your counsel to travel somewhat freely beyond your mere proof or report, because he will almost certainly ask you to give to the court your views upon the points made by the doctors on the other side; he will probably say, "Now, doctor, you heard Mr. B. say he thought this was a case of so-and-so; what do you say about that?" Then is your opportunity to explain why you differ, and to endeavour to do so in a way that will convince the court that your reasons are sound. By doing this you have incidentally made it difficult for the counsel who has to cross-examine you, because you have dealt in advance with most of his material; and if he still persists in putting it the judge gets a bit restive and says to him, "The doctor has already explained that; what's the good of merely putting it to him over again?"

I would like, if time permits, to say a word about the position of doctors as regards what is somewhat vaguely described as "privilege," in so far as it concerns cases that come into the courts. For a witness in court there is no privilege recognized by the law as regards facts which the doctor may have observed or with which he has become acquainted in his capacity as medical attendant. Obviously there cannot be, because it would conflict often with statute law and nearly always with the administration of justice. As to the former, I may instance the Notification of Diseases Act and the Notification of Births Act. As to civil and criminal proceedings, it is apparent that over and over again the ends of justice would be defeated if doctors were allowed to decline, when in the box, to give evidence of the facts which had become known to them.

One can easily understand that in many cases it must be very unpleasant for a doctor to find himself asked to disclose information about his patients; but I fear that he can do no more than appeal to the judge to know whether he is bound to answer. The judge will say Yes; but the doctor will feel that he has done what he can to preserve the confidential relationship between himself and his patient. If he persisted in refusing to answer he would be liable to be committed for contempt of court. Incidentally I may mention that what a witness says in the witness-box is privileged, which means that it cannot be made the subject of an action for slander.

Now may I just try to give you a short summary of my suggestions?

Approach the case with an open mind.

Your examination should be complete—not limited (except in obvious cases) to the part affected or injured, but generally, of the systems and organs; it may later prove to be as important to say that such and such symptoms were absent as that some others were present.

Investigate carefully all the objective symptoms, and if the man complains of some that you do not then see—for example, sickness or passing blood—try to get a chance of seeing them; satisfy yourself as best you can about the subjective ones; make and keep careful records, and date them; and make as sure as you can that you have got the facts right.

When you make your report or give your proof, let it be as intelligible as circumstances permit to the counsel who is to have it: give the dates of your examinations, what you observed on the several occasions (if more than one), and then your conclusions and the reasons for them; ascertain what are the issues or points in the case, and show the bearing of the medical evidence upon them; attend if possible at a conference with counsel, and tell him what are the debatable features, and the probable criticisms or attacks to be expected from the other side.

When the case comes on, be there, stay there, and listen to the opening and the evidence, and jot down for counsel any tips or comments that occur to you. Diagnose the judge, and see what treatment he needs; then give it to him when in the witness-box—for example, clear his mind of some fallacy you have seen him absorb.

When in the witness-box speak plainly and not too fast, and as much as you can towards the judge; it is a good thing as a rule to adjust your pace to the judge's note; watch his pen; keep cool; do not let counsel make you lose your temper; do not argue with him; do not answer questions without being sure you understand them; and

always remember that the judge regards you as there to assist the court in arriving at the true view, and to give your opinions and make your criticisms honestly and impartially. Try to convey to the judge a clear and definite impression of what your decision would be; he wants you to enable him to arrive at a right and a real conclusion, and not to feel that he ought to give a verdict such as a jury once gave where the doctor at an inquest flirted alike with lightning and foul play as being the cause of death, and the bewildered jury's verdict was "Death from a visitation of God under suspicious circumstances."

An Address

ON

CERTAIN PHASES OF LEUCOCYTE ACTIVITY.

DELIVERED TO THE SHEFFIELD MEDICO-CHIRURGICAL SOCIETY

BY

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SECTION I.—PATHOLOGICAL.

IN an article published in the *BRITISH MEDICAL JOURNAL* (January 11th, 1919) it was shown that when washed red cells are ground up, a drop of the suspension of the washed and sedimented debris, when incubated (in a closed cell) with two or three drops of the freshly drawn blood from the same individual, exercises a leucotoxic effect on the native leucocytes varying in intensity according to the degree of concentration of the red cell debris in the incubated blood. The leucocytes emigrate very sparsely from the incubated clot, and those cells which adhere to the slide show marked signs of arrested activity, and cytolysis.

Malaria and Leucocytic Activity.

In *The Leucocyte in Health and Disease* (London, 1904) a description is given of the leucocyte films from the incubated blood of a malaria patient taken during the pyrexial and the apyrexial stages of the disease respectively.

In blood obtained during the rigor or pyrexial stage emigration is very scanty, and the cells are spheroidal and show the cytolytic changes characteristic of poisoned cells. If, however, the same clot be detached from the slide, washed in normal saline, and reincubated on a fresh slide, a much more copious emigration of active cells takes place from the same clot after the removal of the serum. This proves that the scanty emigration in the first instance is not wholly dependent on a reduction in numbers of the white cells.

The findings of different observers as to a rise or fall in the mononuclear and polymorph cell count in various phases of malaria, and in blood drawn from different parts of the body, vary a good deal. The fact, however, that the emigration is very scanty from the incubated blood clot, taken during the pyrexial stage, while it is active from the same clot after the serum is washed away, strongly suggests that the inhibitory influence is in some way due to the presence of the blood serum and not to any lack of leucocytes. If blood be taken from the same patient during the apyrexial stage, normal emigration occurs from the incubated clot.

Induced Malaria.

The induction of malaria by the injection of blood from an infected patient in cases of general paralysis, for therapeutic purposes, has enabled us to carry out observations on malarial patients on a more definite plan.

The emigration picture is first obtained by incubating the blood from a case of general paralysis immediately before the treatment is commenced. A second emigration picture is taken during one or more of the dozen or so rigors which usually follow the injection of the infected blood. A third emigration picture is taken during the stage of treatment by quinine, and a fourth after recovery from the malarial infection. The character of the leucocytic

emigration in this induced form of the disease follows the same lines as in the natural disease—that is, inhibition of emigration during the pyrexial stage and normal activity during the apyrexial stage; and also after recovery by quinine treatment.

Paroxysmal Haemoglobinuria.

I have recently had an opportunity of studying the same problem in a case of paroxysmal haemoglobinuria in a child of 3, in whom, although the paroxysms were accompanied by malaise and the passage of a large amount of haemoglobin in the urine, no rigors and no marked rise of temperature occurred.

The emigration picture obtained from blood during the paroxysm showed only a few leucocytes, surrounded by threads of fibrin and red cells in various stages of degeneration. When the clot was washed in saline and reincubated a much larger crop of active cells was obtained. In the convalescent periods, during which the urine was free from haemoglobin, the emigration picture gradually returned to a more normal condition.

Eason¹ states that a slight leucocytosis is generally present during the paroxysm. If this be so, the failure of the leucocytes to emigrate from the clot cannot be due to a scarcity of these cells in the circulating blood. In order to test this point and to ascertain whether the blood serum during the paroxysm is leucotoxic, three drops of my own blood were incubated in a closed cell with one drop of the patient's blood serum diluted with normal saline. The effect on my own leucocytes, though slightly inhibitory, was not very marked. When, however, my washed red cells, previously treated for one hour with the same serum, diluted with normal saline, were incubated (after rewashing) with my own blood, a striking effect was produced. Only a very scanty emigration of damaged leucocytes occurred, and many of these cells had debris of red cells hanging to them. In a control film, in which the same washed red cells were used without previous treatment with the haemoglobinuric serum, a good emigration field of normal cells was obtained.

This observation shows that the serum of the haemoglobinuric patient exercises its effect on foreign as well as native red cells, at room temperature *in vitro*, and that when cells so treated are incubated (after rewashing) with foreign blood, the red cells apart from the serum acquire a leucotoxic character. This observation is in harmony with the hypothesis put forward by Eason, Landsteiner, and other observers, that the recurring attacks of haemoglobinuria are due to the presence in the blood of a substance (amboceptor) which at a low temperature attaches itself to the red cells. When the local or general temperature of the blood rises, this substance acts as an intermediary body, and links the complement normally present in the plasma to the red cells, and thus brings about haemolysis.

These facts, taken in conjunction with our previous observations on malarial blood, suggest that disintegrated red cells, when damaged, either by invasion by the malaria parasite or as the result of the action of some haemolytic substance, as in haemoglobinuria, do exercise a profound leucotoxic effect *in vitro*; and further, this leucotoxic substance, whatever it be, only apparently becomes leucotoxic after it has been linked up with the red cells of the patient.

In diseases like malaria and haemoglobinuria, in which a mass destruction of red cells occurs periodically, leucotoxic substances are present in the blood serum during the acute phases of the disease. The removal of the blood serum from the clot *in vitro*, and the restoration of the serum to normality during the recovery stages *in vivo*, removes the inhibitory influence, whatever it be, which prevents the emigration of the leucocytes, and which, if present in a more pronounced form, brings about the death of the emigrated cells.

Leucocytic Emigration in Pneumonia.

It will readily be recognized that the emigration picture in diseases which are characterized by a mass destruction of red cells will probably be very different from the emigration picture seen in other diseases, especially those of bacterial origin. In pneumonia, for instance, in the acute febrile stage, the carpet of leucocytes which covers the floor of the cell in which the blood is incubated is a

thick one, the cells are very active, and a large quantity of iodophil substance is liberated. At the same time the increased leucocytosis which is present in acute pneumonia must be borne in mind.

In this connexion some observations by Dr. Gardner-Medwin² suggest that a leucopenia is actually present in the general blood stream during the early days of an attack of acute pneumonia, but that this is succeeded by a leucocytosis at a later stage. However this may be, the emigration picture from incubated blood in pneumonia in the acute stage is a striking one, and is in marked contrast to that seen in malaria and in paroxysmal haemoglobinuria. It may be that in paroxysmal haemoglobinuria a more extreme form exists of a condition ordinarily present in many other diseases (as in pneumonia), where red cells are agglutinated to, and undergo ingestion by, leucocytes in considerable numbers. These conditions can be so modified experimentally by removing the blood serum from the clot that the emigrating leucocytes can be induced to agglutinate and ingest washed native red cells on a wholesale scale.

Leucocytic "Aggressiveness."

If the red clot from which the first crop of leucocytes has been obtained be washed in normal saline to remove the blood serum, and reincubated on a fresh slide with a drop of a suspension of the washed red cells from the blood of the same pneumonia patient, the same phagocytic activity—that is, the aggressiveness—of the same leucocytes to their own washed and native red cells will be much more marked. Almost every leucocyte will be surrounded by a number of adherent red cells, and many will contain one or more ingested erythrocytes. This heightened "aggressiveness" to red cells on the part of leucocytes in the washed clot is not wholly specific in character—that is, it is not limited to native red cells; it extends to the red cells of other individuals, and to non-human red cells. Thus, washed red cells from certain individual sheep, which are sufficiently toxic to inhibit emigration and poison the leucocytes when incubated with human blood, will be readily ingested by the same leucocytes after the removal of the serum from the washed clot when reincubated on a fresh slide.

Moreover, this altered relationship between red cells and leucocytes in a disease like pneumonia may be brought about in normal blood (*in vitro*) by treating the washed red cells of one individual with the blood serum of another individual, or with the blood serum of the sheep, under certain conditions. After rewashing the clot red cells so treated may be readily agglutinated to and ingested by human leucocytes, whereas previously they produced no reaction on the part of these cells, or were so leucotoxic as to prevent emigration.

These and other facts suggest that it is some change on the surface of the red cell which brings about the altered attitude on the part of the leucocytes. We shall consider later whether, in addition to the deposition of this additive substance, some other substance may also not be removed, and whether by this process of addition and subtraction a profound chemical change may not be brought about in or on the surface of the red cell.

The Spleen and Leucocytic Stimulation.

We may regard the lacunar spaces in the spleen and the backwaters in the circulation as regions in which this aggressive action of the leucocytes on the red cells reaches a high level. Thus, in a case of enlarged spleen, in a man (also the subject of syphilis, with a history of previous malaria) in whom the spleen was removed by operation, serum from blood drawn from the finger—that is, the general circulation—when incubated with my own leucocytes and examined under dark-ground illumination, gave an emigration picture in which the individual leucocytes showed only moderate activity, while serum from blood taken from the splenic vein at the time of operation and incubated in the same way had a much more marked stimulatory effect, and greatly increased the pseudopodial activity of my leucocytes.

This comparative observation on serum from the general and the splenic circulation confirms the previous statement that substances which are capable of stimulating the

aggressive activity of leucocytes towards red cells are present in the blood in situations and in organs where, normally, red cells are undergoing disintegration.

In an interesting article on the spleen³ Barcroft has described that organ as a reservoir from which large numbers of red cells are thrown into the circulation under certain conditions. The observations just recorded seem to suggest that substances having a leuco-stimulating character are present in the blood after it has traversed the splenic tissues. It may probably be that, as with bacteria so with red cells, a previous opsonization of the red cells by the blood serum is necessary to enable the leucocytes to ingest them.

It seems clear, however, that it is possible to reduce the leucotoxicity of a given sample of washed red cells *in vitro*, not only by treatment with an appropriate serum, but also by treatment with certain chemical substances.⁴ Further, it is possible to increase the "aggressiveness" of the leucocytes to both native and foreign red cells *in vitro*, just as occurs irregularly in vaccine therapy, and with more uniformity through the influence of a previous infection, as in those diseases in which one attack confers subsequent immunity.

SECTION II.—PHYSIOLOGICAL.

The observations now to be recorded are concerned with the chemical and physical changes which can be observed in leucocytes as the result of, or in association with, the so-called process of stimulation.

One of the earliest signs of commencing activity shown by the white blood cells when removed from the circulation and incubated outside the body is a vibratory movement of the cell granules. This "boiling appearance" is seen in all varieties of leucocytes, but more in some kinds than in others. It may continue under favourable conditions for some hours, and as the liquefaction of the cell substance commences the amplitude of the granular movement increases. At a later stage it slows down, and finally ceases.

If a leucocyte film be prepared from incubated blood in a closed glass cell, the active cells which form a carpet on the floor of the cell do not, for the most part, stain with a 1/2 per cent. solution of ruthenamine chloride in normal saline; or, as this reagent is called, ruthenium red. A few odd leucocytes may show nuclear lobes of a rosy pink colour, but the great majority of the cell nuclei remain unstained. If the film is treated, however, with a 1 per cent. solution of iodine in normal saline, and is then washed in the saline, the majority of the cell nuclei will quickly stain with the ruthenium red. It is possible to bring about the same result by incubating the film in normal saline through which air is frequently bubbled, but the oxidation process takes one and a half to two hours before the cells will react to the stain.

If the film be treated with a weaker solution of iodine (1/2 per cent. in normal saline) for a few seconds only (four to twelve), and the iodine be then quickly washed away, and the film (covered with normal saline) be placed in the incubator for a few minutes, many of the cells will be seen on examination to be exuding colourless droplets. Some of these globules remain attached to the cell for a time, and then gradually dissolve in the surrounding medium. These droplets are composed of substances some of which have a strong avidity for iodine, and stain with it a pinkish-mauve, or, when more concentrated, a port-wine colour. If to the film so treated with a wash of weak iodine the solution of ruthenium red be added, many of the cells still remain unstained. For the most part it is the cells to which these iodophil droplets are attached which do not stain with the ruthenium red. The cells which show the pink-coloured nuclei have probably passed through the first stage of partial oxidation, and have reached the later stage in which the nucleus has become exposed by the removal of the lecithin coat, and under these conditions the nuclear substance combines with the ruthenium red.

Examination of the film at this stage under dark-ground illumination confirms this view. Thus, the iodophil globules can be seen exuding from, or in some cases distending, the cell. The nuclear lobes in these liquefying cells often appear more translucent, as though more liquid

than in cells in which these droplets are absent. The nuclear material can often be seen streaming along one or more of the pseudopodial processes. If a second dose of a stronger solution of iodine (1 per cent.) be floated under the covered glass, the droplets take on the mauve colour, and when the excess of iodine is washed away and the ruthenium red again added all the cell nuclei show the pink stain. What is the significance of this fact—that the cell nuclei do not stain until they have been acted on by the second dose of iodine? We know that the semi-permeable membrane, or the perinuclear meshwork which normally surrounds the nucleus, is composed of a lipid material (chiefly lecithin) in combination with magnesium or calcium, the lecithin component being in a comparatively insoluble form. The first effect of the oxidizing agent, the iodine, is to bring about a change in this lecithin coat. The membrane becomes more soluble and is partly removed, and the nuclear substance is thus exposed to influences which reach the cell from outside. The cell now begins to form iodophil substances in the form of droplets. These droplets consist partly of lecithin in the water-soluble form with traces of cholin split off from the oxidized lecithin, and it is probably this cholin component (and not glycogen as was previously thought) which gives the droplet its capacity to stain mauve with iodine.

This exudation of substances from the cell which have the capacity of lowering the surface tension at the contact face between the cell and the medium brings about the protrusion of dendritic and pseudopodial processes, and eventually results in the cell movements. It also contributes to the surrounding and engulfing of red cells, micro-organisms, and other foreign bodies by the leucocytes. It constitutes, in fact, an essential factor in phagocytosis.

Experimental Evidence.

I must now give some experimental proof that this view of what happens in the cell when activity commences is founded on sound evidence. It is first necessary to establish the fact that the capacity of the nucleus to stain with ruthenium red depends on a denudation of its lecithin coat and consequent exposure of the nuclear material to a water-soluble substance like ruthenium red. The following observations bear on this point.

A leucocyte film, obtained by incubating normal blood in a closed cell, is treated with a faintly acid 1/2 per cent. solution in normal saline of potassium chloro-platinate for one hour. The excess of the platinum salt is then washed away in normal saline and a 0.05 per cent. solution of benzidine base in distilled water is added to the film. A blue-coloured deposit is formed, which reveals the situations in the cell where the platinum salt has combined with the lecithin compound to form a precipitate.

Two points, however, at once arise. First, how do we know that the blue deposit seen in the cell is really a lecithin-platinum precipitate coloured blue by the benzidine, and not some protein or other constituent of the cell? In the first place, the potassium chloro-platinate in neutral solution does not give a precipitate with the globulins or plasma proteins, while it does combine with water-soluble lecithin to produce an insoluble lecithin chloro-platinate in the presence of weak acids. Also reagents like mercury perchloride and iodine, which precipitate serum albumin, do not precipitate lecithin. As long as the lecithin is present in the cell in the form of the magnesium or calcium compound it does not react with the neutral platinum salt. This can be demonstrated in the test tube by taking some lecithin magnesium sediment, suspended in water, and adding the neutral platinum salt in solution to it. No reaction occurs, and no blue deposit when benzidine is added after washing the precipitate. If, however, the lecithin magnesium compound be first converted into the water-soluble lecithin by treatment with sodium chloride, then the soap-like solution so formed precipitates with the chloro-platinate, and, after removing the excess of the platinum salt, this precipitate is coloured blue on the addition of benzidine. The demonstration of the arrangement and location of the lecithin substance in the cell by means of precipitation as a chloro-platinate is of considerable physiological interest, because it serves as an indicator of successive stages of cellular activity.

In the inactive phase, as in the white blood cells circulating in the blood, little or no blue deposit is seen after treating the washed leucocytes by the chloro-platinate-benzidine method, although a few blue granules may appear on the surface of the cells. In the slightly stimulated condition, as in cells which have emigrated from the clot when blood is incubated, the lecithin material becomes water-soluble through partial oxidation. Under these conditions a blue deposit is seen as a granular membrane covering the nuclear lobes when the film is treated with platinum and benzidine.

In cells which have undergone further stimulation and have reached a further stage of activity (as by a wash of weak iodine, or by incubation in a medium, which stimulates dendritic formation and iodophil droplets) the blue material is scattered through the cytoplasm as blue grains or masses, and it may collect as a blue ring at the margin of the cell. Further, since the water-soluble lecithin tends to flow out of the cell in the form of iodophil droplets, it is possible to prevent this material from dissolving in the surrounding medium by treatment with a weak solution of mercury perchloride. In such cases, after treatment with platinum and benzidine, the blue deposit often forms a film over the surface of the exuded droplets.

Treatment of the leucocyte film with a 1 per cent. solution in normal saline of phospho-tungstic acid produces some change or precipitation in the substance forming the iodophil droplets, whereby the droplet stains with basic dyes such as Nile blue after the phospho-tungstic acid has been previously removed by washing.

Experiments have been carried out with other substances which exert a solvent action on the lecithin nuclear membrane without unduly damaging the cell. Thus, cyclo-hexanol dissolves the insoluble magnesium lecithin compound in a test tube.

If a leucocyte film be washed with a 3/4 per cent. solution of cyclo-hexanol (hexhydrophenol) in normal saline, the nuclei in the great majority of the cells at once stain pink with ruthenium red. This reagent, by removing the lecithin coat, exposes the nucleus to the ruthenium salt. A saturated solution of ethyl ether in normal saline, on the other hand, while it dissolves the water-soluble sodium lecithin compound, leaves the insoluble magnesium form untouched; ether also brings about a curious fusiform swelling of the protruded dendrites in the incubated leucocyte film which may be due to surface tension changes which follow the solution of the lecithin, and thus to the retraction of these processes.

Pus Cells.

Pus cells can be used instead of incubated white blood corpuscles. If the liquor puris be removed from healthy pus by washing and centrifuging in saline, the cells so treated can be divided into two groups by the addition of ruthenium red: (a) living cells, which remain unstained; and (b) devitalized or exhausted cells, in which the nuclei take on a pink colour.

On warming the slide the unstained cells commence to throw out dendrites and pseudopods, and eventually form iodophil droplets, while the cells with the stained nuclei retain the spheroidal form of the inactive or the dead cell. Further, if a drop of such living pus in normal saline be incubated for a short time, and then treated by the chloro-platinate-benzidine method, an association can be traced between the distribution of the lecithin compound and the vitality and activity of the cell. The blue deposit appears as a ring surrounding the nuclear lobes in the less active, and as blue masses scattered through the cytoplasm in the more active cells, while in the dead cells, in which the nuclei stain deeply with ruthenium red, the lecithin material is absent, having been washed out of the cell, and no blue deposit is obtained. In the inactive, resting white blood cell, on the other hand, the lecithin substance, although present, is in the insoluble form, hence it fails to react with the neutral chloro-platinate, and consequently no blue deposit is formed with benzidine. The same result has been obtained in the cellular sediment from the cerebro-spinal fluid from a case of pneumococcal meningitis. About one-third of the leucocytes stained pink with ruthenium red, the rest remained unstained. Another portion of the same

deposit was treated by the chloro-platinate and benzidine method. The cells showed very little blue deposit. When the same sedimented fluid was incubated, however, an entirely different picture was obtained.

Three portions of the sediment were taken and incubated in closed cells for a quarter of an hour, for half an hour, and for three-quarters of an hour respectively. Each film of emigrated cells, after washing in saline, was treated by the chloro-platinate and benzidine method. The first film showed a fair emigration carpet of active cells, with some blue material, mostly in the neighbourhood of the nucleus. The second film showed more blue deposit, scattered through the cell. The third film presented the same picture in a more advanced form, and, in addition, blue material was seen in the surrounding medium, starting from points which were probably particles of the lecithin compound which had exuded into the medium.

Just as the capacity to stain with ruthenium red depends on exposure of the nucleus, so the capacity of the lecithin membrane to react with the chloro-platinate can be shown to depend on the water-solubility of the lecithin compound. Thus by testing the capacity of the nuclear substance to stain with ruthenium red, and by testing the distribution of the lecithin compound in the cell by the chloro-platinate benzidine method, some idea can be formed of the stage of activity of the cell.

The rapidity and the completeness with which the nucleus stains with ruthenium red provides a means by which the proportion of living to dead cells can be quickly ascertained in any given sample of pus, and affords a useful criterion of the healthiness of a wound apart from any examination of the number of organisms present, provided the cell has not been exposed to oxidizing reagents which would tend to denude the nucleus.

Oxidation and Contact with Foreign Substances as Factors in Cell Activity.

Before leaving the subject of oxidation some reference must be made to previous observations on this point. Metchnikoff, in his classical researches, dealt with the role played by oxidation in stimulating leucocytic activity. More recently Sir A. Wright⁵ has recorded observations on the bactericidal property of blood obtained under anaerobic conditions. I have recently reinvestigated this question from the point of view of leucocytic activity. If a little saucer-like shelf be made by coating a circle of wire with hard paraffin, melting above 37° C., this little shelf can be let down into a beaker of liquid paraffin, and a drop of blood drawn from the paraffin-coated finger can be placed on the shelf without coming in contact with the air or any foreign substance other than the liquid paraffin. The drop of blood remains for a considerable time as a sphere, standing upon and only touching the paraffin shelf at one point of its convex surface. The beaker is then placed in the incubator. Coagulation is much delayed, though it occurs eventually, as the drop settles down on the shelf, and a droplet of serum exudes from and adheres to the coagulated sphere.

During incubation, and before coagulation takes place, or at any later period if defibrinated blood be used, the still liquid blood can be drawn up in a very fine pipette, and blown out and examined on a slide. The leucocytes, although they have been incubated for a considerable time, will appear as spherical, inactive cells, resembling white blood corpuscles, floating freely among the red cells.

If, before submergence in the liquid paraffin, the drop of blood be received through the air into a drop of normal saline containing a little dissolved glutathione* or other substance which acts as an oxygen carrier, and then placed on the paraffin shelf and incubated as before, the leucocytes on examination will have lost the perfectly regular outline, and will be roughened by projections from the surface resembling immature dendrites. If treated with iodine some of the cells appear of a port-wine colour. Chemical changes have commenced in the cell which, if carried further, would lead to the production of iodophil droplets and dendrites.

* I am much indebted to Professor Sir Gowland Hopkins for the glutathione. This substance, when added to the blood plasma, greatly stimulates leucocytic activity and pseudopodial movement in the incubated blood.

The reason why these do not form, although the cells have access to oxygen, is, I think, owing to the absence of contact with foreign surfaces. No localized reduction of surface tension is produced at any one portion of the surface of the cell. Hence the cells preserve roughly the circular outline. They show, however, a slight tendency to stick together, and to form agglutinated groups of white cells, although they do not adhere to the red cells. These and other observations enable us to say that in the entire absence of oxygen leucocytic activity is delayed, even at a temperature of 37° C.

But although oxidation may set going the chemical changes in the cell which lead to the formation of iodophil droplets and other effects, contact with foreign substances is necessary to bring about those localized surface tension changes on which dendrite formation and pseudopodial movement depend. These and other observations point to the fact that, when incubated under conditions which prevent contact with foreign substances, certain chemical changes are initiated, but that *contact* is necessary to bring about phagocytosis in the full sense of that word.

This is shown in another way. If a slip of glass, previously heated in a flame to drive off the air, be inserted into the blood drop in which the leucocytes are undergoing incubation under the liquid paraffin, the leucocytes at once attach themselves to the glass surface and throw out dendrites and pseudopods.

The Reversible Process in Leucocytic Activity: Cell Recovery.

Although it has not been possible with our present crude methods to demonstrate fully the recurrence of anabolism following katabolism in a very delicate cell like the leucocyte, after cellular activity has reached the stage at which the nuclear membrane undergoes solution, and the nuclear substance stains deeply with ruthenium red, and iodophil substances have been elaborated and dendrite formation has occurred, yet it can be shown that recovery does follow on a less advanced phase of activity.

For instance, living pus cells (which are simply leucocytes which have emigrated from the blood vessels) must at an earlier stage have been sufficiently active and sufficiently mobile, both as regards their nuclear and cytoplasmic substances, to enable them to pass between the endothelial cells lining the capillary wall. When we recover these pus cells, however, from a well drained pleural effusion, or from the surface of a healthy wound, they have returned to the spheroidal shape and inactive condition, although they are by no means dead cells. When washed in normal saline and incubated they rapidly resume activity; they throw out dendrites and pseudopods, and they elaborate iodophil material, and, if activity proceeds further the lecithin membrane may undergo solution, and the nucleus become exposed, and it can then be stained with ruthenium red.

I have already said that in a film of emigrated leucocytes from normal blood a few odd cells will stain with ruthenium red. Are these cells which stain at once dead cells? Are they cells which have wandered on to the surface of mucous membranes, or into the tissue spaces, and have returned by way of the blood stream or the lymph stream to the general circulation by a process of what I have termed "return immigration"? If so they should be found in large numbers in the blood of persons in whom inflammatory foci, or wounds, or catarrhal conditions of the mucous membranes are present.

On the other hand, they may be cells which have become injured in the process of drawing the blood. There are some suggestive facts which bear on this point. In the incubated blood from a patient recovering from pneumonia with a normal temperature, but with some unresolved consolidation at the base of one lung, the leucocyte film of emigrated cells so obtained showed but few cells giving the pink colour. The clot was then detached from the slide, washed, and reincubated on a fresh slide. In this second crop a large number of cells showed pink-coloured nuclei when treated with the same reagent. The second crop of cells from the washed and reincubated clot from the blood of a healthy person remained unstained when treated in the same way. This suggests that the leucocytes in a patient recovering

from pneumonia, even after a week of normal temperature, are in a more unstable condition or are less rich in lecithin than in health—that is to say, their lecithin nuclear membrane is more permeable.

The following observations also throw some light on "cell recovery." A cell containing normal blood was incubated for half an hour, and then allowed to stand at room temperature for twenty-four hours. The emigrated cells, which, if examined immediately after incubation, would have been very active and adherent to the slide by dendritic processes, have now, after twenty-four hours, returned to the spheroidal shape. Some of them have become detached from the slide by the retraction or the rupture of their dendrites, and float freely in the serum as circular cells. The cells still adherent to the glass are nearly all more or less circular in outline; in some of the cells the nuclei stain with ruthenium red, while in others they remain unstained.

If such a film of "recovered" circular cells be washed in normal saline and again reincubated for a quarter of an hour in a mixture of native blood plasma and normal saline, or preferably Marshall's solution, these spheroidal inactive cells quickly resume activity. They throw out dendrites, they adhere to the slide, and they elaborate iodophil material. Moreover, they can again revert to the circular form and again be induced to resume activity a second time by repeated incubation. This observation shows that cells in this inactive phase are not necessarily dead cells. If, as I think, they are cells which, after passing through a katabolic or active phase, have reverted to the resting condition, an important fact will have been established in the life-history of the leucocyte and probably also in that of other cells.

In this matter of the development of acidity by cells when in the active phase it is of interest to note that Rous,⁶ in 1913, showed that fragments of embryonic tissue explanted into plasma coloured blue with litmus turned pink (that is, developed acidity) as growth proceeded, but remained blue if growth was arrested. Peyton Rous has also recently shown⁷ that acid substances are formed within leucocytes and other phagocytic cells under certain conditions during cell activity.

Levaditi and Gabrek⁸ also showed that pieces of tissue stained with neutral red and explanted in plasma become colourless as growth proceeds, while sterile portions turn yellow—that is, remain alkaline.

I have previously shown⁹ that the property of elaborating iodophil substances is not confined to leucocytes. It is present in certain epithelial cells, both in the skin and in mucous membranes, especially those lining the orifices of the mucous canals, also in some marrow cells, in the giant myeloid cells in sarcomata of bony tissues, and in some cancer cells of epithelial origin.

In the light of the observations now recorded it seems probable that this iodophil substance is not glycogen, as was originally thought, but (as shown by the reactions given with platinum salts) that it consists partly at least of lecithin in the water-soluble form, together with cholin split off from the lecithin. Further, it seems probable that the lecithin component is derived from the partial solution of the perinuclear lipid membrane during cell activity, and that cell recovery depends on the lecithin content of the cell, and may be associated with the restitution of this perinuclear lecithin membrane.

If these observations are confirmed, then I think a further step will become possible in the elucidation of the difficult problem of the chemical changes associated with katabolic processes in the life of the leucocyte, and probably also in many other cells.*

REFERENCES.

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* Since this address was given Professor J. B. Leathes, F.R.S., has published his most important Croonian Lectures on "The Role of Fats in Vital Phenomena," in which he describes the essential part played by lecithin in cell metabolism.