cord, pachymeningitis and other organic lesions may cause somewhat similar symptoms. Sustained and marked ankle clonus and rectus clonus would be in favour of organic disease. But they are often absent in the slighter cases of organic disease, and a pseudo-ankle-clonus or pseudo-rectus-clonus may be occasionally obtained in these functional cases, though the clonus is usually less sustained and less marked than in organic disease. Often the "clasp knife" rigidity is of diagnostic service. The rigidity of legs in the extended position at the knee is marked and we have much difficulty in bending the knee at first, but when once it is *very slightly* bent further flexion is easy (as in bending a clasp knife). This form of rigidity would indicate organic disease. Also a Babinski reflex would be definite indication of organic disease.

CONCLUSION.

Certain reflexes are of the greatest service in the differential diagnosis and early recognition of organic disease, and especially valuable in early cases are the Babinski or Oppenheim reflex, and in many cases the loss of the tendo Achillis reflex, since these signs may be detected when other definite changes have not yet occurred.

The chief difficulty occurs when the knee-jerks are not lost, and when ankle clonus, rectus clonus, and clasp knife rigidity are not obtained. In one group of cases the Babinski or Oppenheim reflex is obtained, and this is diagnostic of organic disease. In other cases the plantar

reflex is not of the Babinski type, and it may be lost or feeble. In these the loss of the tendo Achillis reflex would be diagnostic of organic disease.

The following are combinations of diagnostic importance, especially at the onset of a number of organic affections.

Paresis with loss of the tendo Achillis reflex, as in early anterior poliomyelitis—chronic, subacute or acute.

Paresis with loss of the plantar reflex and loss of the tendo Achillis reflex (in many organic diseases).

Paresis with double sciatica and loss of the tendo Achillis reflexes, as in early cauda equina lesions.

Paresis with loss of the tendo Achillis reflex, loss of the vibrating sensation, and pains in the legs, as in early peripheral neuritis.

Loss of the vibrating sensation with very slight incoordination and very slight paresis, with or without a Babinski reflex, as in early combined postero-lateral degeneration of the cord.

Paresis, with Babinski reflex (in many organic affections).

Paresis with loss of the vibrating sensation and Babinski reflex (in the early stages of several organic diseases of the cord).

Root pains, or root symptoms, followed after a period of weeks or months by paresis, as in meningeal spinal tumour.

(In all these early combinations the knee-jerks may be obtained.)

When the diagnosis has been especially difficult, or the symptoms slight and indefinite, the three indications of organic disease which I have found of the greatest service in my own practice have been the Babinski or Oppenheim reflex, the loss of the tendo Achillis reflex, and the loss of the vibrating sensation, whilst other forms of sensation are unaffected.

The value of the Babinski reflex is well known, but the cases briefly mentioned and the points emphasized in this article are, I think, sufficient to indicate that in addition to the well known signs in favour of organic affections we have in the loss of the tendo Achillis reflex, or of the vibrating sensation (whilst other forms of sensation are unaffected), two further signs of much service, and deserving of more frequent consideration in the differential diagnosis and early recognition of certain forms of organic nervous disease.

In certain specially difficult cases, when other usual and well known signs are indefinite or cannot be detected, these two signs are often of great diagnostic value.

AN "IN VITRO" METHOD OF DEMONSTRATING THE "RETURN IMMIGRATION" OF LEUCOCYTES IN BLOOD CLOTS AND IN WOUND TISSUES.

By C. J. BOND, C.M.G., F.R.C.S., COLONEL A.M.S.,

HONORARY CONSULTING SURGEON, NORTHERN COMMAND; HONORARY
CONSULTING SURGEON TO THE LEICESTER ROYAL INFIRMARY;
MEMBER OF THE MEDICAL RESEARCH COMMITTEE.

In articles published in the BRITISH MEDICAL JOURNAL during the last three years¹ I have drawn attention to the phenomenon of the "return immigration" of phagocytes. The main facts established have been: (a) The transportation of pigment particles, previously applied to the surface of granulating wounds, by wandering cells to deeper layers of the granulation tissue. In some cases these pigment-laden cells were recovered from the medullary cavity of exposed bone at some distance from the granulating surface of the wound. (b) These results in the human subject were confirmed by experiments on animals, which also showed in some cases the paths in the tissues traversed by the wandering phagocytes.

Since, however, the question of the possible transference of the pigment granules by the blood or lymph stream arose in all these cases it became important to control these results in the living subject by experiments on excised portions of living tissue, where the circulatory factors could be excluded. In other words, it became necessary to devise some experimental method whereby the only

factor which could be concerned in the transportation of the pigment granules would be the phagocytes themselves.

Experiment I.

Blood is drawn from the finger into a small-bore glass tube and allowed to clot. After coagulation and contraction the clot, if gently detached, lies in a bath of serum. A few drops of a mixture of finely ground carbon particles suspended in normal saline is now added by a pipette to the serum surrounding the clot. The tube is gently rotated so as to bring the suspended pigment in contact with the whole of the outer surface of the clot. The tube with its contained blood clot floating in a bath of carbonized serum is then incubated. After a few hours, varying from six to twenty-four, the clot is removed from the tube and hardened in 5 per cent. formalin. Sections of the cylindrical clot cut at right angles to its length are then stained with methylene blue or Leishman's stain.

In order, however, to save labour in searching for the leucocytes, it is better to centrifugalize the blood before clotting takes place. We then obtain a cell-free zone above, a red corpuscle zone below, and a leucocyte zone between the two. After contraction of the clot has taken place the pigment mixture is added to the serum, care being taken to bring the pigment in contact with the surface of the clot in the leucocytic zone. Fig. 1 shows

FIG. 1.—Section (semi-diagrammatic) of centrifuged blood clot, after incubation in serum with carbon pigment. A, Cell-free fibrin zone. B, Leucocyte zone. C, Red corpuscle zone. D, Carbon pigment.

the appearance of such a pigment-coated clot. The black coating is especially marked in the leucocytic zone. Fig. 2 shows the appearance of a section cut parallel to the length of the clot. In the upper non-cellular fibrin zone a thin coating of carbon pigment is seen covering the outer surface, but no free pigment in the interior of the clot. In the lower red corpuscle zone the same appearance is seen. In the leucocytic zone, however, not only is the pigment thickly deposited on the surface but pigment granules are found in the interior. With a higher magnification (Fig. 2) these granules will be seen to lie in the interior of leucocytes. Of the number of pigment-containing leucocytes which lie on the surface some will be seen to have passed into the interior of the clot, some have made their way upwards into the cell-free zone, while a smaller number will be found lying amongst the red cells in the red corpuscular zone. Pigment granules are not found lying free in the clot, and those that reach the interior must have been carried there by the return immigration of pigment-laden leucocytes from the surface.

The reason for drawing the blood into a moderate-sized tube in preference to the flat emigration tubes used by Sir A. Wright is in order to secure a copious bath of serum, and to prevent the clot from touching the wall of the tube. Leucocytes which come into contact with and adhere to a foreign body like glass fail to detach themselves and are unable to re-enter the clot. In this experiment the blood was allowed to clot in its own native serum. The contracted clot may, however, be removed from its own serum, and, after gentle washing in normal saline, may be dropped into a tube containing serum from another individual, to which also the carbon pigment has been added. In this way the conditions under which the leucocytes pass to the surface, take up the pigment, and

re-enter the clot can be controlled, and the effect of both native and foreign serum on the amoeboid activities of the phagocytes can be compared. Provided that the foreign serum is not toxic to the leucocytes—this fact can be ascertained by incubating a drop of the blood from the individual providing the clot with a drop of serum from the individual supplying the serum in a closed cell, when, if the serum is non-toxic, the leucocytes will emigrate, adhere to the slide, and show the iodophil reaction when stained with iodine—incubation in a foreign serum seems to cause a larger number of pigment-laden cells to re-enter the clot. The effect on return immigration of adding various chemical reagents to the serum can also be observed.

Such are the facts concerning the movements of the leucocytes in an incubated blood clot. This *in vitro* method may also be used for the examination of the movement of the phagocytes in excised portions of different tissues and organs, and especially in the granulation tissue of wounds.

Experiment II.

A wedge-shaped portion of granulation tissue removed during operation from a reamputated stump was placed in a closed cell in normal saline; the free surface was coated with carbon pigment, and the tissue was incubated for a few hours. In another case the granulating surfaces of two

FIG. 2.—Section from blood clot incubated thirty hours in native serum. Shows pigment-laden leucocytes in the fibrin and in the red corpuscle zones. A, Pigment on surface. B, Leucocytes containing pigment. C, Red corpuscles.

portions of tissue previously coated with the carbon pigment were closely applied, and incubated in contact in liquid paraffin. In this way the conditions were reproduced *in vitro* under which secondary closure of a granulating wound is performed, except that the blood and lymph circulatory factors were excluded.

After incubation the tissue was hardened, and sections cut at right angles to the free surface. They show the movements of pigment-loaded leucocytes in a piece of living granulation tissue removed from the circulation and incubated in liquid paraffin. A layer of carbon pigment covers the surface, a large number of the leucocytes on the free surface have taken up the pigment, and of these a certain number will be seen to have travelled from the surface to the deeper layers of the tissue by a process of return immigration. The number of pigment-laden cells found in the tissue will depend, among other things, on the degree of infection—that is, on the healthiness of the wound, and on the density of the tissue to be traversed. These points will be considered later.

Experiment III.

Instead of limiting the inquiry to the activities of the leucocytes already present in or upon the surface of a piece of granulation tissue, we may investigate the same tissue from the point of view of its suitability as an immigration field for wandering white blood cells, both native and foreign.

Some blood from the patient providing the piece of granulation tissue is drawn during operation into a tube, and some of the carbon-mixture pigment added and mixed with the blood. The granulation tissue is then incubated in a closed cell with this carbonized blood, or foreign blood may be used from another individual, or a differ-

entiating method may be employed; thus, the blood, either native or foreign, containing the carbon pigment may be allowed to clot. After coagulation and contraction a disc-shaped section may be cut from the clot 1 or 2 mm. thick. This disc with its contained pigment-laden leucocytes can then be applied to the surface of a piece of granulation tissue, and the two incubated in contact in blood serum in a closed cell. It is not necessary to use the disc of blood clot immediately, for I have ascertained by incubating discs of blood clot in native serum at varying intervals after coagulation that the contained leucocytes will emigrate freely, give the iodophil reaction, and take up pigment particles twenty-four hours and longer after coagulation has occurred.

By using a disc of previously clotted blood the migration of pigment-loaded leucocytes can be concentrated in one area of the granulating surface. Fig. 3 shows a section from granulation tissue incubated in native blood to which carbon pigment has been added before coagulation. The leucocytes loaded with pigment can be seen in the clot surrounding the tissue and numbers of these pigment-laden cells will also be found in the interior of the granulation tissue at a considerable distance from the surface.

The fact that by suitable experiment *in vitro* the return immigration of pigment-laden phagocytes can be demonstrated in granulation tissue in the absence of both the blood and lymph circulations bears on the clinical problem of the influence of sterile and infected blood clots in wounds and the effect of such coagulated blood on the spread of infection.

This *in vitro* method may also be used for investigating different tissues and organs as fields for leucocytic immigration. The relative avidity with which pigment-laden leucocytes enter and re-enter the intercellular spaces of connective and muscular tissues on the one hand and the tissues of organs, such as the spleen or liver, on the other can be ascertained, the disturbing influence of the circulation having been excluded in each case.

Portions of excised living lymph gland have been incubated with this point in view.

Experiment IV.

When a portion of lymph gland is incubated with native blood serum to which carbon pigment has been added, although some of the lymph cells lying on the surface or in the reticulated gland tissue emigrate and adhere to the slide, and although a certain number of these cells ingest pigment particles, they are comparatively few in number. A marked contrast is noted between the behaviour of these small lymph gland cells and the more active white blood cells when incubated under like conditions. While the polymorphs and mononuclears emigrate freely from the clot and ingest pigment particles, the lymph cells, on the contrary, are more sluggish; they preserve their circular outline, adhere with difficulty to the slide, and ingest comparatively few pigment granules.

Also, if a portion of the gland be incubated in native blood to which pigment has been previously added, very little emigration of the loaded leucocytes takes place from the blood into the gland tissue.

Fat tissue has also been examined from this point of view. A piece of excised living omentum was incubated in both native and foreign pigmented blood. In this case carmine was the pigment used. A few leucocytes containing carmine were found in the intercellular spaces of the fatty tissue at some distance from the surface. They were most numerous in the perivascular channels.

Return Immigration in Mucous Membranes.

I have also carried out some observations on mucous membranes which point to the occurrence under certain conditions of a "return immigration" of pigment-laden leucocytes from mucous surfaces. These observations are, however, at present incomplete.

The Size of the Pigment Particles as a Factor in Return Immigration.

The size of the pigment masses ingested by the migrating leucocytes considerably affects the capacity of those cells to re-enter the clot or the tissues. This fact no doubt depends on physical conditions. A phagocyte distended by a large rigid mass of carbon is less able to insinuate itself between the cellular obstacles in its path than a cell containing smaller granules. It is thus possible that the size and the rigidity of organisms ingested by wandering cells may affect the movements of the phagocytes which have ingested them. I have tried feeding phagocytes on substances like magnetic iron in order to ascertain whether such substances (with the containing cells) can be drawn into the tissues in greater volume and to a greater distance by magnetic influence, so far, however, without definite result, although the iron particles are ingested by the phagocytes.

The Varieties of Phagocytes Concerned in Return Immigration.

The examination of the incubated blood clot in pigmented serum affords some evidence of the relative activities of the different varieties of leucocytes in emigration, ingestion, and return immigration. The

observations now recorded confirm the facts already known as to the movements of the leucocytes in the living body. While the polymorphs are the most active in these three directions the mononuclears also take a considerable part. The eosinophil cells with large granules rarely seem to ingest pigment particles.

The Factors Determining Return Immigration.

The outward emigration of leucocytes to the surface of the blood clot seems to be a general movement shared in by all kinds of wandering cells. I have been impressed by the great tendency shown by all white blood cells to get clear of the fibrin and red cells after coagulation has taken place in a blood clot. Sir A. Wright has described leucocytic movement as of two kinds: (1) Eleuthotropic or aimless and general, and (2) chemiotropic in a definite direction. The movements of the leucocytes in "return immigration" are more obscure in character. Of the many thousands of white cells which occupy the leucocytic zone of a blood clot incubated in a bath of serum and pigment only comparatively few pigment-laden cells re-enter the substance of the clot. Of these, the greater number pass obliquely upwards from the surface into the cell-free fibrin zone, and only a few pass into the red cell portion of the clot.

This difference may depend partly on physical conditions. The more open spongy network of the fibrin zone may provide the easier route. The important question still remains: What are the factors which determine whether a pigment-loaded phagocyte shall drop off the surface of the clot into the serum or remain attached to the surface, or whether it shall work its way back into the interior of the clot? The causes are probably both intrinsic and extrinsic. The leucocytes differ in age and vigour, and probably in their capacity to respond to environmental stimuli. The condition of the environment, whether the surrounding serum be a native or foreign serum, the presence of chemical reagents in the serum, all these may influence the result. I have already alluded to the importance of preventing the clot from coming in contact with the wall of the tube by the intervention of a sufficient layer of serum. Leucocytes that have attached themselves to foreign substances become damaged, and seem unable to detach themselves completely. They may crawl for a short distance over the glass surface, but (unlike the amoeba, which is accustomed to the outside world) they do not become free again or re-enter the tissues. No doubt the pigment particles present a foreign surface, but, if not too large, these are englobed by the cells, and the continuity of the

FIG. 3.—Granulation tissue, incubated twenty-four hours with "native" blood and carbon pigment. A, Pigment on surface. B, Leucocytes containing pigment.

protoplasmic surface is restored. This injurious effect of contact with foreign material which cannot be ingested plays an important part in wounds. Living phagocytes which come in contact with a dressing remain adherent to it. Hence the injury produced by frequently removed dressings in a healing granulating wound.

The Influence of Infection on Return Immigration.

In the living body the return immigration of wandering cells is complicated by the presence of a blood and lymph circulation, but here also the pigment-laden cells which re-enter the tissues are comparatively few in number and the inward flow is frequently limited to definite areas on the granulating surface.

Organisms are present in few or in large numbers in or on the granulation tissue of practically all open wounds. This also will influence the movements of the leucocytes, because where organisms are present in large numbers there large numbers of the emigrating cells also perish and are thrown off as pus cells, much tryptic digestion goes on, and few phagocytes survive and return to the tissues.

The complicating element of the organisms and their toxins is, however, eliminated in the case of a blood clot incubated in pigmented serum under aseptic conditions. Here, therefore, we find that a larger proportion of cells take up the return journey. In general terms the relative number of phagocytes which re-enter the tissues after emigration on to the surface of a granulating wound depends largely on the virulence of the infective process, or in other words on the efficiency of the defensive mechanism, that is on the healthiness of the wound.

If the surface of a piece of granulation tissue excised from a moderately infected wound be washed in normal saline and then incubated for a few hours, careful examination with the lens will show collections of pus cells at certain points on the surface. Suppuration, in fact, continues during incubation in the absence of a blood and lymph circulation. Under these conditions, however, there is no uniform secretion of pus over the whole surface. The leucocytes emigrate in certain areas, partly determined by the presence of organisms and partly by ease of transit. In the same way the pigment-laden phagocytes, or as many of them as are not thrown off as dead pus cells, re-enter the tissues in certain areas also.

The Effect of Chemical Reagents on Return Immigration.

The effect of different reagents applied to the surface of the incubated tissue or blood clot during incubation varies considerably, and requires further investigation by the *in vitro* method. A coating of liquid paraffin, or the immersion of the tissue in a bath of liquid paraffin during incubation gives better results than normal saline as far as the activities of leucocytes are concerned. Fewer cells are thrown off as pus cells, a larger number take up the pigment and re-enter the tissues. Gum solutions act somewhat in the same way, but less favourably. These inert substances seem to favour return immigration more than the active antiseptics. I hope to record further observations on the effect of liquid paraffin on the movements of leucocytes later.

The Ultimate Fate of the Phagocytes which Re-enter the Tissues.

In the BRITISH MEDICAL JOURNAL, December 23rd, 1916, I suggested that pigment-laden phagocytes may under certain conditions discharge their pigment loads (possibly by the cytolysis of the cells) and that the pigment granules so liberated may be taken up by the fibroblasts, endothelial and other connective tissue cells. Careful examination of a blood clot in which return immigration of pigment-laden leucocytes is going on will sometimes show little collections of pigment grains lying free in the fibrin network, as though liberated by some cell which had undergone cytolysis. The blood clot, however, contains no tissue cells capable of taking up the liberated pigment. In granulation tissue, on the other hand, pigment granules may sometimes be observed in cells which look like connective tissue cells at some distance from the surface. Since the blood and lymph circulation is absent these carbon granules must have been carried to these situations by wandering cells. These facts bear on the problem of the spread and

the recrudescence of infection, and on the mode of action of the defensive mechanism.

The Importance of the Problem.

Having now shown that a process of "return immigration" of pigment-laden phagocytes occurs under certain conditions in wounds, and probably also on free mucous surfaces, the next step is to show that phagocytes containing ingested organisms behave in the same manner.

If this occurs, as we have every reason to believe, then the importance of the "return immigration" of phagocytes becomes at once apparent. Evidence accumulates (see observations by Peyton and Rous, Kyes, Bull, and others) which tends to show that pathogenic organisms when ingested by phagocytes are not always killed by intracellular digestion. I have previously (see BRITISH MEDICAL JOURNAL, December 23rd, 1916) drawn attention to the association between the spread of infection in certain diseases, the recrudescence of local sepsis in wounds, and the return immigration of phagocytes. The fact now established that return immigration can take place apart from the blood or lymph circulation affords further corroboration of this view.

REFERENCE.

1 BRITISH MEDICAL JOURNAL, June 3rd, 1916; December 23rd, 1916; February 3rd, 1917.

MUSCULO-SPIRAL NERVE DISABILITIES.

BY

ASTLEY V. CLARKE, M.D., COLONEL T.F.R.,

AND

N. I. SPRIGGS, M.D., B.S., F.R.C.S., CAPT. R.A.M.C.(T.),
LEICESTER.

As many and various appliances are now in use for cases of wrist-drop, each presumably with the object of ensuring "complete and continuous relaxation of all the affected muscles over long periods," it seems desirable that precise and definite information should be obtained, anatomically as well as clinically, of the value of the various positions in which the hand is retained by the apparatus.

The positions met with among soldiers and pensioners with this disability may be classified into the six shown in these sketches:

FIG. 1. Complete wrist-drop. No appliance.

FIG. 2. Flat straight splint extending to the palm of hand.

FIG. 3. Flat splint to finger tips. Generally not including thumb.

FIG. 4. Small metal "cock up" splint. (Fingers and thumb free for use.)

FIG. 5. Long "cock up," to support first phalanges, often also thumb.

FIG. 6. Extra long, which supports terminal phalanges and also thumb.

To test these positions anatomically in relation to each individual "muscle relaxation," we have made observations and measurements on adult male cadavera.

The procedure we adopted to estimate quantitatively the amount of "muscle relaxation" in each muscle affected by musculo-spiral disability was:

1. The tendons and muscle bodies on the dorsum of the forearm were exposed and differentiated.
2. A narrow band of annular ligament was left *in situ* to form a "fixed point."
3. The hand was placed in complete wrist-drop (Fig. 1), and in this position a small stitch was placed