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

BY

C. J. BOND, F.R.C.S., HON. COLONEL R.A.M.C.(T.),

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

PART III.—ON THE PRESENCE OF AN IODOPHIL SUBSTANCE IN THE WHITE BLOOD CORPUSCLES IN HEALTH AND IN SOME OTHER CELLS, AND ON THE RELATION OF THIS SUBSTANCE TO PHAGOCYTOSIS AND IMMUNITY.

In the course of this inquiry on Antiseptics, Leucocytosis, and the Healing of Wounds, I have come across certain facts about the iodine reaction in leucocytes and other cells which I think have an important bearing on phagocytosis and immunity. It is, of course, already known that the white blood cells, especially the polymorphs, in some infective diseases frequently give a colour reaction with iodine, which is regarded as evidence of the presence of glycogen in those cells.

The following observations show that even in health a large proportion of the white blood corpuscles normally give, under certain conditions, a colour reaction with iodine, which is closely related to, if not identical with, the so-called glycogen reaction met with in the same cells in disease. The reaction which I am about to describe occurs not only in the white blood cells but in many pus cells, in some of the cellular elements of granulation tissue, in some marrow cells, and in the cells of certain cancerous tumours.

The White Blood Corpuscles and the Iodine Reaction in Health.

If a drop of blood from the finger of a healthy person be incubated for one hour in a Ponder's plasticine cell¹ at 37° C. the white blood corpuscles emigrate out of the clot and adhere to the slide below and the cover-slip above. If the cover-glass and the plasticine cell wall be then removed and the red corpuscles and fibrin carefully washed away with a gentle stream of normal saline, two films of vigorous living leucocytes will be obtained. These films can be mounted in normal saline and a drop of 1 per cent. watery iodine and potassium iodide solution allowed to flow under the cover-slip. All the white blood cells in contact with the iodine will stain a light yellow, but, in addition, a large number (in some fields nearly all) of the cells will show a mauve-coloured exudation of colloid material to one side of the cell. The reaction occurs best in a certain zone of the preparation where the iodine reaches a certain concentration in the saline fluid and where the cells are not stained too deeply by the iodine. Under favourable conditions, the beginning of the reaction can be detected inside the cell. One or more beads of mauve-coloured material collect in the cell, the granular cytoplasm shrinks away from the differentiated protoplasm which forms the boundary wall, the beads run together into larger drops, the limiting membrane ruptures, and the colloidal substance is discharged into the surrounding medium, where it may for a time remain attached to the cell or float away like a coloured oil-drop and eventually diffuse into the surrounding liquid.

Thus far we have described this colour reaction as it occurs in the wet preparation, in the incubated film of emigrated cells to which a watery iodine solution has been added. A second incubated film also freed from clot and red corpuscles is allowed to dry; the leucocytes can then be fixed and stained in iodine vapour, and mounted in iodine gum. The dry film so prepared gives the colour reaction in many of the corpuscles, but in the dry film the iodophil substance takes on a richer stain, and the mauve colour gives place to the mahogany or port wine colour characteristic of glycogen. In healthy emigrated white blood cells the reaction is partly intracellular and partly extracellular, and the iodophil material collects as drops or beads just within or on the surface of the cells. It is already known that in certain diseases a mahogany colour reaction occurs with iodine in some of the leucocytes in a freshly drawn blood film which has not been incubated, and this is an

important distinguishing feature, and serves to mark the boundary line between health and disease.

The Reaction is Characteristic of the Polymorph Cells.

The question arises, Which of the different varieties of leucocytes give the reaction in health? This is a somewhat difficult problem, owing to the fact that nuclear stains prevent the iodine reaction, while the presence of iodine also inhibits the nuclear stain. If, however, a film of normal blood be incubated on a ruled slide and subsequently washed and treated with iodine solution, the leucocytes which give and those which do not give the mauve reaction can be mapped out in a certain square, the cover-slip is then removed, the iodine washed away, and the cells stained by some nuclear stain. It will then be found that the cells which gave the mauve reaction are the finely granular polymorph leucocytes. The large mononuclears and the small lymphocytes do not usually give the reaction, and so far I have not detected it in the eosinophil cells. This is true of healthy blood, and is confirmed by the examination of films of incubated blood in disease. In a child aged 7, suffering from a severe lymphatic leukaemia, a blood count gave polymorphs 8 per cent., large mononuclears 1 per cent., lymphocytes 90 per cent., eosinophils 1 per cent.; in this case the number of cells which gave the iodine reaction was much reduced. In certain cases, however, the mononuclears and the lymphocytes may give the reaction. Incubated films from this patient at a later stage of the disease showed a considerable number of lymphocytes with the mauve-coloured exudation. It is known that the glycogen reaction in disease is confined for the most part to the polymorph cells.

The Iodine Reaction in Leucocytes from Aseptic Closed Wounds.

In a former paper² I drew attention to the essential similarity of the phagocytic problem in the closed aseptic and in the open septic wound. The leucocytes which collect in such numbers in the pigment thread drain in a surgical incision re-enter the tissue spaces or the circulation as primary union takes place, but these are the same cells in origin as those which are thrown off as dead pus cells in the discharge from an open septic wound.

In the first case nearly all the emigrated cells retain their vitality and re-enter the circulation; in the second most of the cells are killed and thrown off as pus cells; a few only re-enter the circulation. If we tease up in normal saline the leucocyte-covered thread from such an aseptic wound and add a drop of watery iodine 1 per cent. solution, nearly all the cells will show the characteristic mauve coloured reaction. (Fig. 1.) These emigrated leucocytes are in fact vigorous living phagocytes of the polymorph class. They have emigrated from the blood vessels into the wound cavity *in vivo*, just as the white corpuscles in the incubated blood film have emigrated from the blood clot on to the slide *in vitro*. The fact that these emigrated leucocytes give the reaction in both cases, although they do not do so in health while circulating in the blood stream, is important in connexion with the further fact that living pus cells also show the same reaction.

Pus Corpuscles and the Iodine Reaction.

If a drop of pus from a healthy granulating wound be diluted with normal saline, and if a drop of 1 per cent. watery iodine solution be allowed to flow under the cover-glass, a considerable proportion of the pus cells will show the characteristic mauve reaction. (Fig. 2.)

A large number of observations have been carried out on samples of pus from different wounds in various stages of healing and under different conditions of treatment. Pus from certain wounds, when examined on separate occasions, may consistently show a high proportion of cells giving the reaction. Pus from similar wounds infected with the same variety of organisms, showing a like tendency to granulation and repair, may fail to give the reaction, or give it only in a few cells. This difference depends largely on the conditions in the wound.

Stagnant pus—pus which has lain for hours in an undrained pocket, or exposed on the surface of a wound—consists largely of dead cells, whereas the pus which continually flows from a well-drained, healthy wound contains

a large number of living phagocytes, and it is the living cells which, when killed with iodine, give the mauve reaction. The iodine reaction thus affords some indication of the vitality of the pus cells. This is shown in another way. If the sero-pus which collects in the lymph-leech contrivance of Sir A. Wright be examined with the iodine solution, a number of the leucocytes which have been aspirated from the walls of the wound and have collected

FIG. 1.—Leucocytes from "phagocyte trap"; aseptic closed incision; fresh preparation in normal saline and 1 per cent. iodine. A, Mauve-coloured iodophil substance; C, red corpuscle. $\frac{1}{12}$ in., B oc.

in the tube will be found to give the reaction even when pus cells from the surface of the same wound fail to do so. Pus aspirated from an empyema or an acutely infected knee-joint may fail to give the reaction when first withdrawn, whereas, if the discharge which flows freely from the empyema or the joint cavity be examined a few days later after efficient drainage has been established, quite a number of the cells will show the mauve-coloured exudation.

The capacity to give the iodine mauve-colour reaction depends largely on the vitality of the pus cell, and pus

FIG. 2.—Pus cells from healthy granulating wound. A, Mauve-coloured iodophil substance; C, red corpuscle. $\frac{1}{12}$ in., B oc.

cells which have lain for some time in a closed cavity in a tryptic medium have undergone partial cytolysis. It is the living leucocyte which has recently emigrated from its natural environment—the blood or lymph stream—which shows the reaction. Of course the

* These semidiagrammatic drawings are taken from actual microscopic preparations; in each case A (shaded area) represents the mauve-coloured iodophil exudation which in some cases (Fig. 2) has broken away from the cell; B (black area) represents the mahogany-coloured substance (glycogen).

conditions to which the cells have been exposed in the wound after emigration are also very important. It is evident that highly concentrated antiseptic solutions which kill the leucocytes will also prevent the reaction, but when these disturbing factors have been eliminated, if a considerable proportion of the pus corpuscles in any wound shows the mauve-coloured iodine reaction, that wound is probably in a healthy condition as far as successful

FIG. 3.—Cancer cells from epithelioma of upper jaw; stained 1 per cent. iodine. B, Mahogany-coloured iodophil substance (glycogen). $\frac{1}{12}$ in., A oc.

reaction to infection is concerned. On the other hand, the absence of the reaction in any one sample of pus from a wound does not necessarily mean that the whole of that wound is unhealthy. The pus cells examined may have lain for hours on the surface and been killed by exposure, or the sample may have been taken from some stagnant pool in some inefficiently drained pocket.

As in the blood cells, the reaction occurs in the polynuclear pus cells. It is much less marked in the mononuclears and in the lymphocytes. This fact must be borne

FIG. 4.—Cancer cells from squamous-celled epithelioma; stained 1 per cent. iodine. B, Mahogany-coloured iodophil substance (glycogen) round nucleus. $\frac{1}{12}$ in., B oc.

in mind in making use of the reaction as an indication of the healthiness of a wound. The acute stage of infection is marked by a flow of polymorphs, at a later stage the mononuclears are more in evidence, and at this stage the reaction would be less marked.

On the whole, efficient drainage and the establishment of a flow of rapidly renewed phagocytes seems to have more influence on the iodine reaction than the nature of the solutions used in the treatment of the wound.

Granulation Tissue and the Iodine Reaction.

It is interesting to find that the iodine reaction is not confined to the cells of the purulent discharge. If a small portion of healthy granulation tissue be removed from a wound and examined in salt solution while alive, and a 1 per cent. watery iodine solution be added, a considerable number of the cellular elements will show the mauve-coloured exudation. The cells which give the reaction are the polynucleated cells; the large mononuclears and the smaller lymphocytes do not show it to the same extent; neither do the fibroblasts and other fixed tissue cells.

The Marrow Cells and the Reaction.

If a small piece of healthy red marrow be examined in 1 per cent. watery iodine solution a considerable number of the marrow cells will show the mauve-coloured reaction. A short incubation at 37° C. will promote this. It is shown by the myelocytes of the bird's marrow, but I have not detected it in the frog's marrow cells. Marrow cells, both avian and human, also have a considerable capacity for ingesting pigment both in the body and when incubated *in vitro*. This is important in relation to the marked tendency to latency of infection and to attacks of recrudescence sepsis which are often associated with gunshot wounds of bone.

FIG. 5.—Leucocytes from incubated blood during streptococcal throat infection. A, Mauve-coloured iodophil substance exuding from leucocytes. $\frac{1}{12}$ in., A oc.

The Nature of the Iodine Reaction in Healthy Leucocytes.

It is, I think, clear from the foregoing observations that we must not regard the colour reaction indicative of the presence of an iodophil substance in leucocytes merely as evidence of a so called glycogenic degeneration, as some observers have supposed. We have seen that healthy leucocytes show a corresponding reaction, and that the distinctness of the reaction—that is, the amount of iodophil substance in the polymorph leucocytes—varies according to the degree of infection. The reaction may take the form of an extracellular mauve-coloured exudation, or of a mahogany-coloured intracellular deposit. In other words, the iodophil substance may exist in the leucocyte in a mobilized form, as in health, or it may be present in a less soluble state, as in some infective diseases.

The question now arises whether this substance which gives the mauve-coloured reaction with iodine in health is chemically the same substance as that which gives the mahogany or port wine colour with iodine in disease. In other words, does the mauve-coloured exudation consist of glycogen? Most observers—Gulland, Wolff, Da Costa, and Burnham—agree that it is the polymorph leucocytes which give the glycogen reaction in dry blood films in septic diseases. We now find that it is the polymorph leucocytes which give the mauve-coloured reaction in incubated blood films in health. Hitherto it has been thought that leucocytes, while circulating in the blood in health, do not give any reaction with iodine, except, of course, the yellow

stain. It is quite true that neither wet nor dry films of freshly drawn normal blood give the iodine reaction, but my observations show that a considerable number of the polymorph leucocytes circulating in the blood in health contain an iodophil substance which becomes mobilized when the blood is incubated, and this substance then combines with any free iodine present.

The same is true of leucocytes which have emigrated from the blood vessels into an aseptic wound. The fact that the cells have migrated and are no longer bathed by the blood plasma seems to render the colloid substance accessible to the iodine.

Whether the reaction shall take the form of the more soluble mauve-coloured extracellular exudation, as in the incubated leucocytes in health, or the less soluble mahogany-coloured intracellular deposit, as in some pus cells, and in the white blood corpuscles in certain infective diseases, seems to depend on the presence of a toxin. The toxin renders the iodophil substance more readily accessible to the action of the iodine, with which it gives in combination a more strongly marked colour reaction when in the intracellular form. The mobilization of this iodophil substance in the leucocytes is probably closely associated with the phagocytic function of these cells.

Wolff³ has suggested that an iodophil substance is present in the circulating leucocytes in health, but that,

FIG. 6.—Leucocytes from incubated blood (streptococcal throat infection); film dried, fixed and stained with iodine vapour and mounted with iodine gum. B, Mahogany-coloured iodophil substance (glycogen). $\frac{1}{12}$ in., A oc.

owing to its extreme solubility, it cannot be demonstrated in a dry film of normal blood, but when rendered more insoluble by the action of certain toxins its presence can be shown. My observations provide an experimental verification of this hypothesis, and show further that the action of a foreign, or at any rate bacillary, toxin is not essential, since the removal of the corpuscles from their natural environment, the blood plasma, will bring about the result. Two films of emigrated leucocytes were obtained from the incubated blood of a healthy person. One film was examined in normal saline to which iodine 1 per cent. solution had been added, and the mauve reaction was obtained. The second film was dried, fixed, and stained in iodine vapour and mounted in iodine gum. Many of the leucocytes in this dry film showed mahogany-coloured beads of colloid exudation. There can, I think, be little doubt, therefore, that the substance which gives the mauve coloured reaction with iodine in the white blood cells in health is either glycogen or a precursor of glycogen, possibly a complex substance, from which, as the result of emigration, the carbohydrate molecule is split off as glycogen, while the protein molecule may be concerned with the elaboration of ferments or antibodies formed by the phagocytes in response to a toxin.

There would seem to be two phases in the response made by phagocytes of the wandering cell type to the action of foreign toxins. The first phase is due to changes in the cell brought about by emigration, and results in the

appearance of an iodophil substance which very readily diffuses out of the cell; the second phase may occur in the phagocytes before emigration, while circulating in the blood stream, and results in the deposit of iodophil material in the cell.

We have already seen that some marrow cells give the mauve-coloured reaction; these are the neutrophil myelocytes, the precursors of the polymorph leucocytes, and, like them, exhibit amoeboid activity and a capacity for ingesting foreign particles.

Cancer Cells and the Iodine Reaction.

Cancer cells from some (but apparently not all) actively growing cancers give the mauve reaction when examined fresh in salt solution and iodine, they also (like some pus cells) give the mahogany reaction when treated with iodine vapour. (Figs. 3 and 4.) Although I have failed to obtain any evidence of any capacity in these cancer cells to ingest pigment particles when incubated *in vitro*,* we must remember that cancer cells retain certain embryonic and parasitic characters, and the presence of an iodophil substance in cancer cells may have reference to this parasitic capacity.

The following experiments show that the iodophil substance in leucocytes is not destroyed by foreign serum. If two drops of blood, each taken from a different individual, be thoroughly mixed and incubated together in the same cell, the mixed leucocytes when treated with iodine show the normal percentage of cells giving the iodine reaction. The foreign serum does not act prejudicially on the formation or the mobilization of the iodophil substance in either case. If a drop of normal blood be incubated with egg albumen the emigrated leucocytes so obtained give the reaction on treatment with saline and iodine.

Although present in the leucocytes of birds and mammals I have not detected the mauve reaction in the incubated white blood corpuscles of the frog either from the blood stream or from the dorsal lymph sac. It may be that the iodophil substance is not present in the leucocytes of cold-blooded animals.

The Iodophil Substance may be Present in Leucocytes in an Infected Area though Absent in Leucocytes drawn from the General Circulation.

In a case of cutaneous erysipelas (streptococcal infection), with marked local and general symptoms, a drop of blood drawn from a puncture in the inflamed area (unincubated) gave a well marked mauve reaction in a number of leucocytes when treated with watery iodine solution. A dried film from the same blood, also from the inflamed area, treated with iodine vapour and mounted in iodine gum, showed the characteristic mahogany-coloured (glycogen) reaction. Blood drawn from the finger of the same patient gave no mauve reaction with watery iodine, or glycogen reaction in a dry film.

The occurrence of iodophilia in the leucocytes from the infected area, while absent from the leucocytes in the general circulation, is a point of interest. It suggests that changes of a defensive kind occur in leucocytes while circulating in an infected area, but that after the corpuscles leave this area and return to the general circulation they revert to the normal condition, and cease to show the iodophil change unless the general toxæmia has been too profound. In connexion with this point, however, it is necessary to remember that the leucocytes which give the reaction may have already emigrated from the capillaries into the tissue spaces and lymph channels before mingling with the blood which escapes from the puncture.

The Association between the Iodine Reaction and Infection.

I have recently had an opportunity of observing the effect of a sharp attack of (streptococcal) throat affection on the amount of iodophil substance in my own leucocytes. Having from frequent observation for some weeks ascertained both the percentage of leucocytes which gave the mauve reaction and the amount of iodophil substance poured out in incubated films of my own blood in health, I was in a position to compare these results with the results obtained from incubated blood films taken during the height of the throat infection. (Fig. 5.) Wet films

* Possibly if incubated in blood plasma cancer cells may exhibit pigment ingestive capacity.

of incubated leucocytes treated with watery iodine solution showed, during the febrile stage, a considerable increase in the number of cells giving the reaction and in the amount of iodophil substance exuded. Films of the same blood incubated, dried, and stained with iodine vapour and mounted in iodine gum showed an abnormal amount of intracellular and extracellular mahogany coloured exudation. (Fig. 6.) Unincubated dried films of the same blood treated in the same way appeared normal. This increased iodine reaction lasted for nearly a fortnight after the temperature had fallen to normal. This observation shows the intimate association between the circulation of bacillary toxins and the occurrence of iodophilia in the leucocytes. It also shows the phases of increased formation of iodophil substance by the phagocytes—first the increase in the extracellular soluble mauve-coloured exudation, then the occurrence of the less soluble intracellular mahogany-coloured deposit. Both these occur before any corresponding change can be detected in the leucocytes in the general blood stream, and they are still present in the incubated leucocytes for some time after the constitutional reaction to the infection has disappeared. This shows the close association which exists between leucocytic activity in response to infection and the formation of an iodophil substance by the phagocytes. A still later stage of this same condition has already been described by other observers in the leucocytes in various septic conditions.

Time alone can show the real significance of the occurrence of iodophilia in the leucocytes in health, and the part played by this iodophil substance in the process of immunization against bacterial, and possibly other toxic substances. It is, however, evident that the problem must be attacked from the chemical side before the metabolic changes in the leucocytes and other cells which lead to the elaboration of this iodophil substance can be fully understood, or estimated at their true value.

I am hopeful that the routine examination of the cells of various kinds of malignant growths at various stages of their development for the presence of iodophil substances, especially undertaken in connexion with the existence or absence of like changes in the leucocytes, may tend to throw light on some obscure points in the metabolism of the cancer cell.

REFERENCES.

¹ *Lancet*, December 12th, 1908. ² *BRITISH MEDICAL JOURNAL*, June 3rd, 1916, p. 777. ³ Quoted by Burnham, *Haemocytes and Haemic Infections*.

Lectures

ON

DISEASES OF THE MALE URETHRA.

BY

FRANK S. KIDD, B.C., M.B. CANTAB., F.R.C.S.,

ASSISTANT SURGEON, LONDON HOSPITAL, AND SURGEON
TO THE GENITO-URINARY DEPARTMENT.

LECTURE IV.—TREATMENT (*Continued*).

GENERAL CONSIDERATIONS, WARNINGS, AND INSTRUCTIONS.

It is advisable to have at hand a small printed card that will fit the waistcoat pocket. On this card should be printed the following instructions.

A. General Warnings.

1. Please remember that until the doctor passes you as "cured" you are infectious to a woman. This is the fact even though you yourself see no sign of urethral discharge. You will think you are cured for a long time before you are really cured, that is to say free from infectious germs.

2. Be careful not to convey discharge on the fingers to the eyes. If you do so you may set up dangerous inflammation and might even lose your sight. If the eyes become inflamed return to the doctor at once for examination.

It is wise to buy a small bottle of lysol or listerine from the chemist and to use a teaspoonful of this in a basin of water wherewith to wash the hands after handling the parts in urinating or injecting.

3. Be careful not to use towels which other persons, especially children, are likely to use. Children may contract the disease from infected towels.

4. If you are using a common bath, try to use it after everyone else has finished. Always pass water before taking a bath. In any case add four teaspoonfuls of lysol or listerine to your bath, and wash your face before you enter the bath. Swill out the bath with fresh water after use.