The leucocyte in health and disease : being an enquiry into certain phases of leucocytic activity / by C.J. Bond.

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IN
HEALTH AND DISEASE

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BEING

AN ENQUIRY INTO CERTAIN PHASES
OF LEUCOCYTIC ACTIVITY

BY

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PREFACE

In deciding to bring the observations and the facts herein recorded before my colleagues and other fellow-workers, and to the notice of physiologists and pathologists generally, I have been influenced by the consideration that, although a very large amount of time and labour has been devoted by many workers during the last fifty years to a study of the many kinds of organisms which may attack the body, less attention has been given to the investigation of the defensive mechanism by which the human organism repels invasion.

There is a growing volume of evidence which points to the outstanding importance of the leucocyte as an essential and integral factor in this problem of defence against invasion by the micro-organisms of disease. Hence I am hopeful that the observations now recorded, which are the outcome of some years of continuous study of the activities of leucocytes in health and disease, may not be without some value in helping towards a fuller understanding of the important part these cells play in the defensive mechanism of the body as a whole.

In carrying out this investigation the routine use of the dark ground method of illumination has been of the greatest assistance. It has enabled accurate observations to be made of changes occurring in the living cell, and as a technical method will no doubt come into more general use by cytologists and pathologists.

I have endeavoured, as far as possible, to control the

observations made on the *dead* leucocyte, as seen in the stained blood film, by a study of the *living* cell. It is only by observations made during life that accurate knowledge of the physical and chemical activities of the leucocyte, as of other cells, can be obtained.

I wish also to record the great assistance I have received from many co-workers and helpers at the Leicester Royal Infirmary and elsewhere, especially from Mr. Frank Young, whose chemical knowledge and advice has been most valuable, and to whom I am also indebted for the micro-photographic illustrations.

C. J. BOND.

LEICESTER,
February, 1924.

CONTENTS

THE LEUCOCYTE IN HEALTH AND DISEASE

SECTION I

INTRODUCTION

THIS investigation may be regarded as a continuation of certain enquiries commenced during the war, and directed to the ascertainment of the influence of antiseptics on the activities of leucocytes and the healing of wounds.

The observations now recorded deal mainly with two stages in the life-history of the leucocyte:

1. While circulating in the blood as a white blood-corpuscle.

2. During and after emigration from the blood-stream as a wandering cell or phagocyte.

The first six sections deal with some of the conditions which affect the emigration of leucocytes when blood is incubated in a closed cell, and with the changes in shape assumed by leucocytes and living pus cells when in contact with a glass surface or with foreign bodies, or with other substances in the blood-serum or fluids in which the cells are incubated.

In Sections I. and II. a description is given, accompanied by micro-photographic illustrations, of the protrusion of fine dendritic filaments by leucocytes under certain conditions.

These appearances have been regarded by some observers as representing changes in shape consequent on

the death of the cell, or as artifacts due to lethal conditions in the environment.

The following facts and observations seem to afford strong, if not conclusive, evidence, however, that these alterations in shape occur during the life of the cell, and that they represent vital processes:

(*a*) These dendritic filaments, like the larger pseudopods, are not seen in dead pus cells when incubated, nor in leucocytes which have been incubated in a highly toxic fluid.

(*b*) Leucocytes which enter on incubation as spheroidal white blood-corpuscles in freshly drawn blood, under favourable conditions rapidly throw out pseudopods or fine dendrites. At a later stage, or sooner, if the conditions become unfavourable to the vitality of the cell, the cell returns just before death to the spheroidal form unless it is fixed by heat or some other reagent while in the active stage. (Figs. 1 and 2.)

(*c*) Cells which have been incubated in a toxic medium or cells which in a normal medium have ingested toxic substances, such as foreign red cells, may, as the result of such ingestion, undergo cytolysis, or they may assume the circular outline, without dendrites and pseudopods, characteristic of the devitalised or dead cell.

(*d*) The great capacity of adaptation to minute irregularities of surface shown by leucocytes during and after emigration described in Sections II. and III., although they are no doubt the result of physical and chemical influences such as changes in surface tension, yet represent responsive reactions on the part of the cell before death rather than coagulative or other changes consequent on the death of the cell.

(*e*) While it is not denied that the protrusion of dendrites and pseudopods may be occasioned by contact

with a glass surface or other foreign substance, it is held that such contact provides a *point d'appui*, an anchorage (not available to the white corpuscle circulating in the blood-stream) whereby the stimulated and emigrating cell can respond by movement physically and also chemically to changes in the surrounding medium.[26]

(*f*) Pus cells, which have migrated on to the surface of a granulating wound or a mucous surface, if the conditions are not such as to kill or seriously damage these wandering cells, may be kept for a considerable time in a test-tube, especially if the liquor puris be removed by washing in saline, and such pus cells will, when incubated in a closed cell in normal saline, throw out pseudopods and dendrites, and will later assume the circular form as death takes place.

(*g*) If a thin fibrin network be prepared by allowing a layer of centrifuged cell-free plasma to coagulate on a slide, and if a drop or two of defibrinated blood from the same individual be placed on the fibrin network and incubated with it in a covered cell, the leucocytes which emigrate from the blood and enter the fibrin scaffolding, although they have not been in contact with the glass slide, will throw out pseudopods and dendrites, and assume the irregular shape. The conditions under which the observation is carried out in this experiment approximate fairly closely to those which obtain in the organism when leucocytes permeate a blood-clot. (Fig. 3.)

(*h*) If a drop of defibrinated human blood be incubated for a few minutes in a closed cell, and if the red cells be then washed away in a gentle stream of warm saline or Ringer's fluid, the leucocyte film so obtained, when examined under dark ground illumination, will show many cells in which the brightly illuminated cell granules within the body of the cell and within the larger dendritic tubular

processes will exhibit rapid oscillatory movement. The whole cell seems to be "boiling." These rapid movements of the granules cease if the cell is killed by heat or by some reagent such as iodine. They occur simultaneously with the solution or partial solution of the cell contents which takes place in these stimulated cells, probably owing to enzyme action.[22 23 24 28]

During this solution of the cell contents and the increased hydrolysis the granules become fewer in number and the oscillatory movements of the remaining granules are of larger amplitude, dendrites and pseudopods are protruded, and the cell passes through an accelerated and heightened phase of physical and chemical activity, in which the droplets of Iodophil and Diffusion substance described in Sections X. and XI. are extruded.

At a later stage many of the dendrites break up into granules or droplets, those which are attached to the glass are broken or detached, the oscillatory movements of the granules cease, and the cell dies in the spheroidal form. It may be that these vibratory movements of the granules are merely Brownian movements, but the fact that they cease with the death of the cell is, however, opposed to this view. In any case, they are associated with the phases of activity which the stimulated cell passes through before death. (Fig. 4.)

If instead of defibrinated blood a drop of whole blood from the same individual be incubated under similar conditions, the cells which emigrate from the clot and adhere to the slide appear smaller and less swollen than the cells from the defibrinated blood, the oscillation of the granules is generally less marked and occurs in fewer cells, and the granules are more tightly packed. The cells, in fact, seem to be in an earlier and less active phase of stimulation.

Individual leucocytes, as already stated, especially when stimulated, often exhibit rapid oscillation of the intracellular granules. This vibratory or Brownian movement is associated with the chemical changes which lead to the solution of the cell contents.[23] It finally ceases with the death of the cell.

These and other facts strongly confirm the view that the protrusion of the finer dendrites, like the larger pseudopods, represents a phase of activity of the living cell.

Thus, when we survey the life-history of the leucocyte as a whole, we come to regard the spheroidal form and the circular outline of the white blood-corpuscle, while circulating in the blood-stream, as characteristic of the resting, the inactive, the anabolic cell.

When, however, the cell comes to rest in contact with the capillary endothelial cells, or tissue cells in an inflamed or injured area in the body, or with the fibrin when the blood is shed, or with the surface of the glass slide on which incubation takes place, it rapidly enters on a katabolic phase of heightened physical and chemical activity. The surface of the cell becomes " sticky," and it adheres to surrounding surfaces and objects. The cell granules exhibit rapid movement, the cell contents and the granules undergo partial solution, and Iodophil and Diffusion substances are formed; these escape into the surrounding medium, pseudopods and dendrites are protruded, the cell moves as a whole, and foreign bodies are ingested, after these have been previously acted on by the cell secretions.

This phase of stimulation and of heightened activity easily, and if the stimulation has been so intense as to injure or poison the cell, rapidly passes over into a lethal or final phase, during which the cell may disintegrate and shed its cell contents and remaining granules into

the surrounding medium, or in which it may die more slowly, in which case the pseudopods and dendrites disintegrate or are retracted, and the cell assumes the spheroidal form and circular outline characteristic of the resting or inactive and also of the dead cell.

Section VII. deals with observations made during the incubation of leucocytes in a glucose saline, or glycogen saline medium, to which insulin in definite volume has been added in one case and not in the other.

The mechanism of the carbohydrate metabolism in leucocytes is thus shown to be influenced by the presence of insulin.

Section VIII. deals with certain physical features and staining reactions peculiar to the eosinophil variety of leucocytes.

Section IX. deals with the phagocytosis of red cells by leucocytes, and the relation between the leucocytes and the erythrocytes in the living blood, both under normal and abnormal conditions.

Section X. deals with the nature of the so-called Diffusion substance which is elaborated by leucocytes under certain conditions. The relation of this substance to the Iodophil substance is described, also the recognition of this Diffusion substance by means of a new technique.

Section XI. deals with the occurrence of the Diffusion and Iodophil substances in certain epithelial and in some cancer cells.

Sections XII., XIII., XIV., and XV. deal with certain facts observed when sheep blood-serum and sheep washed red cells are incubated with human leucocytes.

Section XVI. deals with the use of acetone as an extractive for red cells, and the effect of this acetone extract of red cells on human leucocytes, native and foreign.

Sections XVII. and XVIII. deal with erythro-toxins and erythro-opsonins and their action in health and disease.

SECTION II

EMIGRATION OF LEUCOCYTES: GENERAL CONSIDERATIONS

BEFORE considering certain details, such as the proportional number and the rapidity with which leucocytes emigrate on to a slide from an incubated blood-clot under varying conditions and in different diseases, we must first consider some general problems which concern emigration as a whole.

Differences in Shape assumed by Leucocytes while Circulating in the Blood-Stream and after Emigration.

It is, of course, well known that leucocytes of all varieties, while circulating in the blood-stream, are circular in outline and more or less spheroidal in shape. The question arises whether this spheroidal form so characteristic of the resting cell is the outcome of the absence of stimulating influences, or whether it is due to an inhibitory influence exerted by the blood-plasma on the cell.

It is certainly true that immediately the blood escapes from the bloodvessels and comes in contact with the tissues, or with foreign substances such as a glass slide, the leucocytes, if not too much injured by exposure or other injurious influences, or if not inhibited by thermal changes, commence to show signs of pseudopodial activity.

The same thing occurs during coagulation. The corpuscles entangled in the clot commence to emigrate

through their intrinsic power of movement, and, if not too much inhibited or injured by the serum in which the clot floats, they may, under favourable conditions, exhibit "return emigration" and re-enter the interstices of the fibrin sponge.[1]

Even in highly infected states of the blood, as in streptococcal septicæmia, the observation of both fixed and living, and of stained and unstained films of blood just removed from the circulation shows that the leucocytes are invariably circular in outline, although when blood from such an infected patient is incubated for a few minutes in a closed cell, the leucocytes at once show increased pseudopodial activity and an irregularity of shape above that shown by normal blood under like conditions.

If, however, the stimulation is so intense and overpowering as to become a toxic influence, as may occur just before death in very severe infections, then the leucocytes become poisoned or paralysed, they fail to throw out pseudopods, and they retain the circular form.

Thus it would appear that contact with stimulating and even poisonous substances does not cause changes in the shape of leucocytes as long as these cells circulate in the blood-stream. But as soon as stasis occurs, and the leucocytes come into prolonged and intimate contact with the capillary endothelial cells or foreign substances, then emigration and movement commences, biochemical are reinforced by physical influences, and pseudopodesis leads to an irregular outline of the cells.

Tests of Leucocyte Activity.

I have previously suggested three tests as useful indications of the functional activity of leucocytes when emigrating from a blood-clot after a half-hour incubation of blood in a closed cell.[2] These are:

1. The number and the kind of leucocytes which emigrate, and the rapidity with which they leave the blood-clot and adhere to the slide.

2. The shape assumed by the emigrating cells as affording evidence of pseudopodial activity.

3. The presence, in the form of intracellular droplets or extracellular exudation, of a substance having a strong affinity for iodine "Iodophil" substance giving evidence of the chemical changes going on in the cell.

To these we may now add three other tests as the outcome of recent investigation.

4. The capacity of the cell not only to move by means of pseudopods, but also to protrude large numbers of fibrillar, frequently branching, processes which we may call dendrites.

5. The number of cell granules and the manner in which the cell reacts to and stains with substances like benzidine, orthophenylene diamine, and other reagents in the presence of peroxide of hydrogen.

6. The secretion by the cells of a "Diffusion" substance, probably aldehydic in nature, which reacts with benzidine, and forms an insoluble compound which turns black when this precipitated benzidine substance is oxidised with iodine.

Dark Ground Illumination.

The observation of a leucocyte film under dark ground illumination affords valuable information as to the changes of form shown by leucocytes under different conditions of stimulation.

If the washed living leucocyte film formed on a slide by the incubation of two drops of blood from the finger in a closed cell be examined in normal saline fluid or Ringer's solution under dark ground illumination, many

of the polymorph cells will show a fringe of fine branching processes surrounding the cell.[33] These hair-like processes are very delicate in outline and seem to be tubular in structure. They are formed of an outer wall similar to the outer layer of cytoplasm surrounding the cell. This fine tubular channel is filled with liquid material or cell juice, along which the granules flow from the body of the cell. (Fig. 5.)

The changes in shape which an actively moving leucocyte undergoes during emigration are chiefly (*a*) a flattening and spreading of the whole cell circumferentially, so that it occupies a larger circle and often becomes surrounded by a fringe of fine fibrils, or (*b*) an elongation of the cell so that it assumes a fusiform, or club shape, with the multilobate nucleus usually occupying the larger end.

In cells which have taken on this elongated form the protrusion of dendrites generally occurs at the more actively advancing end. The sides of the cell are comparatively free from fibrils, or a few stunted processes may be present. (Fig. 6.)

The protrusions at the club-shaped end of the cell may take the form of fine hair-like threads, or one or two larger branching processes may extend a long distance (three or four times the diameter of the cell) into the surrounding medium.

There would appear to be some relation between fibril formation and mass movement of the cell as a whole. Thus, in films in which the leucocytes have been stimulated during emigration many of the cells are drawn out into rod-shaped masses, which in the transition from the circular to the rod shape leave a smear indicating the track left on the glass by the flowing movement of the cell, much as a snail leaves behind it a trail of mucus as its foot moves over a smooth surface.

Often the larger or club-shaped end of such an actively moving and elongated cell will throw out a beard or fringe of fine fibrils or streamers, the sides of the rod-shaped mass remaining comparatively smooth.

Observation under dark ground illumination also shows that cells during pseudopodesis, not only throw out bulky processes by means of which the movements of the cell as a whole are affected, but also numbers of finer fibrils resembling antennæ, which act as feelers, and serve also to entangle foreign bodies.

The Relation between the Physical Changes which Accompany Changes in Shape and the Chemical Changes which occur in the Cell.

The leucocyte, when observed in freshly shed blood-plasma, is a colourless cell, circular in outline, and nearly globular in form. It is filled with highly refractive colourless granules. It has the appearance of an inactive quiescent body, passively transported by the currents in the medium. Its inactivity is the expression of a state of chemical equilibrium between the cell and the fluid in which it floats. In a drop of saliva (and occasionally) in a drop of blood taken direct from the finger and examined on a slide in normal saline leucocytes may be seen in which the cell granules appear to be in active movement, oscillating rapidly within the cell. This vibratory condition would seem to be a stage preliminary to the commencement of pseudopodial activity and movement, and to be associated with commencing chemical activity within the cell. The protrusion of pseudopods and fine fibrils may thus be associated with a chemical interaction between the cell and the surrounding medium.

The Part played by the Nucleus in the Changes of Shape assumed by Emigrating Cells.

Although the multilobular nucleus is generally located in the central area of the polymorph leucocyte during its changes of shape, it can also accommodate itself to a restricted area. Thus, in an elongated cell the lobes of the nucleus no longer form a ring or horseshoe, as in the circular cell, but are drawn out into a bead-like string within the rod-shaped body. (Fig. 7.)

One or more of the lobes may flow along a protruding process or cytoplasmic fibril. In some cases a detached single lobe has been seen occupying the club-shaped extremity of the cell, but so far no reproduction of a new leucocyte by splitting of this detached lobe into a fresh nucleus has been observed, although the process suggests the commencement of cell division by fission.

In some cells just before death takes place the lobes of the nucleus appear, by a process of liquefaction, to run together and fuse, forming one single lobe. This may give rise to the fallaceous conclusion that the cell is of the mononuclear type.

SECTION III

EMIGRATION OF LEUCOCYTES: ADAPTATION TO IRREGULARITIES OF SURFACE

Leucocytes, especially the polymorphs, are peculiarly responsive to changes in surface tension.

If some fine lines are ruled with a diamond on the glass slide forming the floor of the cell in which the blood is incubated, the leucocytes, when emigrating from the clot, will occupy the linear grooves so formed.

Each cell sinks or flows into and fills the depression

as an elongated body with perhaps a wisp of fine fibrils projecting from each end of the cell.

Frequently the cells are arranged end to end in close contact, and form a continuous thread of protoplasm along the groove.

If two leucocyte films be prepared on ruled slides, one from defibrinated and one from whole blood, the mode of deposition and the arrangement of the cells will be different on the two slides.

In the film from the defibrinated blood the leucocytes subside immediately and adhere to the glass where they fall. Both the linear grooves and the intermediate spaces are occupied with adherent leucocytes.

In the film from the whole blood, the fibrin threads of the clot provide a scaffolding from which the leucocytes are let down gradually on to the glass, and very probably the chemical changes associated with the process of coagulation provide a further stimulus which causes the cells to flow into and along the grooves before they become firmly adherent to the glass slide. (Figs. 8 and 9.)

In some films nearly all the cells will be found arranged in the grooves, leaving the intermediate areas unoccupied.

It might be supposed that this intimate alignment of the cells along the irregularities of the glass surface in the grooves is the result of chemical influence; for instance, to increased alkalinity of the medium in these situations. It occurs, however, on slides which have previously been strongly heated and cleansed with chemical reagents and distilled water.

The explanation is probably to be found in the peculiar sensitiveness of the living leucocyte to changes in surface tension. A cross-section of one of the grooves on the slide represents one half of a capillary tube; the surface tension would be lower in the groove than on the flat

surface of the glass, and the pseudopods and fibrils would be protruded into and would occupy these areas of lowered tension, and they would carry the body of the cell with them, while the accurate filling of the groove would represent a later stage in the same process.

This power which the leucocyte possesses of accurately adapting itself to the minute irregularities of surface presented by foreign bodies with which it comes in contact, together with its capacity of response to the stimuli which act at a larger distance through chemical disturbances in the medium, are the essential factors which bring the moving cell (or its moving parts) into propinquity and effective contact with red cells, micro-organisms, pigment particles, and other foreign bodies, preparatory to the ingestion of any suitable substances.

SECTION IV

EMIGRATION OF LEUCOCYTES: THE INGESTION OF SUBSTANCES LIKE LIQUID PARAFFIN

In dealing with the ingestion of foreign substances by leucocytes, reference must here be made to the power which these cells possess of ingesting viscous substances like liquid paraffin.

Attention has previously been drawn to the effect of treating toxic red cells with paraffin in promoting the phagocytosis of these cells by leucocytes.[3]

During that investigation it was shown that when normal blood is incubated in a closed cell with a drop of liquid paraffin (or preferably with a drop of a suspension of the small globules formed when liquid paraffin is shaken up with normal saline), many of the leucocytes which

emigrate from the clot and adhere to the slide contain droplets of paraffin. Although we can understand how a leucocyte can surround and englobe a globule of liquid paraffin which has been isolated from the rest of that material in normal saline, it is not so easy to grasp the method by which a leucocyte can itself bite off and isolate a small portion from the mass of liquid paraffin and ingest it. This, however, does seem to occur. (Figs. 10 and 11.)

If a drop of liquid paraffin be placed on the surface of the blood to be incubated in a closed cell, it will spread as a film on the under-surface of the cover-glass which forms the roof of the cell. Some of the leucocytes will pass through this film, if it is not too thick, and adhere to the cover-glass; and some of these adherent cells will contain ingested droplets of paraffin after the remainder of the coating film has been washed away in saline.

By using paraffin coloured by the addition of some soluble non-toxic substance it is possible to obtain leucocytes which contain these coloured droplets, but the cells seem to be repulsed by most of the coloured substances which are soluble in paraffin.

How can soft, non-resisting protoplasmic bodies like leucocytes bite off, as it were, bits or droplets of a viscous substance like liquid paraffin, which has a lower specific gravity than water or blood-serum, and how do these cells make their way through the film of paraffin on to the cover-glass ?

In the case of foreign particles like red cells or micro-organisms, or solid bodies like carbon, the leucocyte protrudes its pseudopods, and flows around the object to be ingested; but, we may ask, can a substance like liquid paraffin oppose sufficient irregularity of surface or resistance to allow of its englobement by leucocytes in a similar manner ?

The addition of colloidal substances to water in contact with oil has the effect of reducing the surface tension at the interface or contact surface between the water and the oil (as shown by Donnan and others).

When the formation of fibrils or dendrites by the leucocytes takes place during emigration, under the stimulus of a changed environment, the digestion or dissociation of some of the cell contents not only initiates the protrusion of the dendrites, it also (by the exudation of colloidal diffusion substances) reduces the surface tension at the interface between the serum which covers the dendrite and the paraffin. As a consequence of this reduction of surface tension the extending dendrite is able to deform the contact surface of the paraffin, and push it before it, until (passing through the film of paraffin) it (the dendrite) comes in contact with the cover-glass. In this way the dendrite perforates the film of liquid paraffin. If now (as is the case) the dendrite terminates in three or four branches, the separate fibres in passing through the film would between them pinch out a disc of liquid paraffin, and this would eventually round itself off into a globule entangled within the fibrils. This globule would then, by extension of the process, eventually become ingested by the leucocyte.

Probably the relationship between the surface of the paraffin film and the skin of the leucocyte is so delicate and capable of such free adjustment of surface tension as to permit of the insinuation of the fine, almost ultra-microscopic protoplasmic threads which observation under dark ground illumination shows *are* protruded from the bodies of leucocytes to long distances. Probably also the peculiar capacity which leucocytes possess of attaching themselves by agglutination to foreign substances and surfaces to be travelled over or through, may be a factor in the process.

It seems clear, however, from these observations that leucocytes can pass through a film of liquid paraffin when emigrating from a blood-clot, and that they can ingest droplets of the paraffin while so migrating.

Incubation with liquid paraffin also causes a richer production of the "Iodophil" substance which (as we shall see later) is formed by emigrating leucocytes. The exuded globules of this substance stain more deeply with iodine and remain for a longer time attached to the cells before dissolving in the surrounding medium in the presence of liquid paraffin and other viscous fluids which retard the circulatory currents in the fluid medium. (Fig. 11.)

The same effect is observed when the fluid in which the leucocytes are incubated contains mucus.

The "Iodophil" substance which is secreted by leucocytes (and by some epithelial cells) in the urethral mucus during recovery from gonorrhœa is peculiarly abundant. It forms droplets which coalesce into globules which do not readily dissolve in the surrounding field, and which stain a rich port wine colour with iodine and also have a characteristic shape. This is only observed in preparations that have remained undisturbed for a considerable period. (Fig. 12.)

SECTION V

EMIGRATION OF LEUCOCYTES: LEUCO-AGGLUTINATION AND THE FORMATION OF "GIANT" CELLS

I HAVE already described the way in which leucocytes tend to flow into and arrange themselves end to end along grooves ruled on a slide forming the floor of the closed cell in which blood is incubated.

It is a noteworthy fact that when healthy blood is

incubated under ordinary conditions the leucocytes which emigrate from the clot on to the slide spread themselves in an even layer. Each cell takes up a position in definite relation to neighbouring cells. Even in films from patients affected with increased leucocytosis it is unusual to find the leucocytes arranged in heaps, one cell overlying the other.

This means that, under normal conditions, the leucocytes are not attached to each other; no clumping of the cells or leuco-agglutination takes place. If, however, a drop of horse serum be added to a suspension of human leucocytes in normal saline, or to a drop of blood diluted with normal saline, especially if the slide be incubated for a few minutes, the leucocytes will tend to run together into clumps, especially in areas around the margin of the cover-glass.

Leuco-agglutination occurs in the same way that bacillary clumping occurs, or that the hæmo-agglutination of red cells takes place in the presence of an agglutinating serum.

This tendency for leuco-agglutination to take place on the addition of horse serum may be associated with the peculiar symptoms which arise when horse serum is injected into the blood-stream in certain individuals.

The leuco-agglutination which occurs on the slide *in vitro* varies in intensity in the blood of different persons, just as the symptoms after the injection of horse serum vary in different individuals.

When washed sheep red cells, which are somewhat toxic to human leucocytes (but not so toxic as to destroy the leucocytes and prevent all emigration), are incubated with human blood in a closed cell, a peculiar effect on the leucocytes is sometimes observed.

Two or three or more leucocytes, each containing

ingested sheep red cells, fuse together into a single mass. Sometimes the outlines of the individual cells which form the syncytium or giant cell can be indistinctly made out; in other cases a mass of cell material, stained yellow with iodine, is seen enclosing single red cells, or groups of sheep red cells, some of which may show signs of decolorisation and digestion. (Fig. 13.)

The noteworthy feature is that this tendency of the leucocytes to fuse together, and thus more efficiently to enclose foreign bodies like red cells, occurs especially when the leucocytes come in contact with highly toxic substances, either dissolved in the serum as in the case of horse serum, or attached to the surface of, or adsorbed to foreign bodies, as in the case of the toxic sheep red cells.

The occurrence of leucocytic clumping is also of interest as illustrating *in vitro* on a slide the early stages in the formation of those aggregates of phagocytic cells which occur during life in the tissues as multi-nucleated giant cells.

In this matter of isolation and occasional conjunction the behaviour of the resting unstimulated leucocyte differs from that of the active emigrating cell, especially when the latter is over-stimulated and partly poisoned by some foreign substance against which it is only partly adapted for effective defence.

SECTION VI

EMIGRATION OF LEUCOCYTES: THE PHYSICAL AND CHEMICAL ACTIVITY OF LIVING PUS CELLS

THE heightened pseudopodial and secretory activity seen in leucocytes which have emigrated from the blood-stream, either into the tissues, during life, or from an incubated blood-clot, is also present in "pus" cells, provided they are not completely devitalised by the bacterial or other toxins present in the liquor puris. Pus cells under such conditions throw out pseudopods and fibrils, and secrete large quantities of "Iodophil" substance.

If some washed sheep red cells which are only moderately toxic to human leucocytes are incubated in a closed cell with two or three drops of the sero-pus from a healthy wound or from a well-drained empyema, the living pus cells will agglutinate the washed red cells to themselves and will ingest them in considerable numbers, although leucocytes obtained direct from the blood of the person supplying the pus cells will be poisoned by the same red cells. They will not emigrate from the incubated blood-clot, or attack the red cells. (Figs. 14 and 15.)

Further, and still more important, if the pus cells are freed from the liquor puris by previous washing in normal saline, they will show an *increased* capacity for agglutinating and ingesting the same red cells. (Fig. 16.)

The same "aggressiveness" of pus cells is also found in regard to human red cells. Here also the phagocytic capacity of the pus cells is increased by washing and the removal of the liquor puris. (Figs. 17 and 18.)

The removal of the liquor puris, like the removal of the serum from the incubated blood-clot, enables the

pus cells and the emigrating leucocytes to ingest the red cells more vigorously.

How can this heightened activity of the leucocytes or pus cells on the removal of the surrounding fluid be harmonised with the established fact that previous exposure of foreign or toxic red cells to the opsonising action of an appropriate serum renders those cells an easier prey to the leucocytes ?

The explanation probably depends on the removal, or the dilution by washing, of the products of cell destruction which have accumulated in the liquor puris or the blood-serum to a poisonous degree.

We have already seen that washing the incubated blood-clot and the removal of the serum in which it floats increases the phagocytic activity of the emigrating leucocytes.

Thus, while fresh serum and healthy liquor puris may aid phagocytosis by preparing the red cells or other foreign material for ingestion, the same fluids, when saturated with the products of tryptic digestion, may act injuriously on the leucocytes and inhibit their activities.

This explanation is in harmony with the facts known about the physiology of other organisms. Yeast cells, when inhibited or poisoned by a high concentration of alcohol in the culture medium, can be restored to renewed activity by the removal of the products of cell metabolism.

These observations show, at any rate, that carefully washed emigrating leucocytes and pus cells can, under favourable conditions, ingest washed red cells which have not been previously treated by an opsonising serum or liquor puris.

The influence of emigration (that is, removal from the blood-stream) in increasing the phagocytic capacity in leucocytes and pus cells is also seen in regard to the inges-

tion of many other foreign substances, organic and inorganic, though in the case of carbon particles Hamburger[32] considers that the presence of blood-serum greatly accelerates or may even be necessary to ensure ingestion.

This heightened phagocytic activity acquired during or after emigration by leucocytes and living pus cell is also, to a certain extent, general and non-specific in character.

Thus leucocytes which have emigrated from a washed, reincubated blood-clot, and washed living pus cells will ingest foreign or toxic red cells which, before emigration, they will only attack after these have been prepared by specific opsonins.

This heightened non-specific activity associated with emigration has an important bearing on certain recently ascertained facts concerning the relationship of specific to non-specific immunity in general.

It must not be forgotten, however, that emigration may be, after all, the result rather than the cause of the heightened activity.

As the result of stimulation by foreign substances present in the blood plasma or serum, an increased chemical and physical activity takes place in the leucocytes or pus cells. When stasis occurs at the localised seat of injury or infection, or in the backwaters of the marrow, or spleen, or other organs, these stimulated cells come to rest in contact with the endothelial cells of the capillary wall; they begin to exercise the pseudopodial activity which leads to emigration, and they protrude the fine fibrils which act as feelers and serve to bring about effective contact with red cells or other organisms.

The same sequence of events takes place during emigration from the incubated blood-clot in a closed cell.

From the clinical point of view the fact that pus cells

are leucocytes which have emigrated from the blood-vessels fits in with our experience of what happens in healthy wounds.

As long as the liquor puris consists of fresh serum and does not become a degraded fluid, saturated with the products of cellular digestion and bacterial toxins, any slight inhibitory influence of the fluid on phagocytosis is outweighed by the advantage of the presence of moderate numbers of emigrating phagocytes having general as well as specific phagocytic capacity, and capable of ingesting and removing red cells or other damaged tissue cells and micro-organisms.

It is probable that the training of leucocytes to increased phagocytic activity by means of vaccine therapy or by auto-immunisation may consist in, or depend upon, the development by exercise of this capacity which leucocytes possess for emigration and of a probable further capacity for "Return immigration," to which latter process reference must now be made.

Emigration and Return Emigration.

In a number of previous communications[4] attention has been drawn to the part played by the return into the tissues, or blood, or lymph-stream, of leucocytes which have previously emigrated on to the surface of mucous membranes or wounds, many of which may have ingested organisms, or red cells, or fat globules, or other foreign substances.

The influence of this return movement of phagocytes in the production of recrudescent sepsis in healed wounds has also been pointed out.[5] The same leucocytic movements which the classical researches of Metchnikoff,[27] Macullum, and many other observers showed as taking

place on mucous membranes and in serous cavities are now known to occur in connection with wounds.

The point, however, with which we are now more immediately concerned is the fact that the heightened capacity for phagocytosis acquired by wandering cells during the process of emigration from the blood-stream is retained and exercised by the same cells after they have returned to the tissues or the lymph-stream during "return immigration."

Looked at from this point of view, living pus cells are leucocytes which have emigrated from the blood-stream on to the surface of a wound, but having emigrated under special conditions, they have acquired increased "Iodophil" substance secreting, "agglutinating," and other phagocytic capacities; they are trained or vaccinated cells, and if, as may be the case, they re-enter the tissues or the lymph or the blood stream, they will have an opportunity of exercising these increased powers. (Figs. 19 and 20.)

SECTION VII

EMIGRATION OF LEUCOCYTES: THE INFLUENCE OF DISEASE AND OTHER ABNORMAL CONDITIONS

THE rapidity with which, and the conditions under which leucocytes emigrate from the incubated blood-clot in different diseases afford valuable information as to the activity of the leucocytes.

Thus, if the blood of a malaria patient taken during, or soon after, the rigor be incubated, and the leucocyte film so obtained be examined in iodised saline, the number of polymorphs which emigrate from the clot and adhere to the slide will be below the normal. In extreme cases

only a few cells will be seen. Further, the cells which do adhere to the slide are more or less circular in shape, they show little sign of pseudopodial activity, and they secrete very little " Iodophil " substance.

If, however, the cover-glass of the cell be removed, and the paraffin or plasticine ring with the contained clot (which stretches as a membrane across the cell) be gently detached with the point of a knife from the slide, and then washed on its upper and lower surfaces with a gentle stream of saline, the red clot can be freed from the serum in which it lies. It is then replaced with its under-surface downwards on a clean slide. The plasticine ring is then firmly pressed down on the glass to exclude air, a drop of saline is added, and a fresh cover-glass is placed over the cell to prevent drying. The washed clot so treated on the clean slide is then reincubated for a quarter of an hour, and the freshly formed leucocyte film examined. A fair number of actively moving leucocytes will be now seen on the slide. The inhibiting serum has been removed and emigration commences.

Although the inhibitory influence of the serum from normal blood when incubated under like conditions is less than in malaria blood, yet a comparison between the leucocyte film obtained from healthy blood on the first incubation and the second leucocyte film obtained from the same clot after washing and reincubation shows that the leucocytes from the washed clot are more active and more phagocytic towards foreign red cells than the leucocytes which emigrate on the first incubation. In the case of normal blood the clot can be rewashed and reincubated several times, and a fair but diminishing crop of living leucocytes obtained up to the third incubation.

The beneficial effect on the leucocytes of the removal of the serum is also seen when human blood is incubated

with toxic sheep red cells. During the first incubation very few cells emigrate, and any which adhere to the glass show signs of degeneration; if, however, the red clot be washed in saline and reincubated with a second drop of a suspension of the same toxic red cells, emigration takes place more freely and ingestion of the red cells begins. The same occurs when human blood is incubated with foreign or opsonised *human* red cells. (Fig. 21.)

In order to induce human leucocytes to phagocytose any given sample of foreign washed red cells, it may only be necessary to wash the clot obtained by incubating normal blood in a closed cell and reincubate the clot thus freed from serum with a drop of a suspension of the washed red cells to be ingested.

These and other facts suggest that the toxic or in-inhibitory influence exercised by serum on the activity of leucocytes in the presence of red cells is due to some reaction which takes place between the serum and the red cells, whereby a substance is formed on the surface of the red cells which, in a concentrated and perhaps unoxidised form, poisons and repels, and in a more dilute, or perhaps more fully oxidised, form stimulates and attracts the leucocytes in the neighbourhood of the red cells.

It may be supposed that the leucotoxicity of the serum is sufficiently accounted for by its antitryptic effect on the leucocytes. While such an explanation might account for the failure of the leucocytes to *digest* the red cells, it would hardly account for their failure to emigrate from the clot, unless, indeed, pseudopodesis and emigration entirely depend on ability to elaborate tryptic digestive ferments on the part of the leucocytes.

The Influence of Temperature on Emigration.

If one or two drops of normal blood be allowed to stand in a closed cell at room temperature 60° F., only a few leucocytes will emigrate on to the slide; while if the cell be kept at a temperature of 80° F. for one or two hours, more cells will emigrate. If the blood be kept at room temperature for twenty-four hours and later be incubated for a short time at 37° C., a good crop of emigrated cells (chiefly polymorphs) will be obtained.

The length of time during which leucocytes imprisoned in a blood-clot in a closed cell at room temperature can retain their vitality seems to be somewhere about forty-eight hours. Some cells will, at any rate, emigrate and adhere to the slide when incubated after this period. Sir A. Wright[26] states that human leucocytes washed in and preserved in normal saline remain alive for twelve hours when kept at room temperature.

The question still remains, however, Why do the leucocytes fail to emigrate freely from blood taken from a malaria patient during or soon after the rigor, though emigration does occur in blood taken in the apyrexial stage ?

The fact that the cells do emigrate from the washed clot after removal of the serum shows that the leucocytes are not all killed in the first case, but that their movements are inhibited by the serum which exudes between the clot and the glass slide; moreover, the few cells which do pass through this layer of serum assume the circular outline characteristic of the poisoned cell.

This leucotoxicity of blood-serum is apparently greater in the pyrexial period, when, as the result of the escape of many malarial parasites, large numbers of red cells are broken up. It is, indeed, probable that the toxicity of

the serum may be due to the broken-up red cells rather than to the malarial parasites.

This peculiar reluctance on the part of the leucocytes to emigrate from malaria blood during the pyrexial stage is also present in other abnormal conditions of the blood, especially those which are associated with periodically recurring pyrexial states.

In a case of Hodgkin's disease in which a seven days' fever recurred every three weeks with intervals of normal temperature, leucocyte films from blood samples taken during the pyrexial periods showed scanty emigration. (Fig. 22.)

It may be thought that the diminished emigration during the fever can be explained by a diminution in the leucocyte content of the blood, a leucopenia present during this period. The free emigration from the washed clot shows, however, that the failure to emigrate during the first incubation is not due to absence of leucocytes, but to some interference with their activity and power to emigrate.*

The scanty emigration film characteristic of malaria and some other blood diseases in the pyrexial stage is very different to the crowded films obtained from the blood of patients suffering from other infective diseases of bacterial origin.

Thus, if one or two drops of blood taken from a patient with lobar pneumonia (in the acute stage) be incubated in a closed cell, the leucocyte film so obtained shows a dense carpet of active irregular-shaped cells. In some cases the number of leucocytes may be sufficient to entirely cover the slide with a pavement of cells in close contact. But even in such cases the cells show a tendency to avoid

* An examination of blood films in malaria cases does not show any marked leucopenia.

superimposition. They seek contact with the glass surface rather than with neighbouring cells. (Fig. 23.)

While in malaria the presence of the blood-serum inhibits the movement of the leucocytes, in pneumonia it stimulates them to increased pseudopodial activity.

It is possible that this different action of the serum in the two cases may be associated with the fact that in malaria red cell destruction is the result of a process going on *in* the blood-stream, whereas in pneumonia the red cell destruction is more gradual and is the result of the entry into the blood-stream of a different kind of toxin formed primarily in the lung tissues. Essentially the problem is probably one of racial or individual adaptation on the part of leucocytes to unusual environmental conditions.

The Effect of General Anæsthesia.

If blood taken from the finger of a patient, who has been under ether anæsthesia for a fairly long period (say one hour), be incubated in a closed cell, the leucocytes in the leucocyte film so obtained show signs of stimulation (see Fig. 6). After prolonged anæsthesia, especially if this has been accompanied with cyanosis, as formerly occurred with the closed methods of inhalation, a number of the leucocytes will have one or two or more red cells agglutinated to them, and some cells will contain one or more ingested red cells. That this tendency to phagocytose red cells is partly due to an increased aggressiveness on the part of the leucocytes can be shown by re-incubating the washed clot with a suspension of normal red cells from a healthy person, when it will be found that these cells also are attacked by the leucocytes from the anæsthetised blood. But while the leucocytes seem to possess an increased aggressiveness to red cells in

general, the red cells of the anæsthetised patient have also been altered and rendered more susceptible to leucocytic attack.

In a later section evidence will be brought forward to show that the change in the red cell is probably the result of an interaction between the red cell and the serum, whereby an additive compound is formed on the surface of the red cell which opsonises the cell and renders it more susceptible to attack.

We know from previous observations[6] on auto-hæmagglutination that prolonged ether anæsthesia increases the auto-agglutinability of the red cells, and we shall see later that erythro-erythro-agglutination—*i.e.*, the clumping of red cells by an agglutinating serum or other agent—although not identical with, is yet related to, erythro-leuco-agglutination—*i.e.*, the clumping of red cells on to and by leucocytes preparatory to phagocytosis.

SECTION VIII

THE LEUCOCYTES AND CARBOHYDRATE METABOLISM: THE INFLUENCE OF INSULIN

If two drops of normal human blood be incubated in a closed cell with one drop of a 0·2 per cent. solution of glucose in normal saline, the free emigration of leucocytes from the clot on to the slide is interfered with. Those leucocytes which do emigrate show signs of injury as well as reduced activity. (Fig. 24.)

If one drop of a dilute solution of insulin (bearing the same relation to the volume of the incubated blood that one unit of insulin bears to the total blood-volume in the body) be added before incubation, then the toxic effect

of the 0·2 per cent. glucose is much diminished, and free emigration of leucocytes takes place on the slide. (Fig. 25.)

When these two leucocyte films so obtained are "fixed" with hot saline, and subsequently treated with benzidine and iodine, a striking difference is seen in the amount of black "Diffusion" substance and stained granules in the two films.

In the film from the blood incubated with glucose alone the leucocytes which adhere to the slide show very few black-stained granules within the cells, or black deposit outside them.

Whereas in the film from the blood incubated with glucose *and* insulin many of the leucocytes are loaded with black granules and numbers are packed with and surrounded by masses of the black deposit.

This is not due to further dilution of the glucose by the addition of the insulin solution, because an extra drop of normal saline was added to the first cell to equalise the concentration of glucose in the two cases.

The same result is also obtained in a rather less striking way when glycogen is used instead of glucose.

It thus seems probable that in normal blood, incubated outside the body with an excess of glucose, the presence of insulin up to a certain proportion facilitates the metabolism of the glucose by the leucocytes and increases the amount of the "Diffusion" substance which results from the intracellular digestion of the glucose by those cells.

Macleod[7] states that "the mechanism by which the blood-sugar is lowered acts in the *tissues* and not in the *blood*. The addition of insulin to blood incubated outside the body does not alter the rate at which the sugar disappears, whereas the addition of insulin to a glucose saline solution perfused through the heart increases the rapidity with which the sugar is removed from that solution."

If we regard the increased amount of "Diffusion" substance formed by leucocytes, when incubated with an excess of glucose in the presence of insulin, as an indication of an increased rate of glucose oxidation, the conclusion would seem to follow that the presence of insulin does facilitate carbohydrate metabolism by leucocytes in shed blood when incubated.

If, instead of adding insulin and glucose to incubated normal blood, insulin alone is added in like proportion, no marked increase in the amount of "Diffusion" substance is seen. The presence of the added insulin, over and above the amount originally present in the normal blood, does, however, cause a deeper rich brown staining of the "Iodophil" substance when iodine is added in the unfixed film. A precipitation may be seen in the globules of the exuded "Iodophil" substance, and pinkish granules appear like condensation points in the droplets.

It may be that the leucocytes while in the circulating blood-stream are not stimulated to increased carbohydrate metabolism by the presence of glucose and insulin in excess, and that only emigrating cells, under the stimulus of coagulation and the other altered conditions which are present in shed blood, act in this way.

It may be that emigrated leucocytes become (from this point of view) "tissue cells" as far as their relation to insulin and carbohydrate metabolism is concerned.

We must, however, remember that there are relatively few leucocytes in the two drops of blood incubated compared with the enormous proportion of muscle cells in the heart tissue through which the glucose saline fluid was passed. Both the rapidity and the amount of the reduction of glucose by such a relatively small number of cells might be difficult to determine.

SECTION IX

THE EOSINOPHIL CELL

THE description to be given in later sections of the formation of "Diffusion" substance and the other physical and chemical activities of leucocytes under different conditions applies to leucocytes as a whole. The eosinophil cell presents, however, certain peculiar features which must now be considered.

Fragility.

It has long been known that the eosinophil is the most fragile of all the leucocytes. It readily undergoes disruption, and liberates its granules into the surrounding plasma or serum when the blood is shed.

This explosive action of the eosinophil cell is well seen in living leucocyte films obtained from incubated blood in which the leucocytes have been stimulated to extra phagocytic activity by the addition of foreign or opsonised red cells, or in diseases like pneumonia. (Fig. 26.)

Treatment of a leucocyte film containing a number of eosinophils by the benzidine and bichromate, or preferably by the benzidine and iodine method does not show any excess of the black or blue deposit representing "Diffusion" substance. On the contrary, the amount of both "Iodophil" substance and "Diffusion" substance formed by eosinophil cells seems to be distinctly less than by polymorphs.

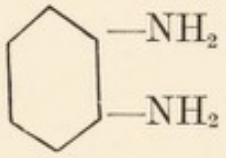
Neither in regard to the phagocytosis of red cells does the eosinophil cell show any marked activity over the neutrophil cells.[31]

It usually throws out one, or perhaps two, main dendrites with which it establishes contact with red cells or other foreign substances. (Fig. 27.)

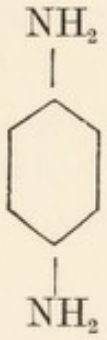
The large characteristic granules which stain yellow with iodine, and rose-pink with eosin, are generally collected at the larger end of the cell, though isolated granules may be seen along the main processes and in some of the finer fibrils.

These granules have a peculiar affinity for some of the aromatic diamines.

If a leucocyte film prepared from incubated normal blood be treated with a few drops of a 1 per cent. solution of ortho-phenylene-diamine in normal saline, and a drop or two of peroxide of hydrogen solution be added, the oxidised ortho-azine substance becomes anchored on to the granules and stains them a deep mauve or brownish-red colour. (Fig. 28.) The granules in the polymorph cells remain unstained. The position of the amino group in the ortho-substance is thus:

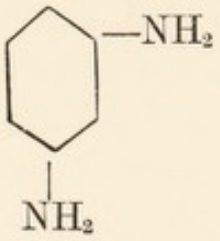


If para-phenylene-diamine be used in which the position of the NH_2 group is thus:



a moderate staining of the granules occurs, but less than with the ortho-compound, while, if meta-phenylene-diamine

is used, little or no staining of the eosinophil granules takes place.



A question arises whether the different staining effect exercised by the three isomeric substances bears any relation to the chemical structure of the substance stained.

However this may be, the way in which the granules in the eosinophil cells anchor the azine compound in the presence of oxygen, and the characteristic colour they assume, provides us with a differential stain by which the eosinophil cells can be picked out from the polymorphs and other cells in the leucocyte film.

It is probable that the large eosinophil granules with their avidity for acid dyes derive some of their activity from the ionisable iron which they contain in association with a proteid substance, as in the case of hæmoglobin.

It can be shown that the eosinophil cells do contain a high iron content by treating a leucocyte film from a case of eosinophilia for twenty-four hours with hydrogen peroxide solution, and then using the Prussian blue method to demonstrate the presence of the ionised iron.[8] [9]

The eosinophil cells in inflammatory tissues in the horse also give an intense iron reaction.

Recent investigations[10] [11] suggest that the real activator of the oxidation process which is so active in the red blood-cells is the ionisable iron which such cells contain.

The highly fragile, explosive character of the true eosinophil cell in incubated blood is also interesting when

considered in relation with the high iron content of these cells.

I have endeavoured to ascertain whether the feeding of polymorphs with red cells produces larger or more numerous granules, or granules which stain with acid dyes or give the characteristic stain with ortho-phenylene-diamine given by eosinophil granules. There is no doubt that leucocytes which contain ingested red cells give a high proportion of pink granules when stained with Leishman's stain. This fact, however, does not justify the conclusion that true eosinophil cells are so formed from neutrophil cells.

In Pernicious Anæmia.

If the blood be incubated from a patient with pernicious anæmia, a leucocyte film is obtained showing a large proportion of cells with granules which give the mauve-coloured reaction with ortho-phenylene-diamine and oxygen.

Further, the number of cells which give the black reaction with benzidine and iodine in the "fixed" film is not increased.

If the same blood be defibrinated before incubation only a few cells are seen giving the mauve reaction, and very few giving the black reaction in the fixed film.

If the red clot be washed in saline and reincubated with a suspension of washed red cells from the same patient, slight erythro-leuco-agglutination and ingestion of native red cells takes place while if washed *foreign* human red cells be used, this ingestion is much more active. The leucocytes are more aggressive to foreign than to native red cells.

The question arises, in pernicious anæmia, as to the true nature of these eosinophilous cells which not only

give the mauve reaction with ortho-phenylene-diamine, but also stain more readily than usual with eosin in a Leishman film.

The evidence does not, I think, justify a final conclusion as to whether they are true eosinophils or polymorphs which, as the result of feeding on the abnormal and effete red cells, have developed an unusual number of eosin staining granules.

As far as is known, a relatively high count of true eosinophils is not characteristic of all cases of pernicious anæmia.

The fact that the eosinophil cells form less "Iodophil" and "Diffusion" substance under appropriate conditions than the polymorphs is in harmony with the known absence of any marked increase in the relative number of eosinophil leucocytes in the ordinary bacterial and coccal infections.

Finally, the elongated form of the eosinophil cell when incubated, and the fusiform shape of its nucleus are also characteristic, although these peculiarities are, of course, not noticed in the dried films obtained from the circulating blood in which all the leucocytes, including the eosinophils, show the globular form under these conditions.

SECTION X

THE PHAGOCYTOSIS OF RED CELLS BY LEUCOCYTES

ATTENTION has been drawn in previous communications[12] to the occurrence of certain consecutive stages in the process of phagocytosis of red cells by leucocytes.

It has been shown that the successful ingestion of animal and human red cells by human leucocytes depends on a chemical and physical reaction in which the process of *erythro-leuco-agglutination* constitutes an early phase.

The first step (in incubated blood) consists in the bringing of the red cell within the sphere of influence of the leucocyte. The red cells no longer float freely in the surrounding medium, but become aggregated around the leucocytes; some become entangled by the delicate fibrillar feelers thrown out by the leucocytes in the direction of the red cells. The red cell either moves towards the leucocyte or the leucocyte moves towards the red cell if this happens to be the more fixed body. (Fig. 29.)

Erythro-leuco-agglutination.

If a drop of a suspension of non-toxic sheep red cells, previously washed in normal saline, be incubated in a closed cell with two drops of human (C.J.B.) blood,[13] many of the leucocytes in the film so obtained will be covered with sheep's red cells closely adhering to the leucocyte.

The agglutinated red cells are easily recognised as sheep and not human cells from their smaller size.

Under favourable conditions and with higher magnification two further facts can be ascertained.

The red cells not only adhere to the leucocytes, but they are frequently deformed into a pear shape with the stalked end of the pear in contact with the leucocyte.

Sometimes, if the serum is agglutinative to the particular red cells, those which float freely in the serum away from the leucocytes may also clump together. Erythro-erythro-agglutination will then co-exist with erythro-leuco-agglutination in the same blood preparation.

Microscopic observation of living leucocytes, especially under dark ground illumination, shows that one or more of the numerous dendritic processes which are thrown out by the cell establishes contact with a red cell—it may

be at some considerable distance, perhaps 20 to 30 μ. Occasionally a main fibre or pseudopod will advance a certain distance and then alter its course by a curve or sharp bend, so as to arrive in the vicinity of a red cell. Actual contact is then established by the fibre or fibrils bending round and closing in on the red cell. Cell granules can often be seen scattered along the interior of these extended fibres up to the fine extremity in contact with the red cell. These granules may be fusiform instead of circular in shape, as though moulded by the narrow channel in the fine tubule along which they travel. (Figs. 27 and 29.)

As soon as effective contact has been established, adhesion and fusion takes place between the leucocyte fibrils and the surface of the red cell. The red cell sometimes becomes coated with a viscous material which can be drawn out into threads.

This mutual stickiness between red cells and leucocytes is in marked contrast to the indifference and ease with which native red cells roll over and round native leucocytes in incubated films of normal blood, in which there is no phagocytic activity.

As soon as it has been even partially ingested, the red cell begins to lose its characteristic yellow colour through abstraction or destruction of its hæmoglobin.

Non-toxic sheep red cells which have been ingested by human leucocytes appear as decolourised circles or "ghost" cells. Nothing, apparently, is left of the red cell but the waxy decolourised envelope, which appears as a bright ring within the cytoplasm of the leucocyte.

The presence of these decolourised "ghost" cells within a leucocyte affords valuable evidence of the phagocytic activity of that cell towards any given red cells.

These red cell remains not only show up in fresh films of leucocytes (which have been incubated with

ingestible red cells) after staining with 1 per cent. iodised saline, but they can also be recognised as pale patches or vacuole-like and unstained areas in dried and fixed films from the same blood after staining with Leishman and other stains. (Figs. 30 and 31.)

In blood taken from patients with acute pneumonia and some other diseases in which there is a tendency to auto-erythro-erythro-agglutination and auto-erythro-leuco-agglutination,[14] leucocyte films obtained by incubating such blood in a closed cell will give a number of leucocytes which contain the remains of digested red cells.

Certain Changes in the Shape of Leucocytes which follow the Ingestion of Red Cells.

If a leucocyte film obtained by incubating human blood with either (*a*) non-toxic or opsonised *sheep* red cells or (*b*) foreign or opsonised *human* red cells be examined in weak iodised saline, those leucocytes which have agglutinated to themselves and have ingested a number of red cells will assume a more or less circular outline, while cells not so engorged will still exhibit the irregular outline characteristic of pseudopodial activity.

There seems to be some association between the globular shape and the engorged condition in these fully fed phagocytes.

We know that the globular form is characteristic both of the inactive condition which the leucocyte assumes in the circulating blood-stream, and of the devitalised condition, such as that which follows emigration in a highly toxic wound. The dead pus cell is circular in outline.

Leucocytes which have ingested numbers of toxic red cells or virulent organisms also show signs of poisoning.

They cease to put out pseudopodia or fibrils. They become stationary. They assume the globular form. They stain badly with iodine and other stains. They show, in fact, signs of commencing cytolysis. Further, as we shall see later, their effective sphere of influence, as shown by the reduced amount of " Diffusion " or " Iodophil " substance which they secrete, is also diminished.

This assumption of the circular form by fully fed leucocytes is liable to cause some misconception in certain cases.

Thus it sometimes happens that when blood from a case of acute disease like pneumonia is incubated, many of the leucocytes in the film so obtained will look at first sight like swollen red cells. Each leucocyte has a red cell seated on and accurately fitting its upper surface; the leucocyte has taken the form of the round red cell, the contour of which it has accurately followed in applying itself to the cell. The actual presence of the red cell on the leucocyte can sometimes only be detected by noting the projection of some processes or fibrils from the leucocyte extending beyond the spherical border of the red cell.

Such are some of the physical or physico-chemical changes which take place in leucocytes during the process of erythro-leuco-agglutination, the process by which the leucocyte establishes effective contact with the red cell preparatory to ingestion.

We must now consider some of the more peculiarly chemical factors concerned in phagocytosis.

SECTION XI

THE "DIFFUSION" SUBSTANCE SECRETED BY LEUCOCYTES AND ITS RELATION TO THE "IODOPHIL" SUBSTANCE PREVIOUSLY DESCRIBED

In previous communications[14] it has been shown that in normal blood drawn direct from the finger and incubated in a closed cell, many of the cells which form the leucocyte film contain droplets or they exude globules of a colloidal material lying just without, but attached to the cell. This liquid substance has a great affinity for iodine, and when treated with an iodised saline solution, stains a mauve or red-brown colour, according to the degree of its concentration.

I have called this material "Iodophil" substance in order not to prejudge its chemical composition. It has some of the properties of glycogen.

If, however, instead of using iodine alone, a living leucocyte film (especially one obtained from blood incubated with foreign red cells) is rapidly fixed (without drying) with a few drops of hot normal saline just below the boiling-point, and is then treated for half an hour with a 0·05 per cent. solution of benzidine base in normal saline to which a few drops of acetone have been added, and then examined (preferably by D.G. illumination) in saline, many of the leucocytes, especially those which contain ingested red cells, will be surrounded with a precipitated substance, which can also be seen as granules in the cytoplasm of the cells.

Further observation shows that this substance which is secreted by the leucocytes reacts with the benzidine to form an insoluble compound.

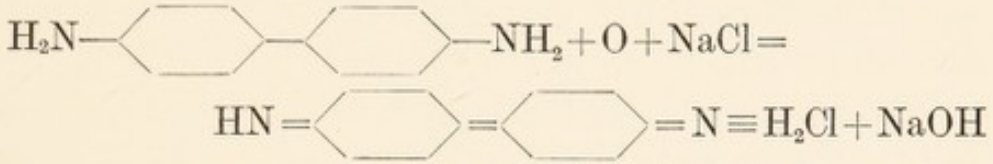
This condensation product can be converted into a coloured substance by washing away from the film the uncombined benzidine solution with normal saline, and then adding to the film a $\frac{1}{2}$ per cent. solution of sodium bichromate in normal saline. This oxidises the benzidine precipitate compound and turns it blue.

A better method is to use a 1 per cent. solution of iodine in normal saline. This carries the oxidation of the benzidine compound a stage further, and it becomes black or brownish-black in colour.

The two reactions may be represented thus:

1. The Bichromate Reaction.

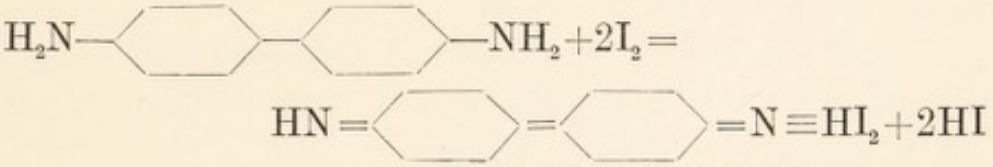
Benzidine.



Blue Oxidation Product.

2. The Iodine Reaction.

Benzidine.



Black Oxidation Product.

These oxidation products of benzidine can be also readily produced in a test-tube by adding the bichromate solution or the iodine or ferric chloride to a solution of benzidine base. It is, of course, the substance formed in the well-known benzidine blood test, the oxidising substances used there being hydrogen peroxide and hæmoglobin.

The use of iodine as the oxidiser also has the advantage

of greater permanence, it stains the cytoplasm of the leucocyte yellow, and any "Iodophil" substance that may be formed after the removal of the benzidine solution a pinkish-red.

Under favourable conditions a leucocyte film so fixed and treated with benzidine and iodine shows a number of active cells elongated in form, some with larger pseudopodial processes, and some surrounded with finer fibrils. The cytoplasm of these cells is stained a brownish-yellow by the iodine, and the multi-lobar nuclei of the polymorphs, the kidney-shaped nuclei of the mono-nuclears, and the fusiform nuclei of the eosinophil cells can be readily distinguished.

Many of the cells, especially the polymorphs, contain numerous brown-black granules which stand out against the yellow background of the cytoplasm.

In some cells these black granules aggregate together and coalesce into rounded or irregular-shaped black masses.

In others the cytoplasm shows at its circumference one or more caps of this black material, while the remainder of the cell is free from deposit.

Small black dots or granules of the same material are occasionally seen along the line of one or more of the pseudopodial processes or fibrils.

Those cells which contain the remains of ingested red cells often show signs of commencing disintegration. The black granules escape from the cytoplasm and form a cloud-like deposit of black material which surrounds the cell.

Other cells show more advanced autolytic changes and rupture, the disintegrated body of the cell being barely visible in the centre of the black cloud. (Figs. 32 to 35.)

The Nature of this "Diffusion" Substance which combines with Benzidine and is coloured Black by Benzidine and Iodine.

Although its exact chemical nature has not yet been accurately determined, this substance which diffuses from the leucocytes is probably aldehydic in character. If so, it may be derived from the digestion of the glycogen or preglycogen granules in the leucocytes. Laboratory experiments show that certain bodies of the aldehydic series—*e.g.*, formaldehyde, furfural, and perhaps other substances—combine with benzidine to form insoluble compounds which, when collected and washed, can be oxidised into blue or black compounds resembling that formed in the leucocyte film under similar conditions.

The limitation of the black deposit to the interior of, or to the immediate neighbourhood of the leucocytes depends on the fact that the uncombined benzidine left in the solution (after the permeation of the cells by the benzidine base) has been washed away by a stream of saline before the iodine is added to the film.

The *black* colour produced by the oxidation of the benzidine precipitate by iodine and the *blue* colour produced by oxidation by the sodium bichromate depend on the stage to which the oxidation is carried. The more permanent character of the black-coloured iodine deposit probably depends on its greater insolubility.

The Relation of the "Diffusion" Substance which is coloured Black with Benzidine and Iodine, to the "Iodophil" Substance coloured Mauve or Pinkish-Red with Iodine alone.

The two substances resemble each other in physical characters. Both take the form of a granular deposit, or

an exuded colloidal mass, or liquid droplet, which, although slowly soluble in the surrounding watery medium, remains for a time attached to the leucocyte.

The preservation of the exuded material as a mass or droplet of fluid can be aided by altering the density of the medium, by the addition of gum or some other mucoid substance. Thus the Iodophil substance secreted by the leucocytes in the muco-purulent urethral discharge following gonorrhœa remains preserved in the iodised mucoid medium for some weeks as large coalescent globules of red-brown material, which show characteristic notched edges. (See Fig. 12.)

In the same way the "Diffusion" substance which combines with benzidine and is stained black by the addition of iodine tends to aggregate into globular masses, and dissolves slowly in the surrounding medium on standing for some time.

The "Iodophil" substance gives some reactions characteristic of glycogen. The black "Diffusion" substance may be a further product of carbohydrate cleavage. It may be that the pink "Iodophil" globule represents the mucoid moiety and the black "Diffusion" substance the aldehydic moiety of a single complex body.

The elaboration by the leucocytes of both substances seems to be equally favoured by any stimulation of leucocytic activity during incubation as by the addition of foreign or opsonised red cells. The ingestion of foreign red cells brings about commencing cytolysis in the engorged leucocytes, with a tendency to disruption of the cell and the disappearance of the cell granules.

It is true that in some leucocyte films a few cells may be seen which contain black "Diffusion" substance granules, and also an exuded globule or globules of mauve-coloured "Iodophil" substance. This coexistence of

"Diffusion" substance and "Iodophil" substance, giving different staining reactions in the same cell, may depend on the fact that the exudation of the "Iodophil" substance occurred after the benzidine had been washed away from the film and before the addition of the iodine.

Previous fixing of the film by hot saline certainly favours the formation of the black deposit after treatment with benzidine and iodine. It does not seem to have the same marked effect on "Iodophil" substance treated with iodine alone, though it renders this more concentrated and gives it a deeper pink colour. The moist heat may, by hydrolysis, dissociate the "Diffusion" substance from the cytoplasm of the cell.

The Nature of this "Diffusion" Substance: Is it an Oxydase?

It may be supposed that this "Diffusion" substance now described as secreted by stimulated leucocytes is merely one of the many oxydases which are present in leucocytes, as in many other living cells, and that the oxydase becomes more active as the cell undergoes cytolysis. The following observation, however, does not support this simple conclusion:

If a living, unfixed leucocyte film be treated with a solution of benzidine base in normal saline and with peroxide of hydrogen, some of the cells will show a few fine blue granules in the cytoplasm.

The same blue granules can be seen in the unfixed film when sodium bichromate is used to oxidise the benzidine.

This deposit of the blue benzidine compound in the presence of oxygen does, no doubt, indicate the presence of some oxydase in the cytoplasm of certain leucocytes.

But if the leucocyte film be fixed by boiling saline solution before the benzidine and oxygen are added, no blue granules appear; the cytoplasm of the cells remains colourless, as, indeed, we should expect, knowing the effect of boiling on oxydases.

It is, on the other hand, in the film "fixed" by boiling saline solution that the black deposit formed by the "Diffusion" substance with benzidine and iodine is chiefly found.

Further, the droplets of the mauve-coloured "Iodophil" substance appear in the fixed as in the unfixed film on treatment with iodine alone without benzidine. This we should expect if the two substances represent the same material in a different stage of hydrolysis. Hence the "Iodophil" substance and the "Diffusion" substance do not represent merely one of the many oxydases present in living cells.

The Action of the "Diffusion" Substance on Red Cells and its Influence on Erythro-leuco-agglutination and Phagocytosis.

Under favourable conditions in leucocyte films obtained from blood incubated with foreign or opsonised red cells, after treatment with benzidine and sodium bichromate or iodine, some of the red cells which have come in contact with, or have been agglutinated to, or ingested by leucocytes will themselves be stained blue, or will be stippled with blue or black patches, while the red cells which float in the medium away from all contact with the leucocytes retain only the yellow iodine stain.

This colouring or coating of the red cells suggests that the "Diffusion" substance secreted by the leucocytes and liberated by them into the surrounding fluid combines with or forms an additive compound on the surface of

those red cells with which it comes in contact, and prepares them for ingestion by the leucocytes.

Experiments have also been carried out with yeast cells. These, when washed in normal saline, are toxic to human leucocytes when incubated with whole blood in a closed cell. If the yeast cells, however, have been previously washed in an isosmotic solution of lithium chloride they become ingestible. Leucocyte films so incubated and then fixed with hot saline, if subsequently treated with benzidine, iodine, and erythrosin, show under favourable conditions yeast cells which have been ingested by, or acted on by, leucocytes as black bodies, while the yeast cells floating free in the medium are stained pink.

SECTION XII

THE "DIFFUSION" SUBSTANCE IN EPITHELIAL AND SOME OTHER CELLS

In previous communications[15] I have shown that the "Iodophil" substance formed by leucocytes is also present in certain young and functionally active epithelial cells in the Malpighian layer of the skin, and especially in the epithelial covering of the chief mucous channels entering the body, the lips, mouth, tongue, naso-pharynx, rectum, and genito-urinary passages. (Fig. 36.)

If the "Diffusion" substance, which is also liberated by leucocytes during phagocytosis, is identical with or closely related to the "Iodophil" substance previously described, then this "Diffusion" substance should be also present in epithelial cells under similar conditions.

In Normal Epithelial Cells.

If the urethral mucous membrane just within the meatus be gently swabbed with a probe covered with cotton-wool soaked in normal saline, the surface epithelial cells so obtained can be transferred to a drop of saline on a slide, and after fixing in boiling saline, can be treated with benzidine and iodine in the manner described when dealing with leucocytes.

Some of the epithelial cells so treated will show black granules or a black cloudy deposit in the cytoplasm; others will give the mauve or pink-brown colour characteristic of "Iodophil" substance, while in other cells the black deposit and the mauve-coloured substance may be present together.

This suggests that the material which gives the black colour with benzidine and iodine may be the same substance as that which gives the pink-brown colour with iodine, but perhaps in another phase or more fully hydrolysed condition.

In Cancer Cells.

The occurrence in large amount of "Iodophil" substance in some cancer cells of epithelial origin—*e.g.*, epithelioma of the lip and tongue—and in the cancer cells which have invaded the lymph glands, draining the affected area, has previously been described.[15] (Figs. 37 and 39.)

The same substance has also been demonstrated in the large multi-nucleated cells found in the so-called myeloid sarcomata. (Fig. 38.)

Although these latter cells are not epithelial in origin, but appear in meso-blastic tumours, they have some generic relation to and community of descent with the marrow cells, from which the mother cells which give rise to the leucocytes also spring.

It is therefore a matter of interest, both from the point of view of cell heredity in general and of the ancestry of the cancer cell in particular, to find that "Iodophil" substance is elaborated (as far as present observation goes) only by those cells in sarcomatous growths which arise from marrow cells from which also the leucocytes derive their origin.

The "Iodophil" substance is not found in the stroma cells of connective tissue origin which form the ground substance of these growths, nor is it found in the connective tissue cells of the skin, but only in certain epithelial cells which form the covering of the papillæ.

In a case of epithelioma of the floor of the mouth, with extensive secondary deposits in the cervical lymph glands, scrapings form the glands, examined in iodised saline and iodised gum solution, showed a number of cancer cells which contained in their cytoplasm *black* granules and *black* deposit, although in this case no benzidine and only iodine had been used. (Fig. 40.)

Here, apparently, a substance had been formed in the cancer cells which stained black instead of yellow with iodine. The black colour in this case was not due to the presence of any iodide of benzidine or other oxidised benzidine compound, but to some phase of the "Diffusion" or "Iodophil" substance which gave a black reaction instead of a mauve reaction with iodine alone suggestive of starch.

In regard to this point the following observation is of interest:

If some of the material which collects at the edges of the gums, or from the cavity of a carious tooth, be examined on a slide in iodised saline, a sample of the bacterial flora of the mouth will be obtained, but among the many kinds of cells and organisms which stain yellow with the iodine, a bacterium may occasionally be found, singly or in

chains, which stains a deep *blue-black* with the iodine in the saline. These organisms which give this blue-black colour with iodine have not at present been identified culturally or morphologically.

The fact, however, that they give the *blue-black* reaction with iodine characteristic of starch suggests that they may contain carbohydrate material in some form.

These cancer cells in the lymph glands, while giving the blue-black colour with iodine, had come originally from cancerous tissue growing in the floor of the mouth, exposed to the action of the saliva and other buccal secretions to which the blue-black staining bacteria had also been exposed.

The occurrence of the same colour reaction with iodine in certain bacteria and in some epithelial cancer cells suggests the presence of a similar carbohydrate substance in both cases. This substance may be the same or closely allied to the substance which has now been described as "Iodophil" or "Diffusion" substance, and which is formed by stimulated leucocytes, by normal epithelial cells, by epithelial cancer cells, and by the multi-nucleated cells of myeloid sarcomata, and by certain marrow cells.

The "Diffusion" Substance in Pus Cells.

When describing the pseudopodial and phagocytic activities of living pus cells, the fact that these cells secrete considerable quantities of "Iodophil" substance was mentioned.

If a few drops of fresh sero-pus from a healthy wound or the washed pus cells from a slightly infected wound are incubated in a closed cell, numbers of the pus cells which adhere to the slide will contain droplets of "Iodophil" substance, or they will show exuded globules of the same substance in the surrounding medium.

Further, if such a film of living pus cells be "fixed" with hot saline and then be treated with benzidine and iodine in the way previously described, many of the cells will contain the black granules or the black deposit characteristic of the "Diffusion" substance formed by leucocytes under like conditions.

The living pus cells are, of course, emigrated leucocytes, mostly polymorphs, and they retain the physical and the chemical capacities characteristic of such cells.

SECTION XIII

THE TOXIC ACTION OF SHEEP BLOOD-SERUM ON HUMAN LEUCOCYTES

If a drop of the fresh serum pipetted off defibrinated and centrifuged sheep's blood be incubated in a closed cell with two drops of human blood, the sheep blood-serum poisons the human leucocytes. Very little emigration takes place from the clot, and the cells show signs of cytolytic degeneration.

Unlike the toxic effect of certain washed sheep red cells, this leuco-toxic action of sheep blood-serum is present in the blood of *all* sheep so far examined.

Heating the serum for one or more hours at a temperature of 56° to 60° C. greatly diminishes the toxic effect.

Saturating the serum with repeated doses of washed human red cells and pipetting off the serum after each dose also removes the leuco-toxicity.

It is also diminished by allowing the serum to stand for forty-eight hours in contact with native sheep red cells.

A control sample of serum pipetted off the centrifuged

red cells immediately after defibrination, and allowed to stand forty-eight hours, retained its leuco-toxicity.

The leuco-toxicity is also diminished by CO_2 gas. Serum through which CO_2 or alveolar air has bubbled intermittently for some hours is less toxic than control serum.

SECTION XIV

THE TOXIC EFFECT OF WASHED SHEEP RED CELLS ON HUMAN LEUCOCYTES

The toxic effect of the red blood-cells obtained from the defibrinated blood of certain individual sheep after removal of the serum has been previously alluded to.[16]

If a drop of a suspension of sheep's red cells previously twice washed in normal saline be incubated with two drops of human blood, the leucocytes do not emigrate from the clot, and such few cells as do adhere to the slide show marked cytolytic changes.

This leuco-toxicity of the washed red cells from the defibrinated blood is present in a majority, though not in all sheep.

The effect ranges from marked poisoning, in which the leucocytes are cytolysed and no emigration occurs, through intermediate stages in which a few leucocytes are seen on the slide with a few red cells adhering to them, to a smaller non-toxic group, in which large numbers of leucocytes migrate, and many are coated with sheep red cells, some of which are ingested.

Why different sheep should vary in the leuco-toxicity of their red blood-cells after the serum has been removed by washing is unknown. I first thought it might depend

on fatigue, or over-driving, or prolonged fasting before the animals were killed, or possibly that it might be associated with some dietetic factor connected with the change from winter to summer pasturage. Up to the present, however, no such causal factor has been discovered. The variability is, however, of interest when considered in relation to the effect of disease in the human subject. Disease frequently heightens the leuco-toxicity of human serum to human leucocytes, or it may opsonise human red cells (probably through the serum) and render them ingestible by native leucocytes. Reasons will be advanced later for thinking that the effect on the red cells induced by disease is conveyed to the red cells by the altered serum.

The appearance presented by a film of human leucocytes engorged by the phagocytosis of sheep's non-toxic red cells is very striking. Some of the leucocytes contain ingested red cells, while larger numbers are surrounded with a ring or coating of adherent sheep red cells, which can be distinguished from the human erythrocytes by their smaller size. From their peculiar appearance I have called these leucocytes which are surrounded by adherent red cells "mulberry cells." (See Fig. 21.) The agglutination of the red cells to the leucocytes is not the result merely of clumping of the sheep's red cells by the human serum; my own serum is non-agglutinative to sheep's red cells. It is the outcome of a true erythro-leuco-agglutination, some of the factors concerned in which have already been described.

Seeing that sheep's red cells which are toxic to human leucocytes retain their leuco-toxicity after twice washing in normal saline solution, it becomes important to test the effect of washing the same red cells in isotonic solutions of other salts.

The following were used:

	Per Cent.	*Toxicity of the Salt to Human Leucocytes in the Concentration Used.*
Sodium chloride	0·9 (standard):	– Tox.
Lithium chloride	0·64	– Tox.
Potassium ,, ...	1·15	V.S. Tox.
Magnesium ,, ...	3·1	,, ,,
Calcium ,, ...	3·4	Slightly Tox.
Barium ,, ...	3·75	Tox.
Strontium ,, ...	4·1	Tox.

Seeing that the calcium, barium, and strontium salts used in the concentration necessary for isotonicity with the red cells are highly poisonous to human leucocytes, it also became necessary to rewash the red cells so treated in normal saline solution, and thus standardise the result of the treatment and carry out the incubation with the leucocytes under comparable conditions.[32]

It was found in general that the chlorides of lithium and potassium standing nearest to the sodium salt had little effect in reducing the leuco-toxicity of the sheep's red cells, the chlorides of the alkaline earths Ca, Ba, Str diminished it, while magnesium chloride stands in an intermediate position, sodium chloride 0·9 per cent. being taken as the standard.

Different samples of sheep's red cells, however, gave different results with the same salt. In most samples of red cells some one (or more) salt was found, previous washing with which, followed by subsequent washing with normal saline reduced the toxicity of the red cells suspended in it. In the case of one very toxic sample of sheep red cells lithium chloride was the only salt which materially reduced the toxicity to human leucocytes.

Sheep cells also differ from human red cells in their reaction to other substances. The effect of previously washing *sheep* red cells in an isotonic solution of sodium hippurate is to increase the toxicity of the cells to human

leucocytes, while washing *human* red cells (previously ascertained to be toxic) with the same solution renders them less toxic to the same cells.

As elsewhere mentioned, there are reasons for regarding this leuco-toxicity of the washed sheep's red cells to human leucocytes as the outcome of some interaction between the red cells and the sheep's blood-serum.

In the case of the hæmagglutination reaction, Calvin and Coulter state[17] that the hæmagglutination of the red cells is intimately associated with the precipitation of the euglobulins of the blood-serum. "A condensation of the protein euglobulin occurs on the surface of the red cells."

I have shown that the repeated washing in normal saline of previously hæmagglutinated red cells removes the additive substance which glues the cells together, and renders them reagglutinable by a fresh dose of the same serum. The cells are shaken apart fairly easily.

The leuco-toxic substance, on the other hand, which is formed on the red cell by interaction between the cell and a toxic serum is removed less easily, and, as we shall see later, this applies also to the material which coats the red cells after treatment with an opsonic serum, and which renders such treated cells more easily ingested by native leucocytes.

SECTION XV

THE LEUCO-TOXIC ACTION OF SHEEP BLOOD-SERUM ON HUMAN RED CELLS

The toxic effect which sheep blood-serum exercises on human leucocytes, incubated with it, can be transferred under special conditions to human erythrocytes, thus: If washed human red cells are allowed to stand for one

hour in the toxic blood-serum of certain individual sheep, the human red cells so treated when rewashed with normal saline become toxic to the native leucocytes when a suspension of the treated red cells is incubated with the same human blood.

Effect of Heat.

If the sheep's serum, however, has been previously detoxicated by heating for one hour or more at a temperature of 58° to 60° C., then the human red cells treated with it are opsonised and made ready for ingestion by the same native leucocytes.

Red cells so treated no longer repel and poison the leucocytes, but are drawn to and ingested by them.

This brings us to the important problem of the opsonic action of sheep blood-serum and of blood-serum in general.

SECTION XVI

THE OPSONIC EFFECT OF SHEEP BLOOD-SERUM ON HUMAN RED CELLS

We have already seen that the leuco-toxic effect of fresh sheep blood-serum on human leucocytes can be handed on to human red cells, whereby red cells so treated also become leuco-toxic.

We have also found that the erythro-toxic, like the original leuco-toxic, capacity can be removed by heat, and that sheep serum so treated loses its toxic capacity and becomes opsonic to the red cells.

The same opsonic capacity is present in the fresh blood-serum of certain individual sheep, if not too leuco-toxic.

It can be shown thus:

One volume of a suspension of human (C.J.B.) red cells previously washed in normal saline is allowed to stand for one hour or more in sheep blood-serum diluted with an equal volume of normal saline.

The serum is then pipetted off and the red cells are washed twice in normal saline.

One drop of this suspension of the rewashed red cells is then incubated with two drops of human (C.J.B.) blood in a closed cell.

The C.J.B. leucocytes in the film so obtained show signs of stimulation and exhibit increased pseudopodial activity. They agglutinate to themselves many native red cells, and they ingest a number of them.

This phagocytosis of opsonised native red cells becomes even more marked if the red clot be detached from the slide, rewashed in saline, and reincubated on a clean slide with a second dose of the opsonised red cells. (Fig. 41.)

The removal of the serum facilitates phagocytosis.

The opsonic effect of sheep serum on human red cells, although not entirely, is markedly specific in action.

Thus, if a drop of the suspension of C.J.B. red cells so treated be incubated with the blood of another individual, the signs of leucocytic stimulation and the phagocytosis of the foreign red cells are much less marked.

When dealing with the effect of acetone extract of red cells, facts will be given which suggest that the specificity of the above reaction is due to the deposit of an additive substance, having specific qualities, on the surface of the red cells from the sheep's blood-serum.

Carrell[18] found that heated blood-serum made a better culture fluid for heterologous than for homologous fibroblasts. It would seem that fresh serum contains some substance which is injurious to native tissue cells and

native red cells, and that this substance is removed by heating. The hæmo-opsonins for human red cells which exist in the blood-serum of certain individual sheep, like the corresponding hæmo-agglutinins (but unlike the leuco-toxins), are thermo-stable.

Heating for one hour or longer at a temperature of 56° to 60° C. does not destroy, but increases, the opsonic capacity of the serum.

As we have already seen, heating may convert a serum which in the fresh state is too leuco-toxic to be opsonic when used with human red cells, into an opsonic serum.

The hæmo-opsonins, like the hæmo-agglutinins and the hæmo-toxins, can be removed by treating the serum with repeated doses of washed human red cells.

The capacity to opsonise human red cells to human leucocytes does not depend on the power to hæmagglutinate the same red cells. Thus a certain sheep's blood-serum may opsonise human red cells without agglutinating them, while another serum may be strongly hæmagglutinative but non-opsonic to the same cells.

There seems, however, to be some association between the opsonic and the hæmagglutinative action.

Both are removed by saturating the serum with repeated doses of red cells at about the same stage of saturation.

But while the hæmagglutinins are present in full strength in the fresh, unheated serum, the hæmo-opsonins are developed by heat and increase in a serum which has been pipetted off and allowed to stand at room temperature in the absence of red cells.

It is clear, however, that some interdependence exists between the leuco-toxic action of sheep's blood-serum on human leucocytes and the opsonic effect of sheep blood-serum on human red cells.

Thus a highly leuco-toxic serum may render human red cells (treated with it) also leuco-toxic to the same leucocytes even after they, the red cells, have been washed and the toxic serum has been removed. Whereas the same serum after heating (or a less leuco-toxic fresh serum), instead of making the red cells toxic to native leucocytes, opsonises them and prepares them for ingestion. The opsonic would therefore seem to be a diluted or mitigated phase of the leuco-toxic action, the toxin being converted into an opsonin by heat.

I have already stated that only the fresh blood-serum of certain individual sheep has the power to opsonise human red cells.

Samples from the blood-serum of twenty-nine sheep were tested; of these, ten were opsonic and fourteen non-opsonic to the same human red cells. Of the non-opsonic group, thirteen rendered the red cells toxic to the leucocytes and three other samples hæmolysed the human red cells.

Hæmo-opsonins, like hæmagglutinins, seem to be absent from the blood-serum of young lambs up to the age of about three months.

At three months they begin to appear in the blood of individual lambs.

On the other hand, the washed red cells of quite young lambs (like the washed red cells of many adult sheep) are toxic to human leucocytes.

Guinea-pigs' blood-serum, in the case of individual guinea-pigs, shows a slight opsonic effect on human red cells.

Fowls' blood-serum has no opsonic effect, except very slightly after heating.

Ox serum is very slightly opsonic, moderately toxic, and frequently hæmolytic.

This opsonic action is not limited, of course, to the blood-serum of animals; it is also present in the blood-serum of certain human individuals, as we shall see when dealing with disease.

SUMMARY OF SECTIONS XIII., XIV., XV., XVI.

Thus the evidence points to a graded effect. Sheep's blood-serum is toxic to human leucocytes, and in certain individual sheep renders washed human red cells treated with it toxic to native leucocytes. If heated, however (or if the less toxic fresh serum from certain individual sheep is used), it may opsonise the red cells to the same leucocytes.

A substance (or substances) is apparently formed on the surface of the red cell, as the result of a reaction between red cell and serum, which in one form, possibly a too concentrated or unoxidised form, repels and injures, and in another form, possibly a more dilute or more fully oxidised form, attracts the same leucocytes.

This explanation of the formation of an opsonic out of a toxic material and its deposit on the surface of the red cell, from which it can be washed away or removed by appropriate treatment, seems to be supported by evidence.

Another explanation would be that the action of the toxic serum consists, not in the formation and deposit of a toxic substance on the surface of the red cell, but in the removal of a protective surface covering from the red cell by the serum. This removal allows the hæmoglobin and the other globulin contents of the red cell (representing its endo-toxin) to exude, and to come in contact with and poison the leucocyte.

In the same way opsonisation would consist, not in the deposit of a less toxic substance as an attractve coating

on the red cell, but in a less complete or a partial denudation by which the contained endo-toxin can escape in less volume or in a less concentrated and a less poisonous form.

It is not suggested that escaped hæmoglobin by itself is poisonous to the leucocytes. Observation shows that it is not, but the iron in the hæmoglobin is accompanied with certain globulin constituents which, there is evidence to show, may act as a foreign protein, and thus bring about a state of leuco-toxæmia analogous to that produced in anaphylactic shock by the injection of horse serum.

The mechanism by which toxic effects on the one hand, and opsonic effects on the other, are exercised on human red cells by the same serum under different conditions is also paralleled to a certain extent by the action of the same serum on sheep's red cells.

If a substance (toxic or opsonic) be formed on the surface of red cells by appropriate serum treatment, we might expect to recover this substance in solution by washing the treated red cells in normal saline.

Experiment shows that while the hæmagglutinins can be so removed, the hæmo-toxins and the hæmo-opsonins are not so easily dealt with. This may be due to the fact that the latter are less soluble in normal saline, or less easily detached from the red cell.

There are some suggestive facts, however, which bear on this point which will be described in the next section.

SECTION XVII

THE ACTION OF ACETONE ON RED CELLS AND OF ACETONE EXTRACT OF RED CELLS ON LEUCOCYTES

FOLLOWING on the publication of Professor Dreyer's address on diaplyte vaccines,[29] I endeavoured to test the formaldehyde acetone method of extracting lipoidal substances on red blood-cells.

It was found[19] that if sheep's washed red cells (previously found to be toxic to human leucocytes) were allowed to stand for some hours in formaldehyde 1 per cent. solution in normal saline, then washed and placed in acetone 5 per cent. solution in normal saline, and then again rewashed in normal saline to remove the free acetone, and subsequently incubated with the same human blood, the red cells so treated lost their leuco-toxicity.

Treatment with formaldehyde alone does not detoxicate the red cells; its value seems to consist in holding the red cells together while the acetone acts on the enveloping membrane and extracts the lipoidal substance. The degree to which the extracting process should be carried is determined in the case of the red cell by the occurrence of hæmolysis. This should be avoided, the object being to retain the original shape and general constitution of the red cell.

The detoxication of the red cell by acetone seems to depend on the removal (as we shall see later) of some substance from the red cell which remains in solution in the acetone liquid.

If washed human red cells are used instead of sheep's red cells, and the acetone extract so obtained is evaporated almost to dryness, to drive off the acetone, and the deposit

is redissolved in a volume of water equal in volume to the acetone solution before evaporation, an extract of human red cells in normal saline is obtained which exercises an opsonic effect on human red cells.

If one drop of this extract in normal saline is incubated with two drops of blood from the individual supplying the red cell extract, the leucocytes will show signs of phagocytic activity towards their own native red cells. Many leucocytes will be surrounded with a ring of agglutinated red cells, and a certain number will contain ingested red cells. The extract opsonises the red cells to their native leucocytes. (Fig. 42.)

If, however, a drop of the same extract be incubated with the same quantity of the blood of another individual, less opsonic effect on the red cells will be produced.

Further experiments will be undertaken to test this specific character of the acetone extract of red blood-cells, and to ascertain whether it is individual in action and limited to the red cells and leucocytes of the same person, or whether it extends to the blood group to which that individual belongs.

The extract not only specifically opsonises the red cells in the presence of native serum when incubated with the native blood, but if washed red cells are treated with the extract in solution and then rewashed in saline they too are opsonised, and when incubated with native blood they are ingested by the leucocytes.

Thus toxic sheep's red cells are detoxicated and human red cells are opsonised by acetone extract from corresponding red cells.

But sheep's red cells so detoxicated can be again rendered toxic to human leucocytes by resuspension in the toxic sheep's blood-serum. After rewashing to remove

the serum they again become toxic when incubated with the human blood.

Guinea-pigs' red cells, on the other hand, which have been detoxicated by treatment with formalin and acetone are not made retoxic by treatment with the *same* sheep's serum.

These facts suggest that the toxic capacity of the red cells depends on the formation by the serum of an additive compound on the surface of the red cells which is specific in character.

Treatment of red cells by the acetone method also increases their agglutinability (after rewashing) to an appropriate or agglutinating serum, and may, under certain conditions, render red cells auto-agglutinable—that is to say, they clump together when treated with native serum.

The agglutinin which reacts with the agglutinogen of the red cell is dissolved in the serum, and the substance which is formed by the reaction can be removed from the surface of the clumped red cells by agitation in normal saline. The close relationship which exists between hæmo-agglutinins and hæmo-opsonins would suggest that the hæmo-opsonic substance could also be removed from the red cell in the same way; the latter, however, is much more difficult to remove.

It is a suggestive fact that the change which is brought about on the surface of a red cell by opsonisation also increases the hæmagglutinability of the same cell.

The importance of the interactions which take place between the blood-serum and the red cell, and the highly specific character of this reaction in relation to leucocytic activity, become evident from these observations.

It may be that the position of stable equilibrium present between the red cells and the leucocytes in the living blood is also the result of a reaction between the red cells

and the blood-plasma, whereby protective additive substances are deposited from the plasma on the surface of the red cells, which protect these cells against leucocytic attack under normal conditions.

Acetone Extract and the "Diffusion" Substance.

Washed sheep's red cells after treatment with a 5 per cent. acetone solution in normal saline are rewashed in normal saline, and a drop of the suspension of red cells so treated is incubated with human (C.J.B.) blood.

The leucocyte film so obtained, after fixation in boiling saline, is then treated with benzidine, followed by iodine, in the manner previously described. A striking picture of the stimulating effect of the opsonised sheep red cells on the leucocytes is presented. Many of the leucocytes contain numerous black granules, and much intra- and extra-cellular black "Diffusion" substance is seen. This material also coats many of the red cells which have become adherent to, and have been ingested by the leucocytes.

In a control film, obtained from the same blood incubated with the same sheep's red cells (but treated with normal saline instead of acetone solution), and then fixed and treated with benzidine and iodine in the same manner, fewer black granules are seen, and much less black "Diffusion" substance is formed, and the red cells retain their yellow colour.

Thus the presence of sheep's red cells which have been previously extracted with acetone, when incubated with human leucocytes in the presence of human blood-serum, stimulates the leucocytes to produce larger quantities of a secretion ("Diffusion" substance) which acts on the red cells and prepares them for ingestion by the leucocytes.

SECTION XVIII

ERYTHRO-TOXINS AND ERYTHRO-OPSONINS IN DISEASE

Human Red Cells.

An attempt must now be made to bring the facts ascertained experimentally in regard to sheep's red cells into correlation with the changes which occur in human red cells as the result of disease.

Attention has already been drawn to the special character of the leucocyte film which is obtained from the incubated blood from a pneumonia patient in the acute stage of the disease.

Such a film consists of a thick carpet of emigrated cells. The phagocytosis of the native red cells may be only slight in the first incubation; the reincubation, however, of the rewashed red clot with another drop of a suspension of the washed red cells from the same patient will result in a second crop of leucocytes, many of which will show marked erythro-leuco-agglutination, and will also contain ingested red cells. (Figs. 43 and 44.)

The removal of the native serum and the presence of the washed native red cells stimulates the phagocytic activity of the leucocytes.

But this increased "*aggressiveness*" of the leucocytes is not limited to native washed red cells, for if another red clot from the same pneumonic blood be incubated with foreign washed red cells from a healthy person, the pneumonia blood leucocytes will also agglutinate and ingest these healthy red cells, though perhaps in rather less degree.

The leucocytes from the pneumonic patient also show

the same phagocytic *aggressiveness* towards washed *sheep* red cells.

Thus sheep's washed red cells which are toxic to, and therefore not phagocytosed by, the leucocytes from a normal human blood are frequently freely ingested by the leucocytes which emigrate from the washed red clot obtained from pneumonia blood.

This fact shows that the increased *aggressiveness* towards native red cells acquired by leucocytes during an attack of pneumonia in the acute stage is not wholly specific in character—that is to say, it extends also to other varieties of red cells.

Further, this increased phagocytic capacity to red cells does not disappear with the crisis and the end of the pyrexial stage of the disease. It persists in some cases for three weeks, perhaps longer, and extends well into the convalescent stage.

I have previously shown[20] that the auto-hæmagglutinative reaction (that is, the hæmagglutination of native red cells by native blood-serum in the shed blood) persists for two or three months after an attack of pneumonia, and only disappears entirely after nine months. It becomes, therefore, of interest to find that the increased "aggressiveness" of the leucocytes to native red cells in the shed blood on incubation also persists for some weeks, probably months, after an attack of pneumonia.

But this increased leucocytic "aggressiveness" to red cells is not limited to pneumonia.

In a case of spleno-medullary leucæmia in which a blood-count showed a large increase in myelocytes and large lymphocytes, with a largely reduced hæmoglobin percentage volume, two drops of blood incubated in a closed cell gave the same packed leucocyte film as in the pneumonic blood, but not the same excessive production

of "Iodophil" substance as is seen in pneumonia in the acute stage.

The washed red clot, however, when reincubated with foreign (C.J.B.) washed red cells, presented a marked picture of leucocytic "aggressiveness."

Many of the large myelocytes were surrounded with rings of adherent pear-shaped red cells, and a number of leucocytes contained partially decolourised and digested red cells.

Eosinophilia.

In the incubated blood from a case of eosinophilia (70 per cent.) active emigration of the eosinophils from the blood-clot occurred. The cells, when stained with iodine, were seen packed with the same large granules which give the rosy pink colour with eosin. These cells emigrated actively upwards on to the coverslip, as well as downwards on to the slide forming the floor of the cell, and many showed a tendency to shed their granules into the surrounding medium.

These eosinophil cells showed little erythro-leuco-agglutinative tendency, neither did they contain many ingested red cells.

When, however, this eosinophilic red clot was washed and reincubated, a different picture was seen. The film now consisted of a second crop of different cells, not eosinophils, but chiefly polymorphs, with some mononuclears; many of the polymorphs contained ingested red cells.

In another case of eosinophilia (9 per cent.) the same result was obtained in a less marked form.

The washed red clot from this patient, when reincubated with a suspension of washed foreign human red cells, showed considerable phagocytosis of the foreign cells.

Thus the eosinophil cells are markedly pseudopodial

and are the first to emigrate, but they show rather less "aggressiveness" to red cells than the polymorphs.

This is important in relation to the nature and function of these eosinophil cells.

I have already stated that, while it is possible by feeding polymorphs on opsonised red cells to increase the eosin staining affinity of these cells, they are not themselves converted into true eosinophils by such diet. The eosinophils, in other words, seem to be a distinct variety or race of leucocytes, and not merely polymorphs which have acquired eosin staining granules as the result of feeding on hæmoglobin.

Pernicious Anæmia.

The "emigration picture" is different in this disease, and tends rather to resemble that seen in malaria.

The tendency to emigrate from the incubated blood-clot is less than in normal blood, even after taking into consideration the leucopenia which is often present in this disease.

There is frequently marked erythro-leuco-agglutination and ingestion of native red cells, both in the film obtained at the first incubation, as well as after the second incubation of the rewashed red clot.

Foreign washed red cells are also agglutinated, and to some extent ingested by the leucocytes in this disease.

The amount of phagocytosis of the abnormal washed native red cells in a case of pernicious anæmia by *normal* human (C.J.B.) leucocytes is difficult to determine, because these abnormal red cells, even after washing in normal saline, are frequently toxic to normal leucocytes, and very little emigration may take place when normal blood is incubated with such abnormal and toxic red cells.

SECTION XIX

ERYTHRO-TOXINS AND ERYTHRO-OPSONINS IN DISEASE

Human Blood-Serum.

In Section XV. we have seen that the heated serum from sheep's blood (and also the fresh, unheated serum from certain individual sheep) exercises a marked opsonic effect on washed human red cells, and renders them ingestible by native leucocytes.

The same is true of the blood-serum of patients suffering from certain diseases.

Thus, if normal human (C.J.B.) red cells previously washed in normal saline are allowed to stand for two hours in the blood-serum (diluted with an equal volume of normal saline) from a patient suffering from pneumonia in the acute stage, the red cells will be opsonised, and when rewashed and incubated with native human (C.J.B.) blood, they will be agglutinated to and ingested by their own native leucocytes. (Fig. 45.)

There is some evidence to show that the blood-serum from a patient with pneumonia also opsonises the *native* red cells from the same pneumonia blood, for if these red cells are previously treated in the same way with the pneumonia blood-serum, and are then rewashed and re-incubated with the pneumonia blood from the same patient, they too are ingested by native leucocytes. (Fig. 46.) This effect, however, is difficult to estimate because, as we have seen, the leucocytes in pneumonia have already been stimulated and their erythro-ingestive capacity to native red cells has been heightened even in the absence, or the partial absence, of blood-serum, as we saw in the

rewashed and reincubated red clot experiment. This opsonic effect of pneumonia blood-serum is not necessarily exercised on *all* red cells treated with it. Thus it may fail to detoxicate toxic sheep red cells to human leucocytes, or to render *toxic* human red cells ingestible by human (C.J.B.) leucocytes.

The opsonic effect of pneumonia blood-serum on foreign red cells, like the auto-hæmagglutinating action on native red cells, and the heightened "aggressiveness" of pneumonia leucocytes to both foreign and native red cells, persists into the convalescent stage of the disease. It also persists longer in relation to *foreign* than to *native* red cells.

This power of pneumonia blood-serum to opsonise foreign red cells to their own native leucocytes does not necessarily co-exist with any marked "aggressiveness" in the pneumonia blood leucocytes.

It was absent in the blood-serum of one pneumonia patient, although the leucocytes from the washed and reincubated blood-clot from the same patient showed marked phagocytic capacity towards foreign red cells.

This shows that stimulated leucocytes can, under favourable conditions, deal with native and foreign red cells with little aid from any specific hæmo-opsonins present in the blood-serum—that is, in the incubated shed blood.

Further, the loss of hæmo-opsonic capacity in the blood-serum, like the loss of "aggressiveness" in the leucocytes, occurs earlier during convalescence in relation to *native* than to *foreign* red cells.

This means that the normal biochemical equilibrium between native leucocytes, red cells, and blood-serum is re-established early in the blood-stream, and before the corresponding equilibrium with foreign red cells is reached.

Other examples of the absence of erythro-opsonins in the blood-serum co-existing with erythro-phagocytic activity of the leucocytes have been seen in other diseases.

There can, I think, be little doubt that further investigation into the incidence and co-existence of erythro-opsonins in both blood-serum and leucocytes to both native and foreign red cells in different diseases and at different stages of the same disease would throw further light on the immunity problem.

It is also important to compare the erythro-opsonic capacity of the blood-serum with that of certain transudate fluids in the same patient.

Thus in a case of enlarged spleen (Banti's disease) the blood-serum contained a substance which opsonised washed foreign red cells to their own native leucocytes. It also auto-agglutinated native red cells.

The ascitic fluid from the same patient did not opsonise foreign red cells to nearly the same extent, neither did it clump the native red cells.

Both the blood-serum and the ascitic fluid from this patient, however, contained a substance or substances which were highly stimulating to my own leucocytes. Many of the cells in the incubated film assumed a very elongated shape and showed excessive pseudopodial activity. The stimulating effect was less marked with the native leucocytes from the patient's own blood.

In another patient with jaundice and ascites, due to cancer of the liver, my own washed red cells were opsonised by the blood-serum, but were rendered toxic by the ascitic fluid to their own leucocytes.

This fact further illustrates the close association that exists between toxins and opsonins.

But the stimulating influence of foreign blood-serum on normal leucocytes not only varies in different diseases,

it also varies according to the organ or area of the circulation from which the blood is obtained. Thus, in a patient with a large spleen and splenic anæmia, one drop of serum from the blood from the splenic vein, withdrawn at the operation of splenectomy, was incubated with two drops of normal human (C.J.B.) blood. The leucocytes in the film so obtained showed marked stimulation. They assumed the elongated rod-shape, and the nuclear lobar movement characteristic of heightened activity. A second drop of the serum obtained from the blood of the finger of the same patient during the same operation showed a less stimulatory effect. The cells were more circular in outline, and there was less movement of the nuclear lobes. (Figs. 47 and 48.)

This result is of interest when considered in relation to the recorded observation[20] that the auto-agglutinative reaction between serum and red cells also differs in degree in blood samples taken from different parts of the body.

The Erythro-opsonic Action of Syphilitic Blood-Serum on Red Cells.

A preliminary investigation in a few cases suggests that the opsonic capacity of a given blood-serum to render washed foreign red cells digestible by their own native leucocytes does not depend upon a complement deviating factor such as is present in the blood-serum of syphilitic patients.

Neither, apparently, does the blood-serum from a syphilitic patient detoxicate washed toxic sheep's red cells, or render them ingestible by human leucocytes more than the blood-serum from a non-syphilitic individual.

In conclusion, we have already seen that fresh untreated sheep's blood-serum is toxic to human (C.J.B.)

leucocytes. In previous communications[21] attention has been drawn to the fact that the blood-serum of different individuals, even in health, varies in leuco-toxicity to human (C.J.B.) leucocytes.

The same is markedly true of the blood-serum in different diseases.

All gradations are found between non-toxicity and indifferent action through a stimulatory effect, in which both the pseudopodial and the phagocytic activities of the leucocytes are increased, to an inhibitory action, and finally to the poisoning and destruction of leucocytes through cytolysis.

SECTION XX

GENERAL CONCLUSIONS

THE main conclusions which emerge from these observations would seem to be:

1. The importance of the form and outline assumed by leucocytes under different environmental conditions as indications of the vitality and of the physical and chemical activity of the cells.

2. Stimulated, emigrating leucocytes are capable of protruding very fine fibrils or dendrites as well as the larger pseudopods by which cell movement is brought about.

These finer fibrils are revealed under dark ground illumination with higher magnification.

Stimulated leucocytes—that is, cells which have emigrated from the red clot during the incubation of blood in a closed cell—frequently show, under dark ground illumination, an oscillatory movement of the cell granules. This movement is also seen in living pus cells. It varies

in intensity with the chemical activity going on in the cell, and ceases with the death of the cell.

3. The lobes of the nucleus in the multi-lobate polymorph cell take up a different position in the cell relatively to each other and to the protruding processes as the cell changes in shape. A single lobe may travel along an extending fibre and become detached from the rest of the nucleus.

4. Leucocytes, especially polymorph cells, are capable of delicate adaptation to the finest surface irregularities of any substance or object with which they come in contact.

The essential factor in this delicate process of adaptation seems to be extreme sensitiveness of the cell cytoplasm to changes in surface tension.

5. The ingestion of viscous substances like liquid paraffin can be explained by this capacity of response to changes in surface tension on the part of the leucocytes and of the fine cytoplasmic processes which they extrude.

6. The formation of " giant cells " by the coalescence or leuco-agglutination of a number of leucocytes can be observed under certain conditions *in vitro* on a slide.

The favouring conditions seem to be the running together of engorged or partially poisoned cells, each containing, in the experiment observed, ingested, toxic, or foreign red cells.

7. Living pus cells, capable of movement, exhibit many of the characters shown by leucocytes when emigrating from the red clot during blood incubation.

The liquor puris, like the blood-serum, is inhibitory or poisonous to the living pus cell in proportion as it contains the products of cell disintegration or bacterial toxins.

Washed pus cells, like washed emigrated leucocytes,

can phagocytose foreign or toxic red cells without any previous preparation or opsonisation of those cells by serum or liquor puris.

8. The fact now demonstrated that living pus cells, in common with emigrated leucocytes, possess an increased capacity for the ingestion of foreign substances like red cells, and for the secretion of "Iodophil" and "Diffusion" substances, is of importance in relation to "return immigration," or the process by which leucocytes, which have wandered on to the surface of mucous membranes and wounds, re-enter the tissues, or the lymph, or the blood-stream.

9. The relative number of cells which emigrate from the incubated blood, the rapidity with which they adhere to the slide, and the different shapes assumed by the emigrating cells vary under different conditions, and in the blood of patients with different diseases.

In malaria, in blood taken during the rigor, emigration may be scanty, while in pneumonia, in the acute stage, a rich crop of emigrating polymorphs is obtained.

This varying migratory and secretory activity depends primarily on the state of the blood-serum—that is to say, the attitude of the leucocytes to the red cells, when whole blood is incubated with native or foreign or otherwise toxic or opsonised red cells, depends apparently on some reaction between the red cells and the serum, whereby additive toxic or opsonic substances are formed on the surface of the red cells by the serum.

10. Saturation of the blood with ether during prolonged anæsthesia, especially if cyanosis accompanies the administration, renders the red cells more or less auto-agglutinable by their own blood-serum *in vitro;* it also tends to make the red cells more or less ingestible by the native leucocytes when such blood is incubated, and it also

produces an increased *aggressiveness* on the part of the leucocytes to the red cells in the incubated blood.

Here also the result seems to depend primarily on a reaction between the red cells and the blood plasma or serum.

11. A comparison between leucocyte films obtained from normal blood, after incubation with glucose and insulin, and films of the same blood incubated with glucose alone, after treatment of the washed and fixed films with benzidine and iodine, shows that the presence of insulin increases the amount of diffusion (or ? aldehydic) substance elaborated by the leucocytes when incubated with glucose.

This observation has important bearings on the mechanism of carbohydrate metabolism by leucocytes.

12. The use of ortho-, para-, and meta-phenylene-diamine substances in conjunction with peroxide of hydrogen affords a useful means of differentiating the eosinophil cells from other leucocytes in the incubated film.

Ortho-phenylene-diamine with peroxide stains the eosinophil granules a rich mauve colour.

Although it is possible by feeding the leucocytes (in incubated blood) on suitable or suitably prepared red cells, to produce an increase in the number and size of the eosin staining granules, it is not certain that the cells in which this increase has occurred become true eosinophil cells.

The life-history of the eosinophil cell and its relation to other varieties of leucocytes requires further study.

13. The first stage in the phagocytosis of red cells by leucocytes depends upon erythro-leuco-agglutination or the secretion of a substance or substances by the leucocyte which secures mutual *adhesion* between the phagocyte and the red cell.

This stage is followed by the *ingestion* and, under favourable conditions, by the *digestion* of the red cell.

The presence of "ghost" red cells, or partially or wholly decolourised red cell remains in leucocytes, affords an indication of erythro-phagocytic activity by such leucocytes in the incubated blood. These remains are found in the leucocytes in the incubated blood of patients suffering from different diseases and at different stages of the same disease. In pneumonia these red cell remains can be detected as inclusion products in the leucocytes in the incubated blood in the post-pyrexial and convalescent stages of the disease.

14. The use of the benzidine and iodine method on the fixed leucocyte film shows the formation by stimulated leucocytes (*e.g.*, after incubation with prepared and foreign red cells) of a special secretion which I have called "Diffusion" substance. This substance appears to be aldehydic in character, and to be closely related to the "Iodophil" substance previously described as secreted by leucocytes under certain conditions.

This "Diffusion" substance is not merely one of the ordinary oxydases present in many living cells.

It is exuded into the surrounding medium, and acts on red cells exposed to its influence, and prepares them for *agglutination* and *ingestion* by leucocytes.

It is also present, like the "Iodophil" substance, in some normal epithelial cells of certain mucous membranes, in some cancer cells of epithelial origin, in the multinucleated giant cells of myelomata, and in certain marrow cells.

15. Observation with a standard human blood when incubated with sheep and with native and foreign human red cells, in health and disease, throws some light on the process of leucocytic stimulation.

The toxic effect of sheep washed red cells and human red cells on human leucocytes seems to depend on the kind of substance which is formed by the serum with the red cell.

The power of the blood-serum (sheep and human) to render sheep or human red cells leuco-toxic can be removed by heating the serum to 56° to 60° C. for one hour.

16. In some diseases, of which pneumonia may be taken as a type, the leucocytes from the incubated blood which emigrate on the floor of the closed cell exhibit a heightened phagocytic capacity or aggressiveness to both native and foreign red cells.

Leucocytes which emigrate from the washed and re-incubated clot from the same blood show a still more increased aggressiveness to the same red cells.

This heightened leucocytic *aggressiveness* is not limited to the acute pyrexial stage, but extends into the convalescent stage of the disease.

17. Along with this increased leucocytic *aggressiveness* to red cells, the blood-serum in pneumonia also acquires an increased power of rendering foreign and native red cells, previously treated with it, either toxic to, or under certain conditions more readily ingested by, the same leucocytes.

This capacity of the same blood-serum to render red cells leuco-toxic on the one hand, or leuco-attractive on the other, seems to depend on a different stage in the reaction between the serum and the red cell—that is, on different substances or different oxidation phases of the same substance formed from the serum on the red cell.

The erythro-opsonic capacity of a blood-serum under certain conditions is not removed, but is increased, by heating the serum to 56° to 60° C.

The evidence suggests that the erythro-opsonins and the erythro-toxins present in the blood-serum in certain diseases are intimately associated. The erythro-opsonins may, indeed, be derived from the erythro-toxins.

18. Substances can be extracted from the envelopes of washed red cells by treatment with acetone and some other reagents, which, when evaporated down and redissolved in normal saline, and added to the incubated blood of the same individual, render the red cells ingestible by the native leucocytes.

This effect of red cell extract in opsonising red cells seems to be partly specific in character, and more or less limited to the blood system used in the preparation of the extract.

19. Evidence has been given in Sections I. and III. to show that both the oscillatory movement of the cell granules and the protrusions of dendrites into the surrounding medium are the result of changes in surface tension at the interface between the granules or the processes and the medium in which they are immersed.

Further evidence (see Sections I., II., III., X., XI.) suggests that a dendrite or pseudopod tends to move towards adjacent objects according to the nature of those objects—that is, according to the chemical constitution of the substance which forms their surface coating.

In other words, the directional flow of a given dendrite depends primarily on changes in surface tension in the medium. The chemical changes which produce these alterations in surface tension depend upon the presence or absence, or on the degree of concentration of the diffusion substance in different areas of the medium.

When any body or substance is present which is capable of combining with, or of forming an insoluble additive product with, the diffusion substance present in solution,

this brings about a further liberation of diffusion substance by the leucocyte or the dendrite in that area of the medium, and, as a consequence of reduction in surface tension, to a continued protrusion of the advancing cell process in the same direction.

In this way red cells or micro-organisms or other foreign bodies may perhaps be said to "attract" dendrites and leucocytes.

FIG. 1.—HUMAN DEFIBRINATED BLOOD INCUBATED: FILM UNFIXED, MOUNTED IN NORMAL SALINE. (D.G. × 750.)

Shows leucocytes with dendrites; oscillation of granules.

(Page 2.)

FIG. 2.—HUMAN DEFIBRINATED BLOOD INCUBATED AFTER FORTY-EIGHT HOURS' STANDING: FILM UNFIXED, MOUNTED IN NORMAL SALINE. (D.G. × 750.)

Leucocytes dead, circular in outline; granules not moving.

(Page 2.)

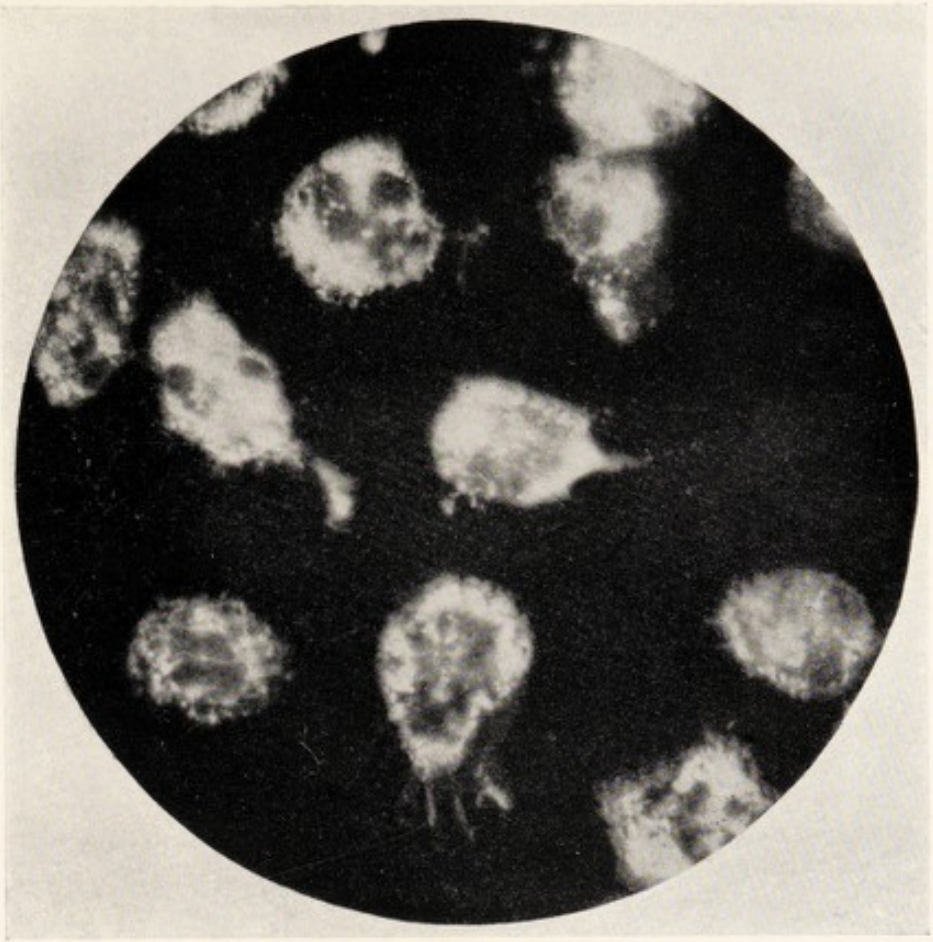


FIG. 1.

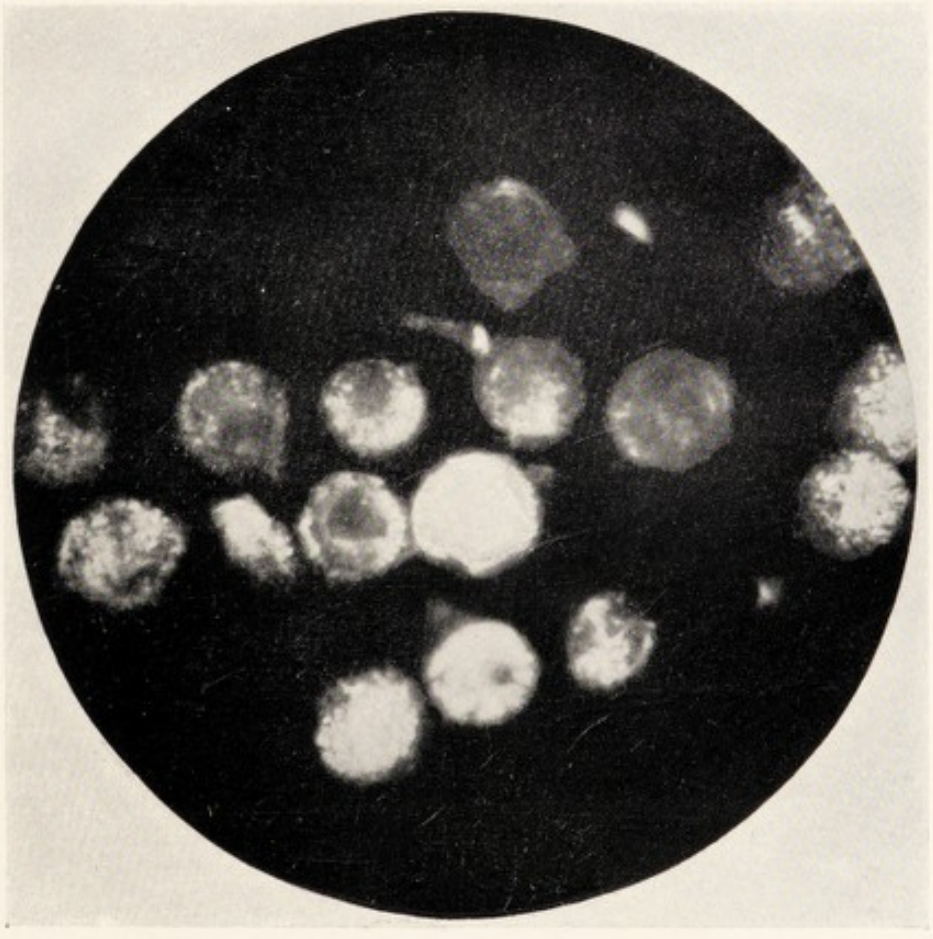


FIG. 2.

FIG. 3.—HUMAN FIBRIN NETWORK INCUBATED WITH NATIVE DEFIBRINATED BLOOD: FILM WASHED WITH IODINE SALINE. (D.G. × 690.)

Shows leucocytes with dendrites adhering to the fibrin scaffolding.

(Page 3.)

FIG. 4.—HUMAN BLOOD INCUBATED (D.G. × 1060), MOUNTED.

Shows terminal fusion of dendrites from individual cells, and establishment of inter-cellular fibular connections.

(Page 4.)

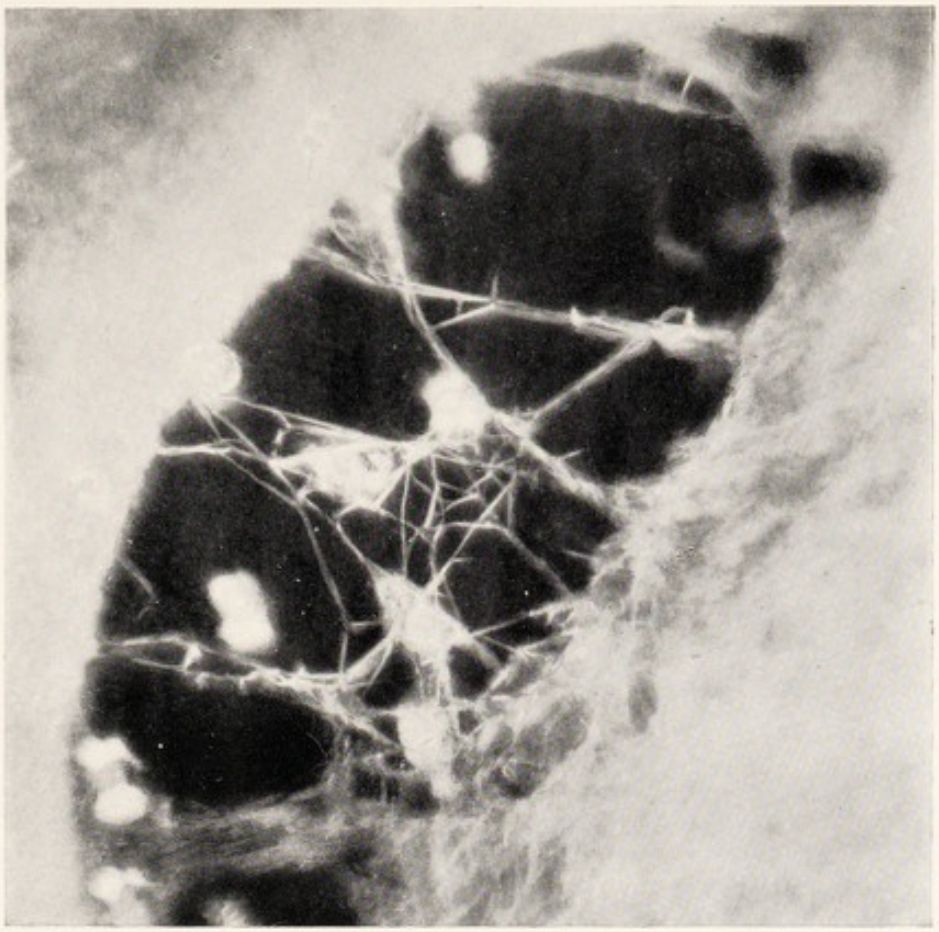


FIG. 3.

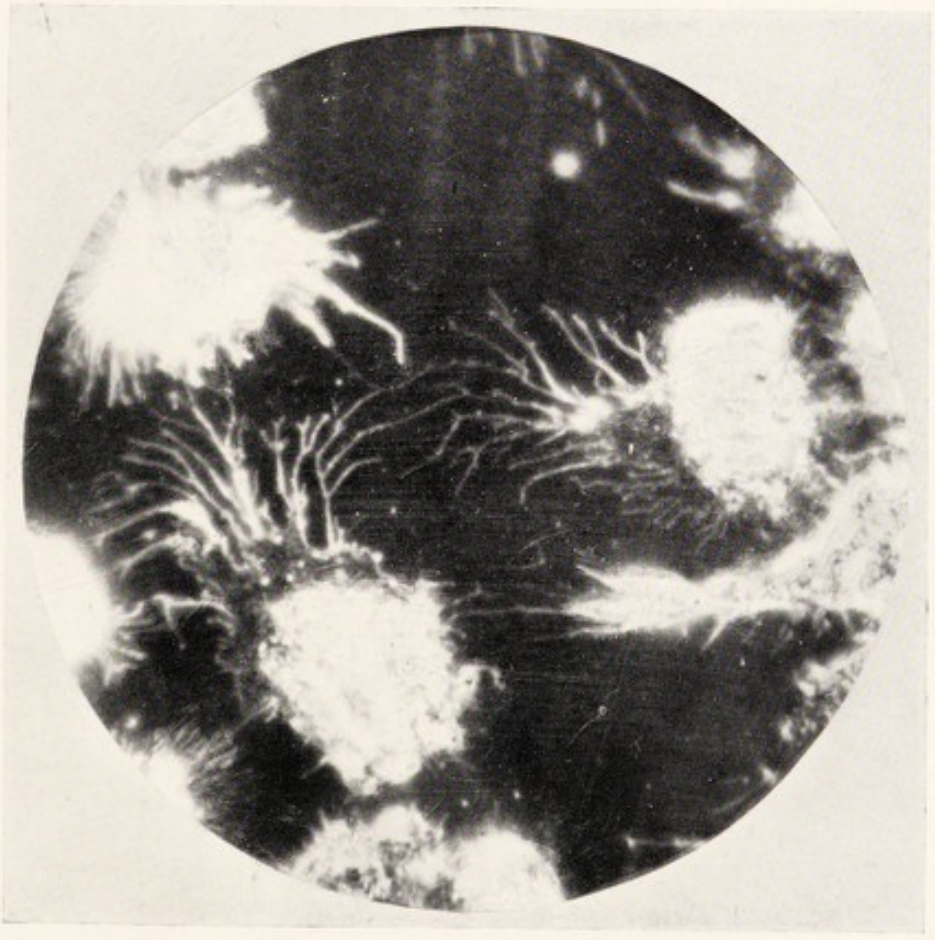


FIG. 4.

FIG. 5.—WASHED RED CLOT FROM HUMAN INCUBATED BLOOD: CLOT REINCUBATED WITHOUT BLOOD-SERUM. (D.G.×1,060.)

Shows stimulation of leucocytes with protrusion of dendrites.

(Page 10.)

FIG. 6.—INCUBATED BLOOD FROM A PATIENT AFTER HALF AN HOUR'S ETHER ANÆSTHESIA. (D.G.×960.)

Shows highly stimulated leucocytes, club-shaped ends, with dendrites.

(Pages 10 and 29.)

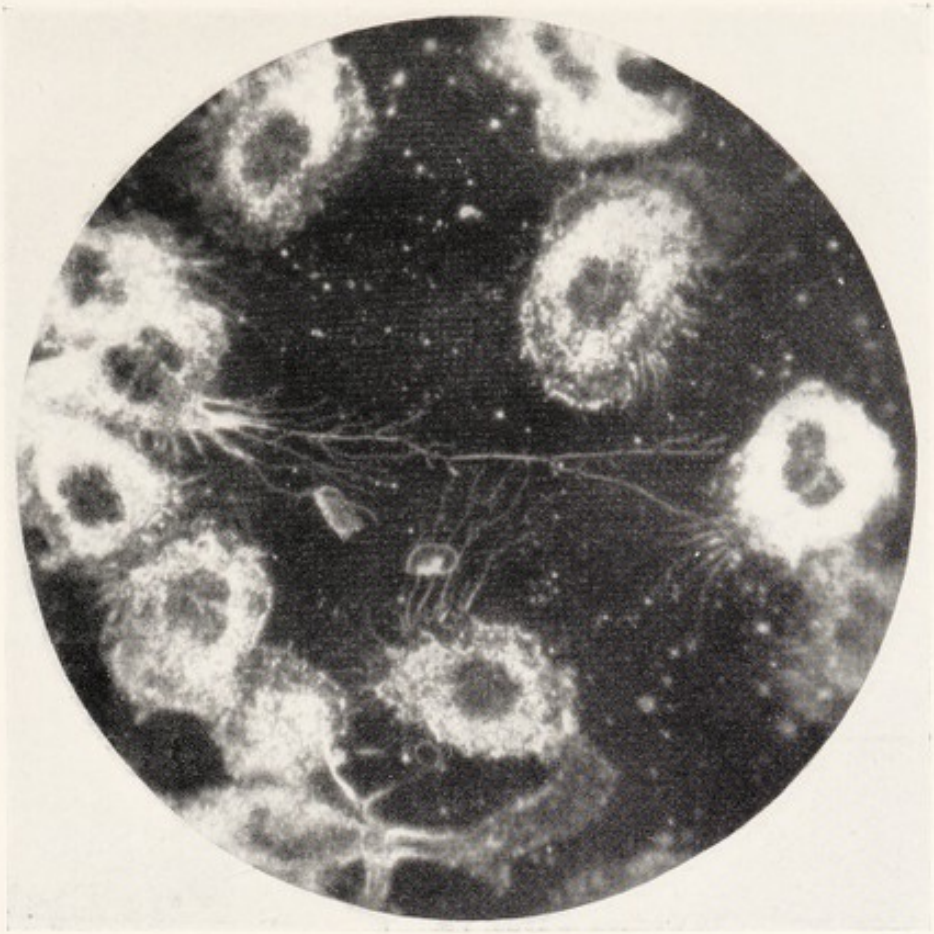


FIG. 5.

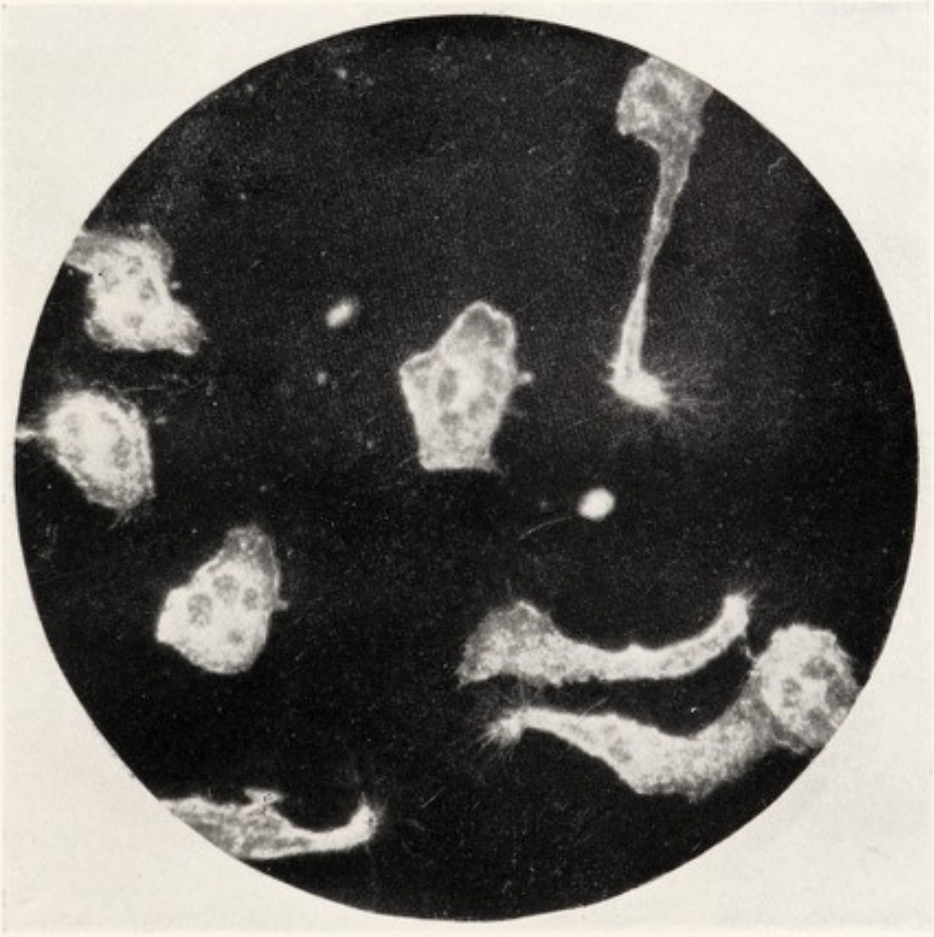


FIG. 6.

FIG. 7.—HUMAN BLOOD INCUBATED, RED CLOT REINCUBATED. (D.G. × 790.)

Shows the migration of the lobes of the nucleus in polymorph leucocytes in active movement.

(Page 12.)

FIG. 8.—WASHED RED CLOT FROM INCUBATED BLOOD REINCUBATED ON A RULED SIDE. (× 170.)

Shows the alignment of the emigrating leucocytes along the ruled grooves.

(Page 13.)

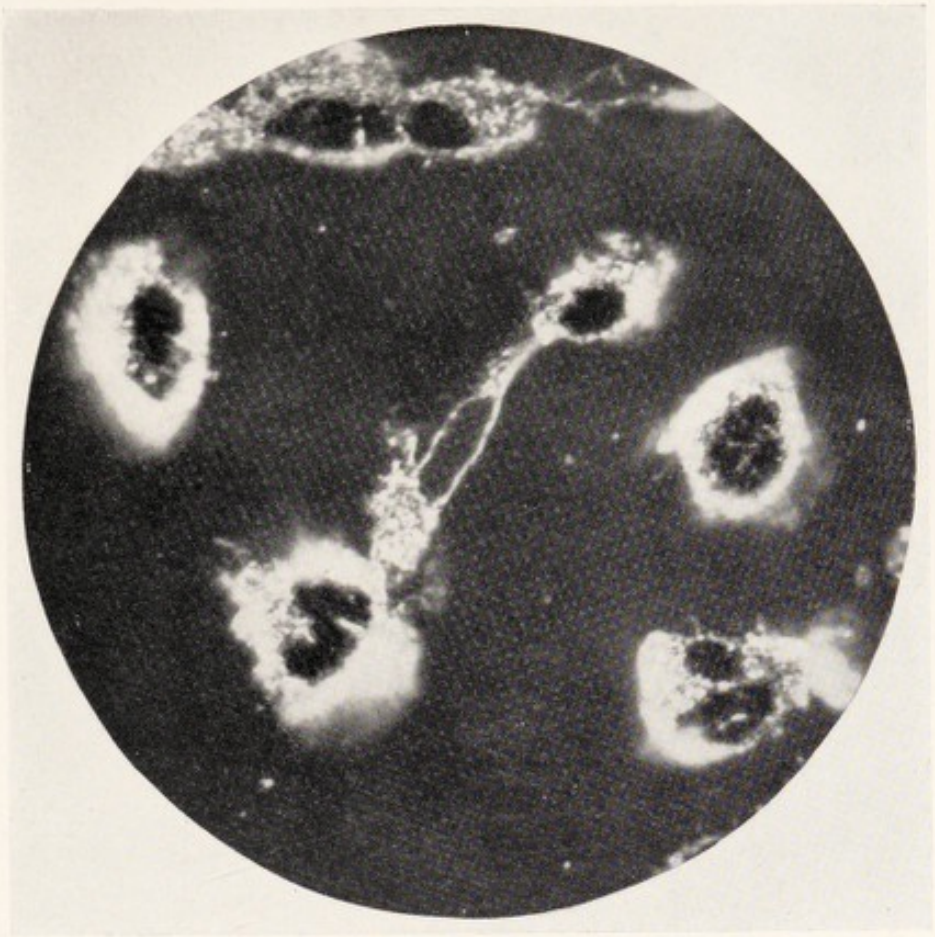


FIG. 7.

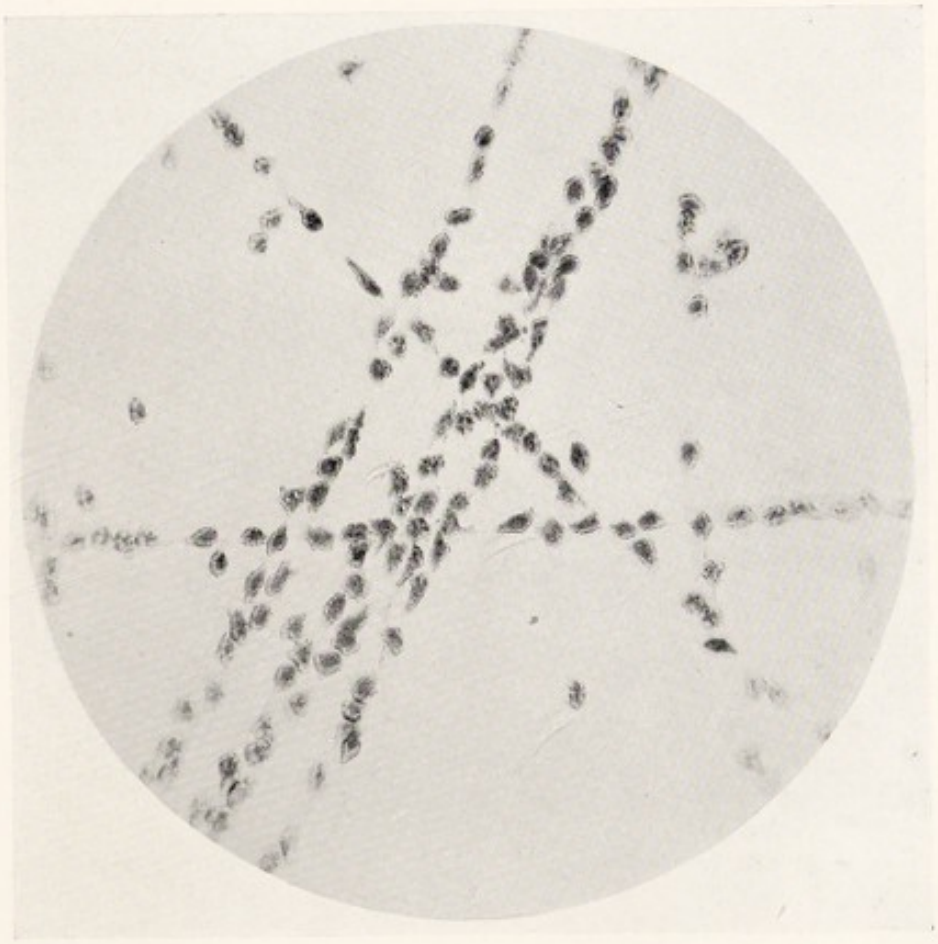


FIG. 8.

FIG. 9.—THE SAME WASHED RED CLOT FROM INCUBATED BLOOD REINCUBATED ON A RULED SIDE. (×690.)

(Page 13.)

FIG. 10.—HUMAN BLOOD INCUBATED WITH A SUSPENSION OF DROPLETS OF LIQUID PARAFFIN IN NORMAL SALINE. (×1,460.)

Shows ingested droplets of liquid paraffin.

(Page 15.)

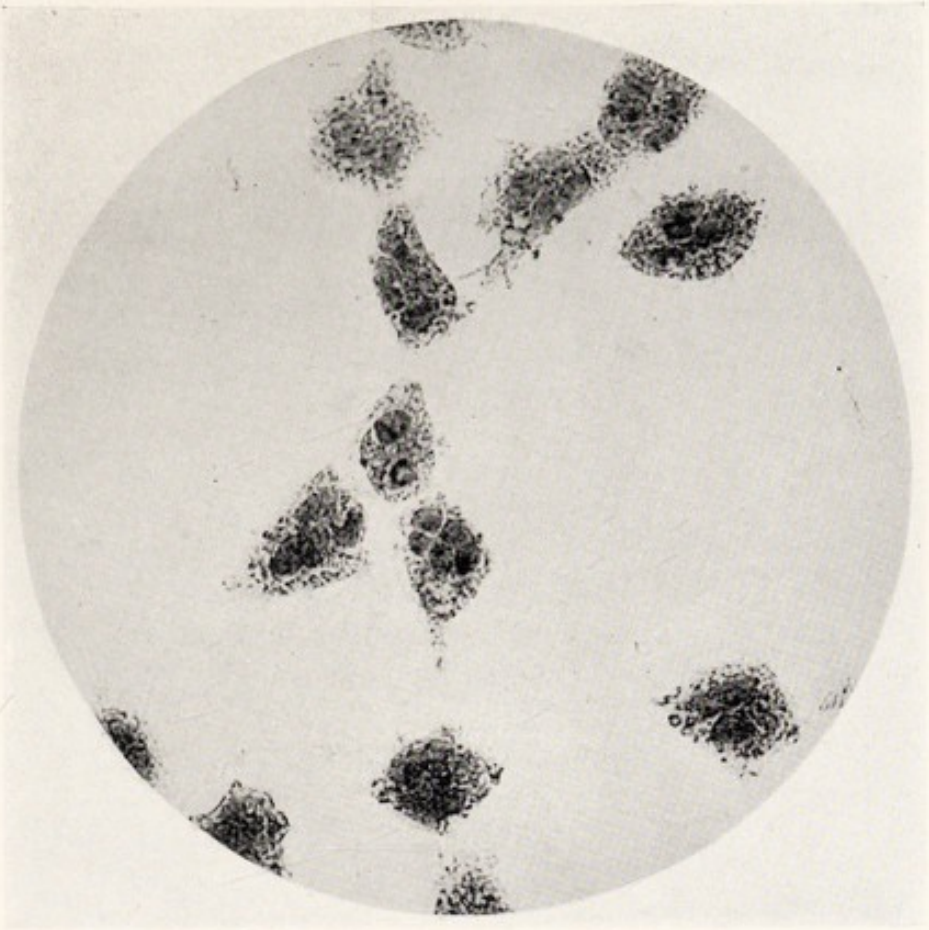


FIG. 9.

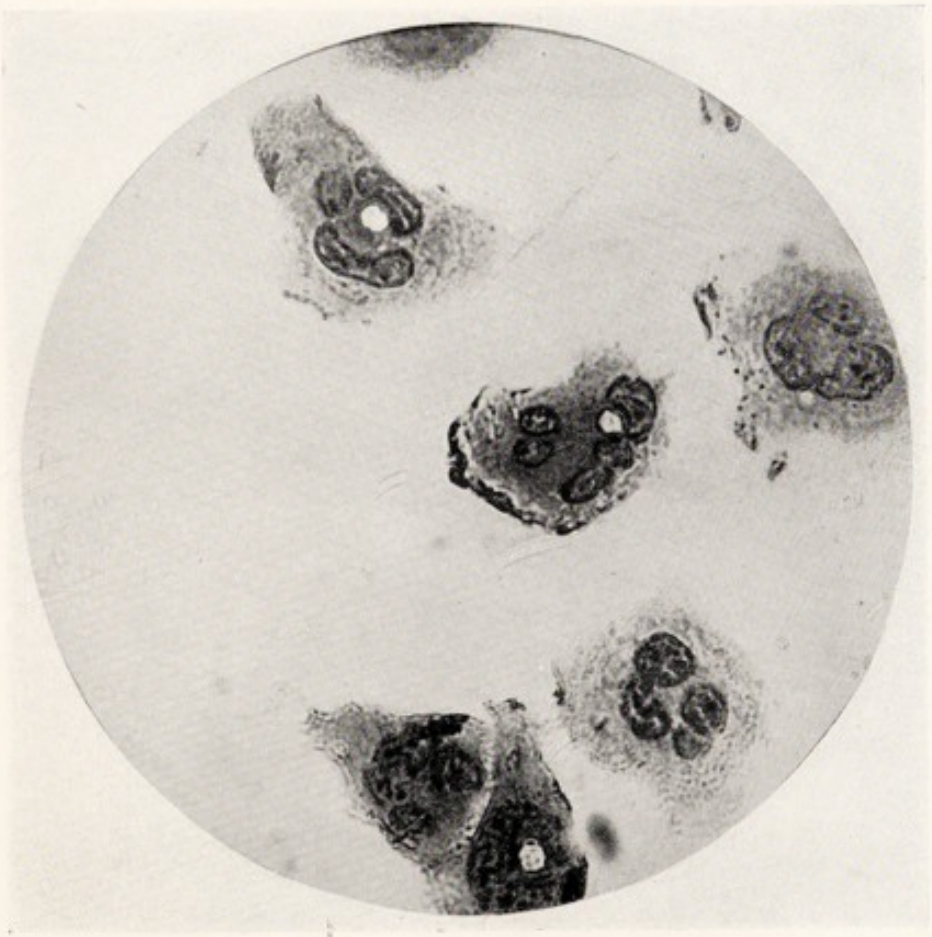


FIG. 10.

FIG. 11.—HUMAN BLOOD INCUBATED ON A FILM OF LIQUID PARAFFIN. MOUNTED IN IODISED SALINE. (×370.)

Shows exuded globules of "Iodophil" substance attached to the leucocytes, A

(Page 17.)

FIG. 12.—URETHRAL PUS (AC. GON.) MOUNTED IN IODISED GUM SALINE. (×450.)

After standing three months shows extra-cellular spheres of "Iodophil" substance with characteristic notched edges.

(Pages 17 and 46.)

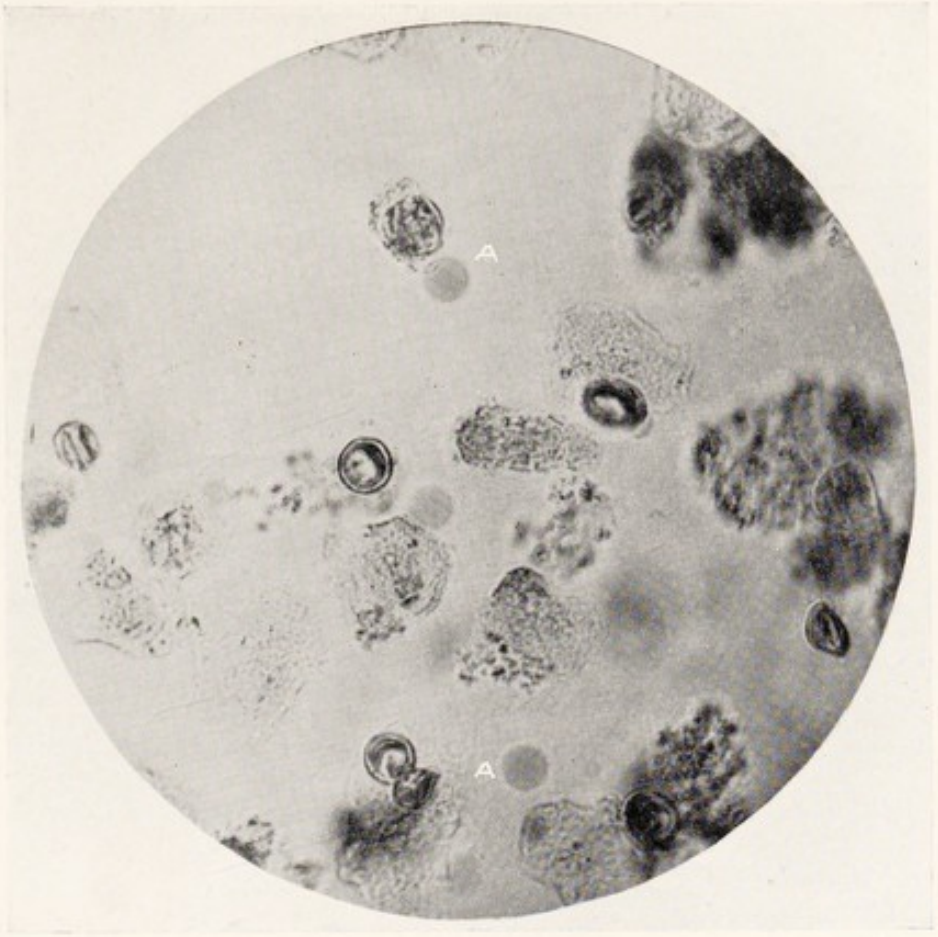


FIG. 11.

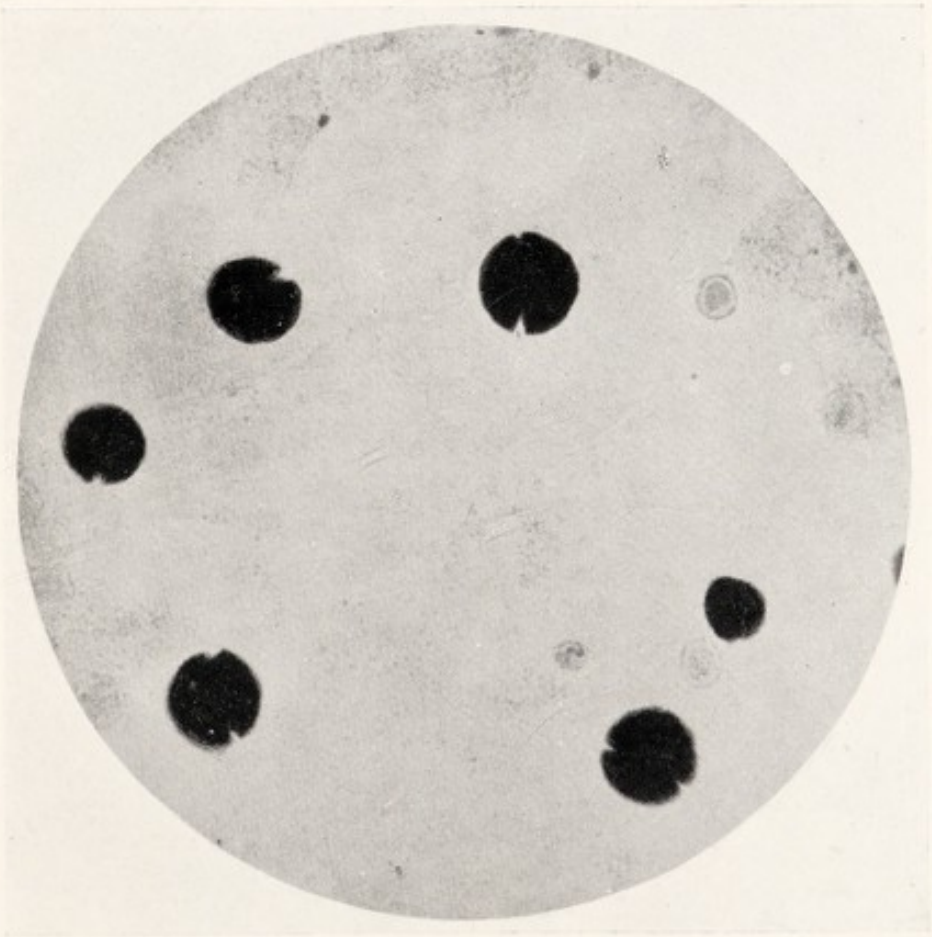


FIG. 12.

FIG. 13.—HUMAN BLOOD INCUBATED IN A CLOSED CELL WITH LECITHIN 1 PER CENT. SUSPENSION IN NORMAL SALINE. (×570.)

Shows formation of "giant cells."

(Page 19.)

FIG. 14.—SERO-PUS FROM A CASE OF PNEUMOCOCCAL SYNOVITIS OF THE KNEE INCUBATED WITH SHEEP TOXIC WASHED RED CELLS: IODINE SALINE. (×780.)

Shows agglutination and ingestion of sheep red cells by pus cells in the presence of liquor puris.

(Page 20.)

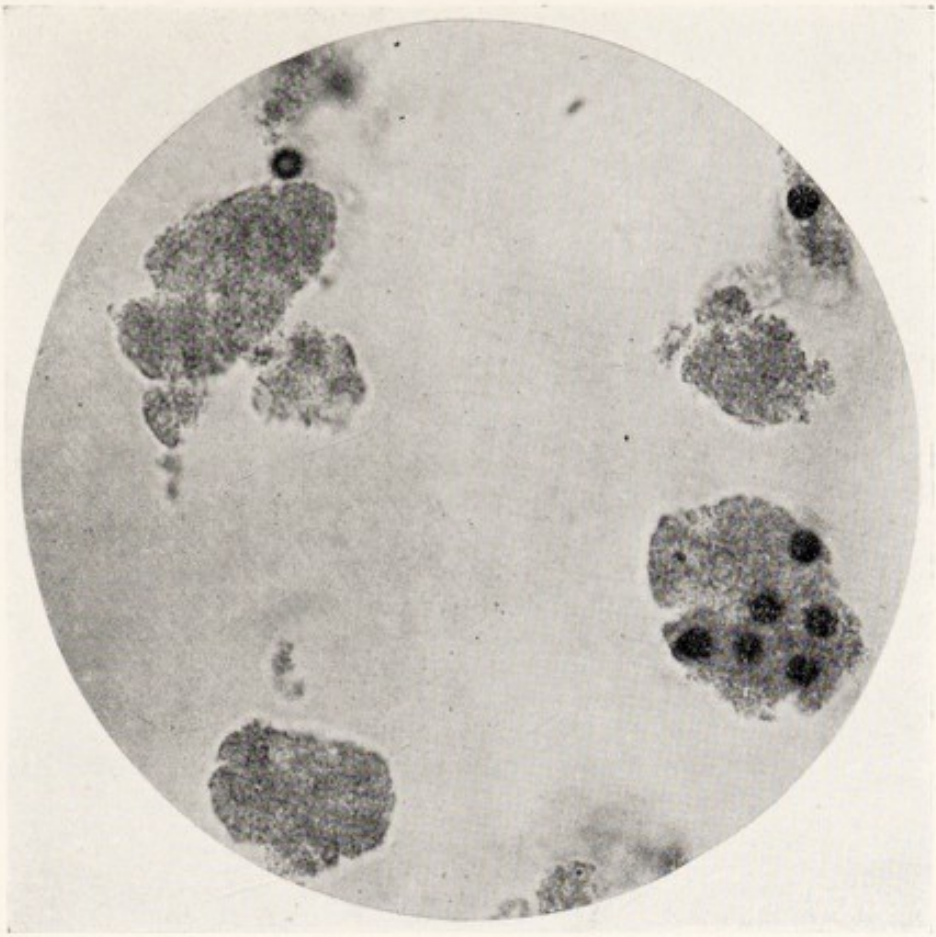


Fig. 13.

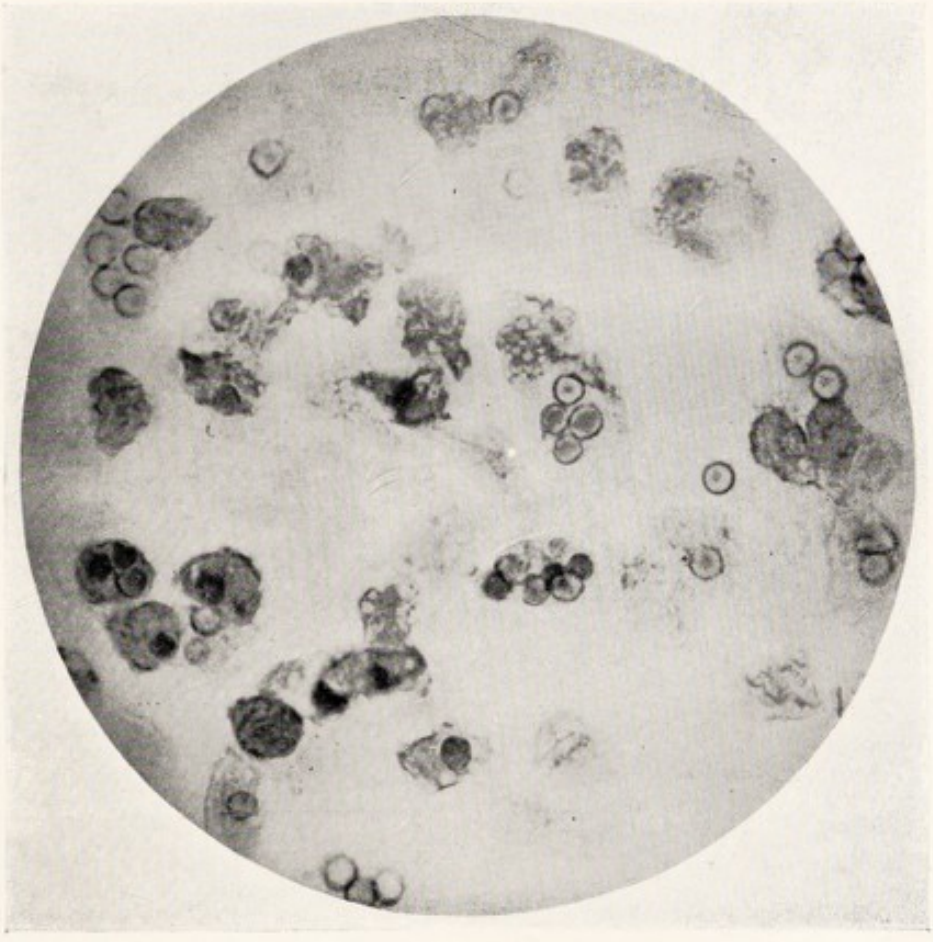


Fig. 14.

FIG. 15.—SERO-PUS FROM A WELL-DRAINED EMPYEMA INCUBATED WITH SHEEP TOXIC WASHED RED CELLS: IODINE SALINE. (×640.)

Shows marked leuco-agglutination of sheep red cells.

(Page 20.)

FIG. 16.—WASHED PUS CELLS FROM THE SAME SERO-PUS (FIG. 15) INCUBATED WITH THE SAME SHEEP RED CELLS: IODINE SALINE. (×640.)

Shows marked leuco-agglutination of sheep red cells.

(Page 20.)

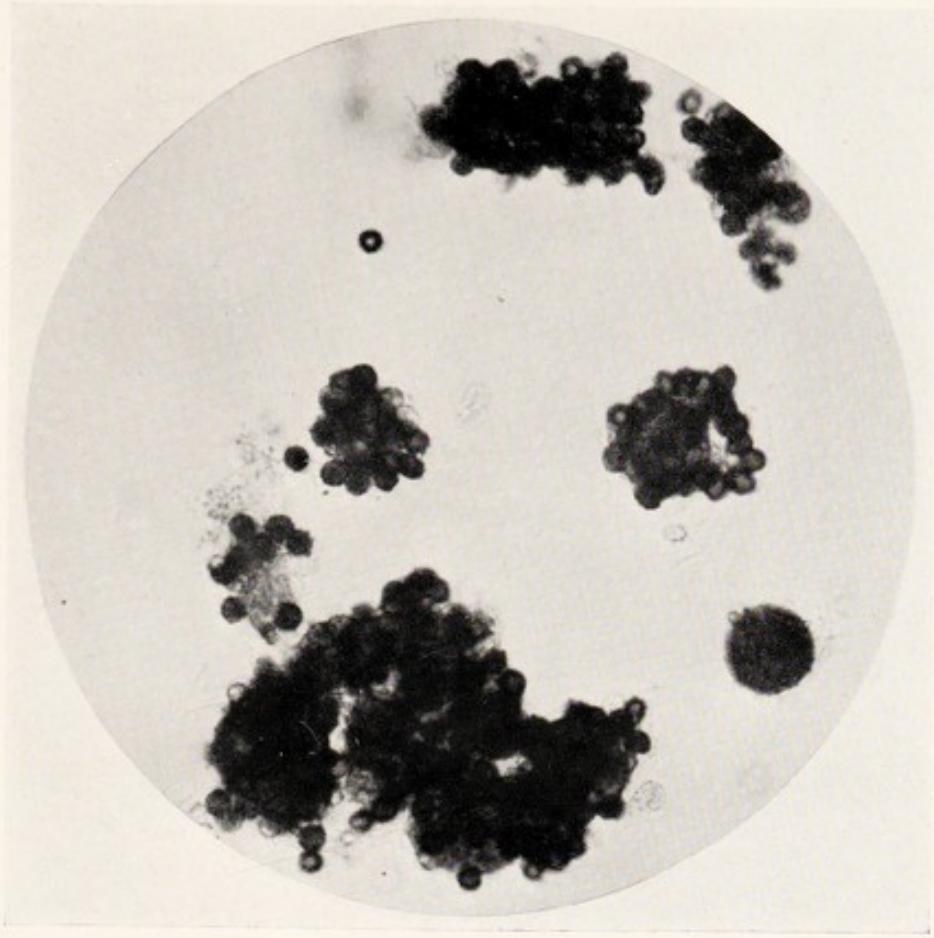


FIG. 15.

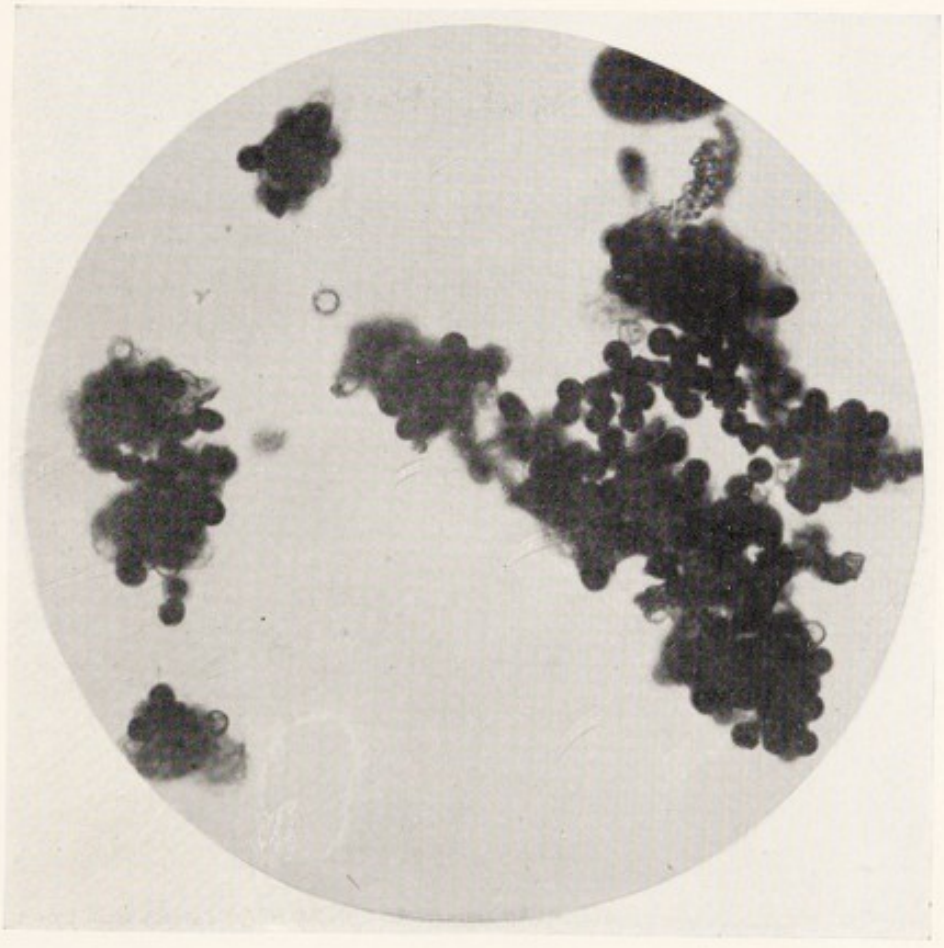


FIG. 16.

FIG. 17.—SERO-PUS FROM THE SAME PATIENT INCUBATED WITH HUMAN WASHED RED CELLS: IODINE SALINE. (×640.)

Shows leuco-agglutination of human red cells.

(Page 20.)

FIG. 18.—WASHED PUS CELLS FROM THE SAME SERO-PUS INCUBATED WITH THE SAME HUMAN RED CELLS: IODINE SALINE. (×760.)

Shows some leuco-agglutination and ingestion of human red cells, but less than with sheep red cells.

(Page 20.)

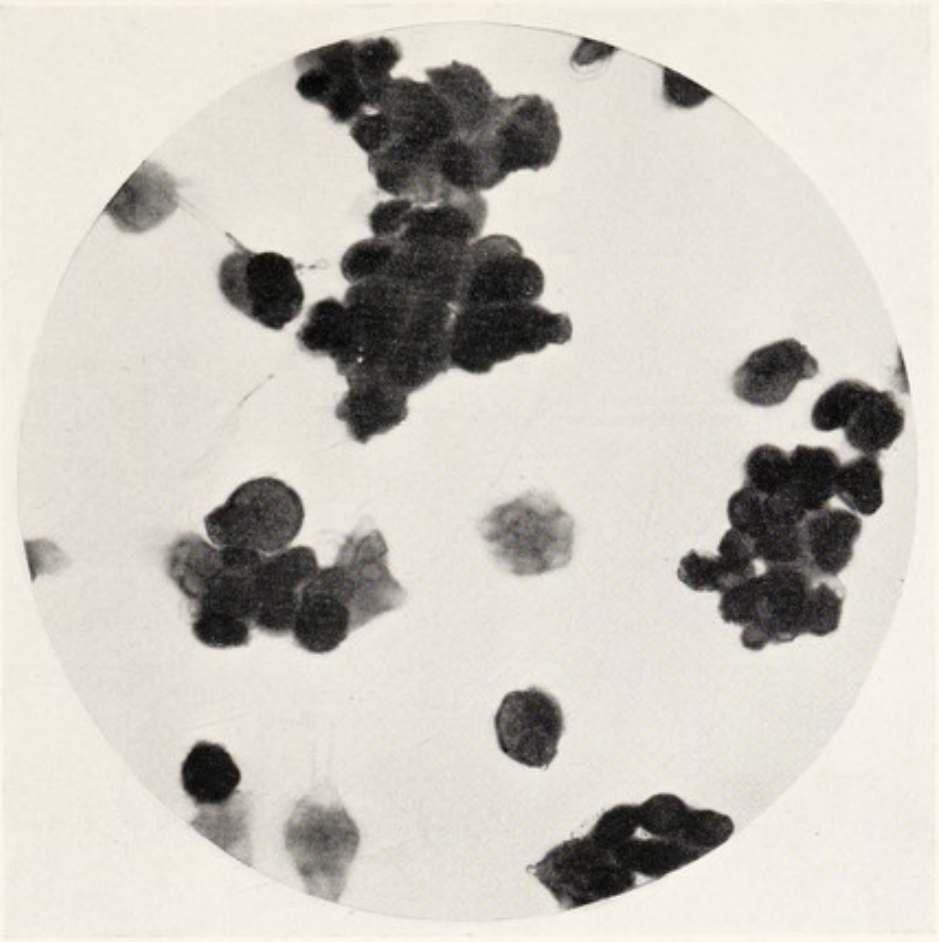


Fig. 17.

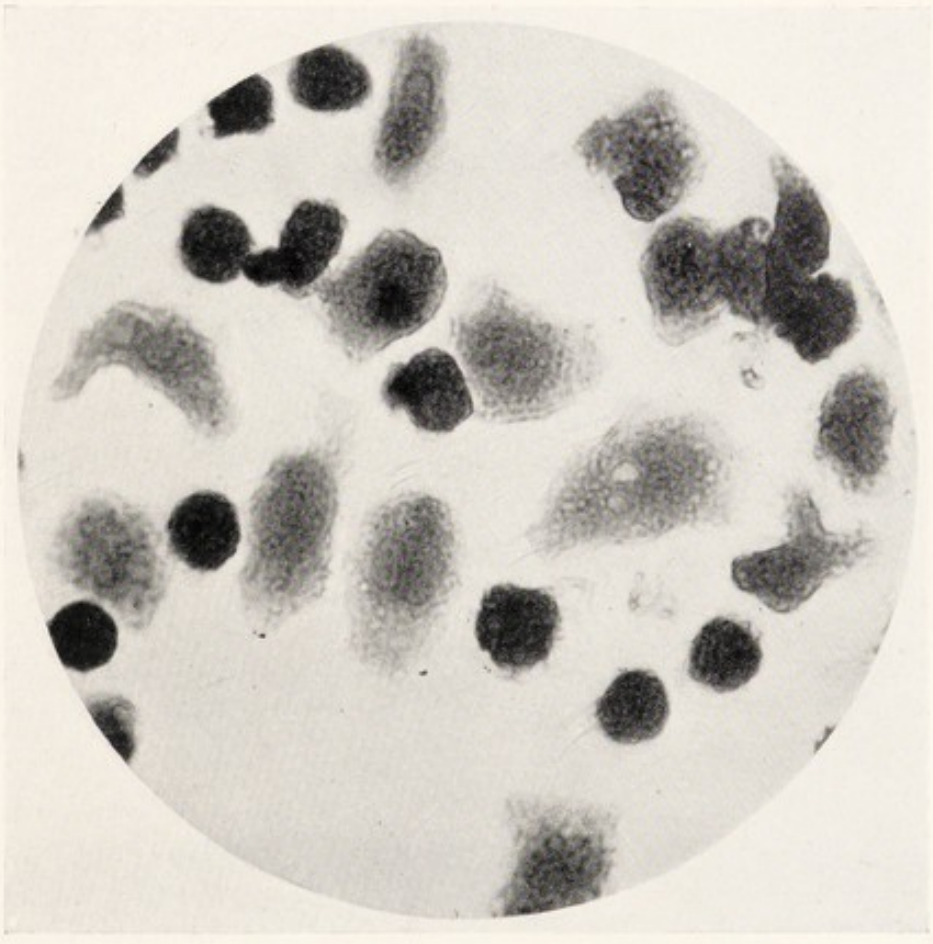


Fig. 18.

FIG. 19.—SECTION THROUGH THE GRANULATING SURFACE OF A RE-AMPUTATED STUMP PREVIOUSLY DRESSED WITH STERILISED RICE STARCH, MOUNTED IN IODISED GUM SALINE. (×146.)

Shows the presence of starch grains in the "return immigrated" leucocytes in the tissues.

(Page 24.)

FIG. 20.—SECTION OF BLOOD-CLOT AFTER INCUBATION IN SERUM CONTAINING STARCH GRAINS: IODINE SALINE. (×640.)

Shows starch grains in the leucocytes which have re-entered the red clot from the serum.

(Page 24.)

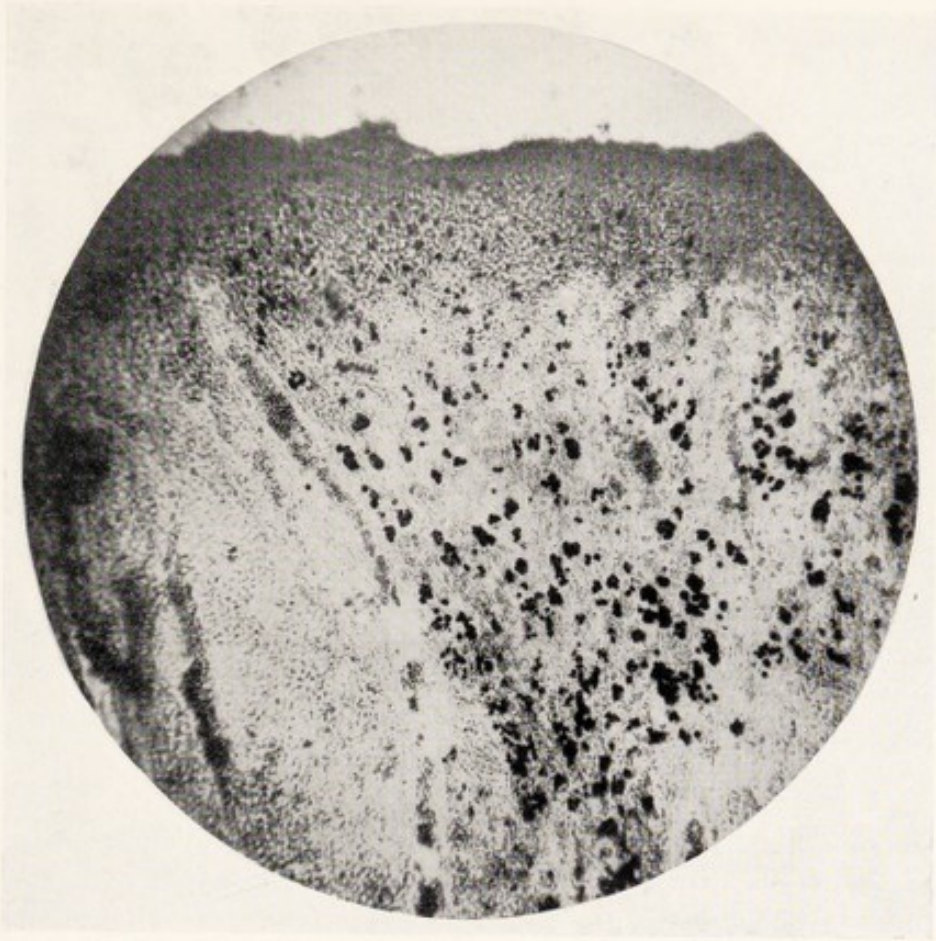


FIG. 19.

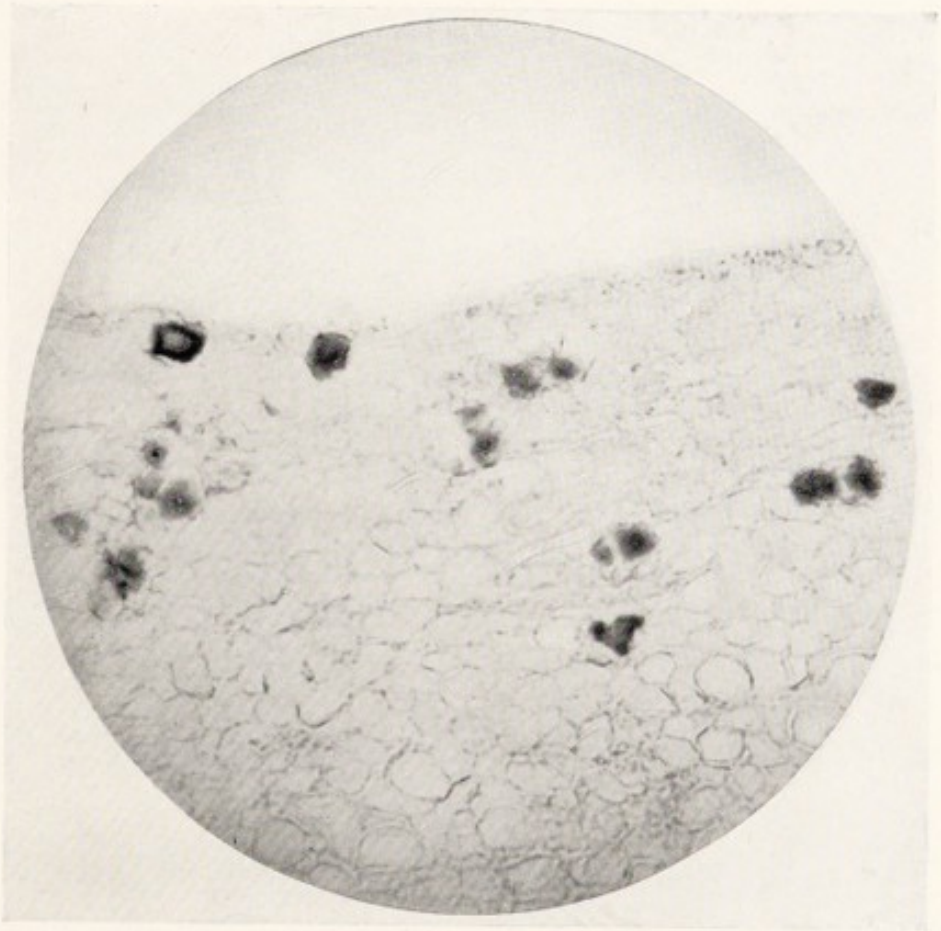


FIG. 20.

FIG. 21.—A WASHED RED CLOT FROM HUMAN INCUBATED BLOOD REINCUBATED WITH A SUSPENSION IN NORMAL SALINE OF SHEEP TOXIC WASHED RED CELLS: IODINE GUM SALINE. (×450.)

The leucocyte film shows phagocytosis of the toxic sheep red cells by the emigrating leucocytes.

(Pages 26 and 55.)

FIG. 22.—LEUCOCYTE FILM OBTAINED FROM INCUBATED BLOOD FROM A CASE OF HODGKIN'S DISEASE DURING THE PYREXIAL PERIOD: IODINE SALINE. (×780.)

Shows scanty emigration of circular devitalised leucocytes.

(Page 28.)

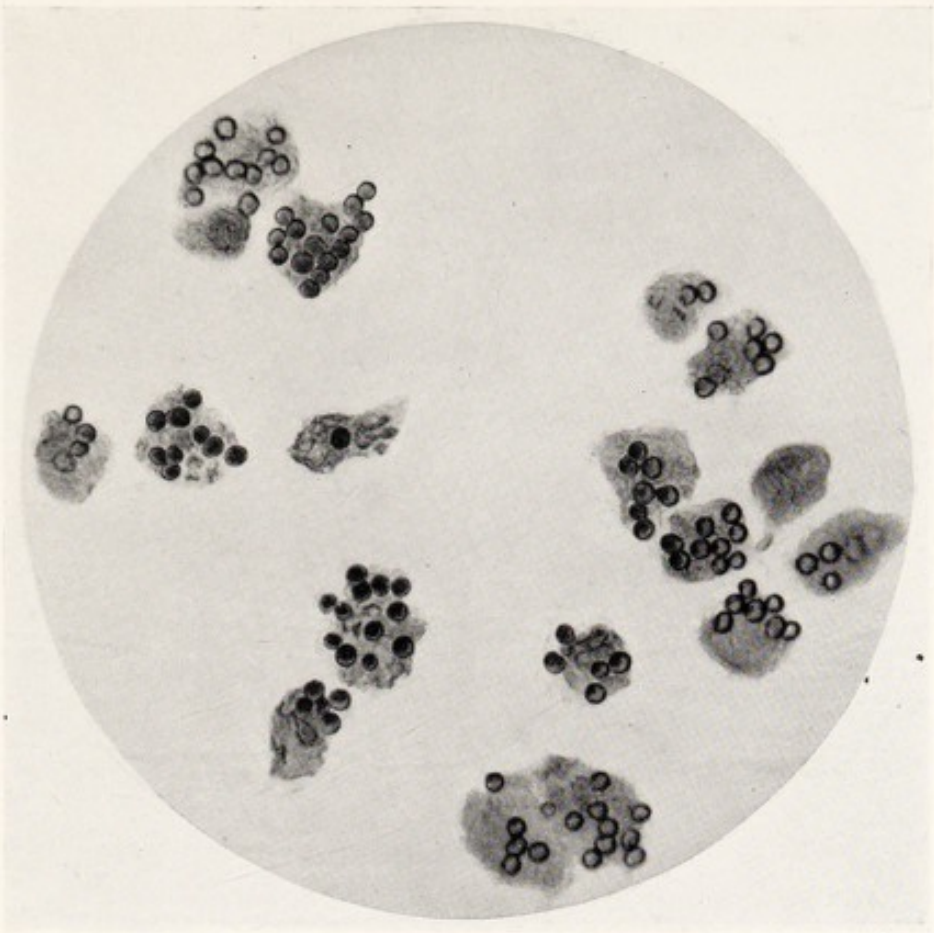


FIG. 21.

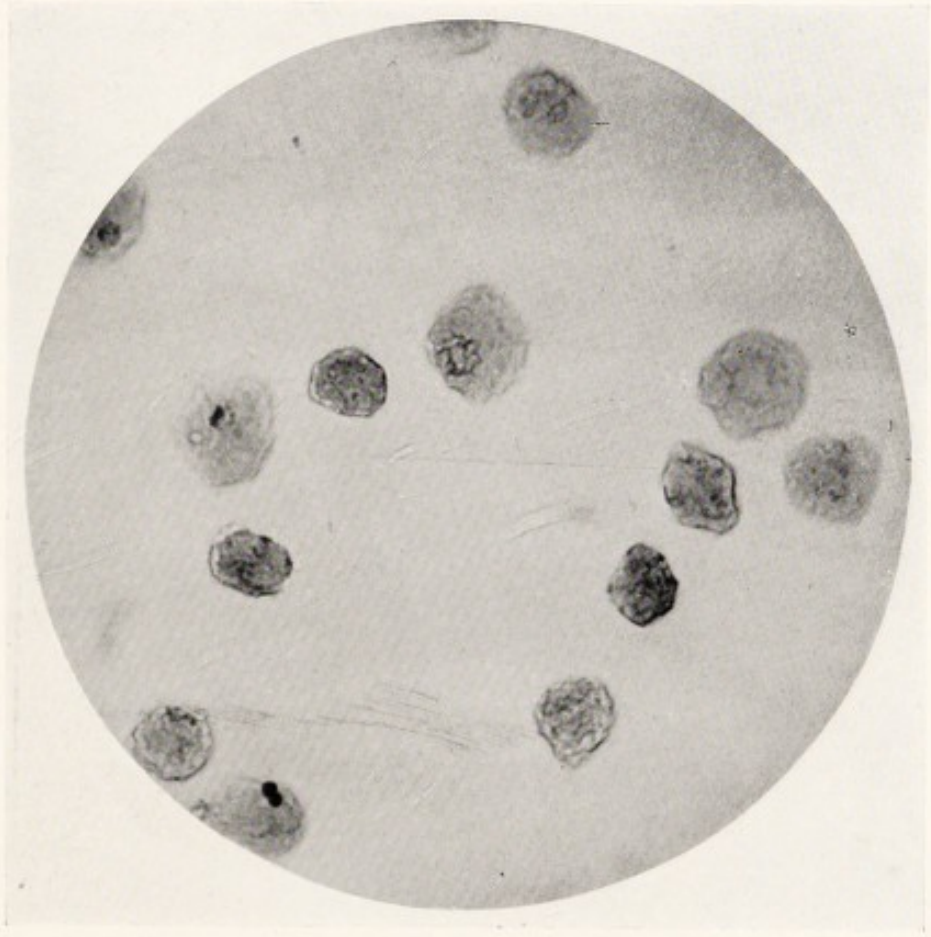


FIG. 22.

FIG. 23.—INCUBATED BLOOD FROM A CASE OF PNEUMONIA. FIFTH DAY OF THE DISEASE: IODINE SALINE. (×960.)

Shows a thick carpet of emigrated leucocytes with active pseudopodesis and excess of "Iodophil" substance.

(Page 29.)

FIG. 24.—HUMAN BLOOD. TWO DROPS, INCUBATED WITH ONE DROP 2 PER CENT. GLUCOSE IN NORMAL SALINE AND ONE DROP NORMAL SALINE: IODINE SALINE. (×420.)

The leucocyte film shows very little black "Diffusion" substance intra- or extra-cellular.

(Page 30.)

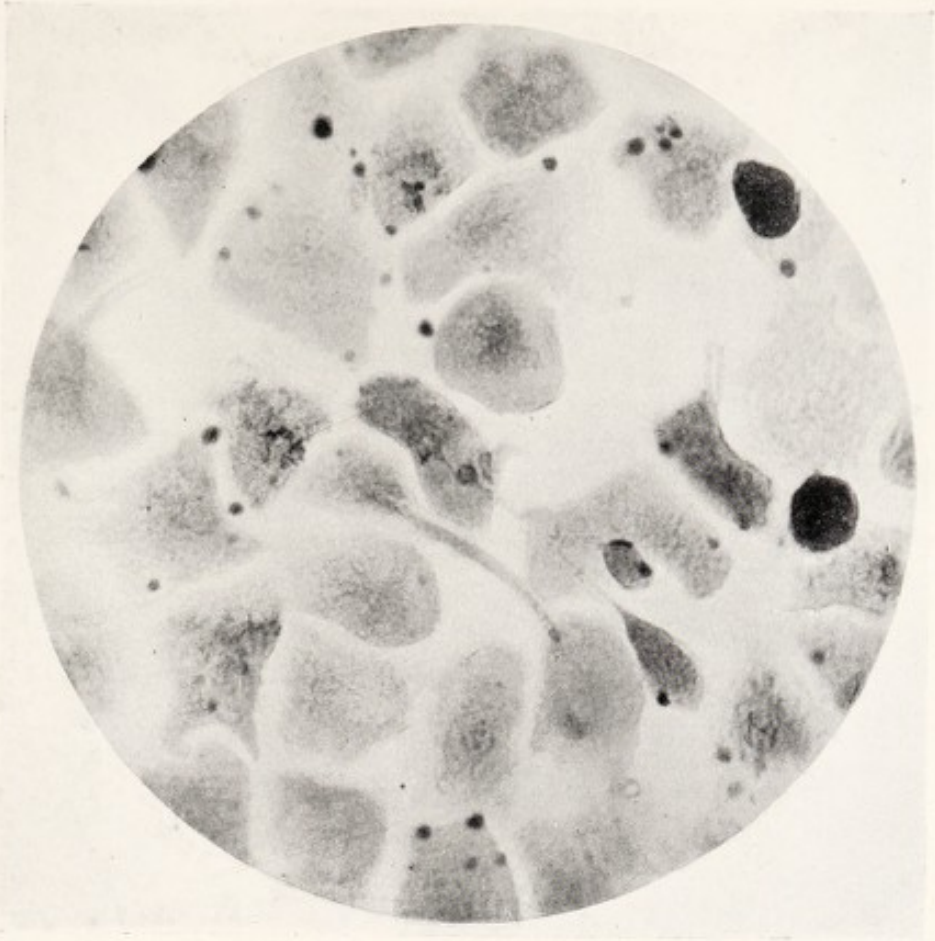


FIG. 23.

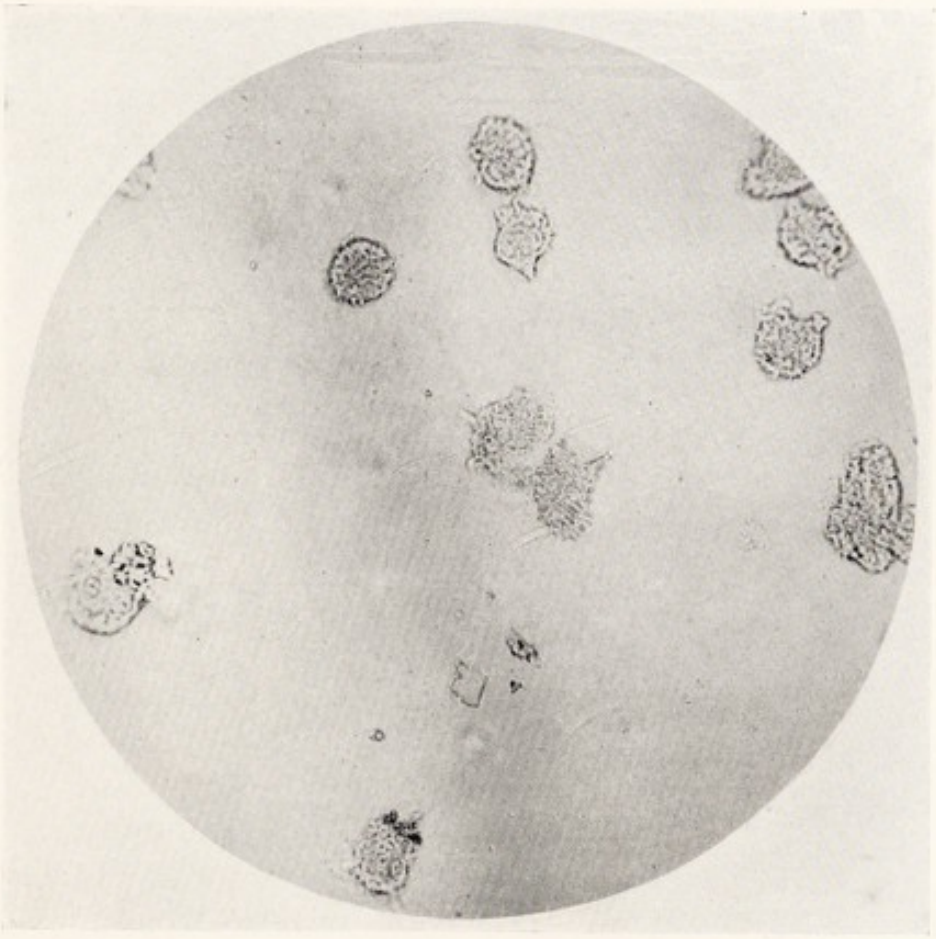


FIG. 24.

FIG. 25.—HUMAN BLOOD INCUBATED WITH ONE DROP 2 PER CENT. GLUCOSE IN NORMAL SALINE AND ONE DROP INSULIN IN NORMAL SALINE: IODINE SALINE. (×420.)

The leucocyte film shows much black "Diffusion" substance in the leucocytes.

(Page 31.)

FIG. 26.—BLOOD INCUBATED FROM A CASE OF PNEUMONIA IN THE CONVALESCENT STAGE: IODINE SALINE. (D.G.×780.)

The leucocyte film shows two disintegrating "bomb" eosinophil cells, also between these a leucocyte with an ingested red cell surrounded by a haze of "Diffusion" substance.

(Page 33.)

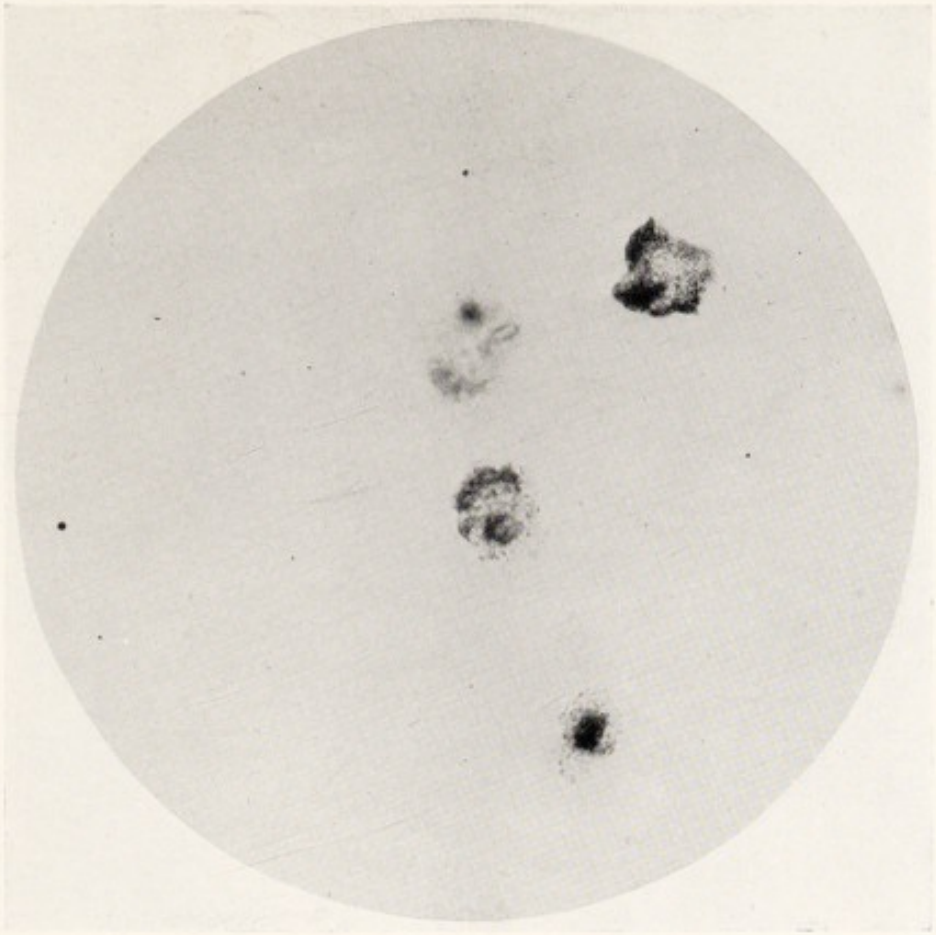


FIG. 25

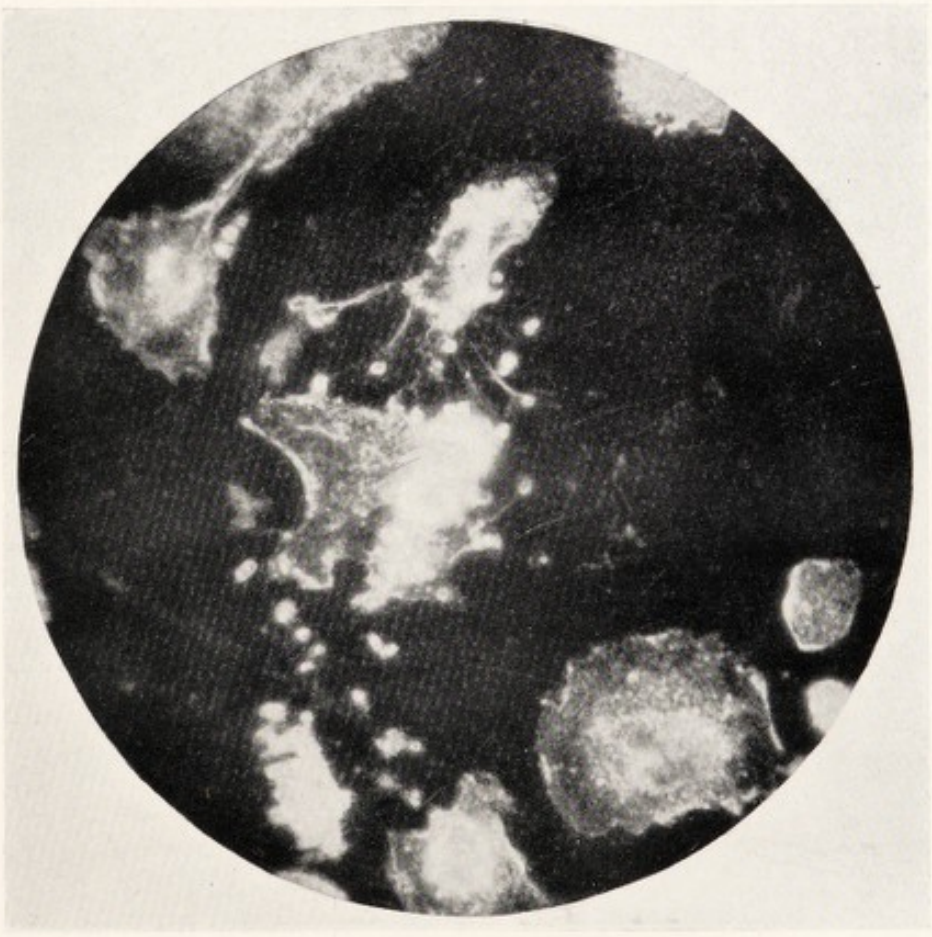


FIG. 26.

FIG. 27.—HUMAN BLOOD INCUBATED WITH ONE DROP OF ACETONE EXTRACT OF SHEEP WASHED RED CELLS: IODINE SALINE. (×1,160.)

The leucocyte film shows an eosinophil cell attacking two native red cells.

(Pages 34 and 39.)

FIG. 28.—BLOOD FROM A CASE OF EOSINOPHILIA 30 PER CENT. INCUBATED: NORMAL SALINE. (×700.)

The leucocyte film of living cells after treatment with ortho-phenylene-diamine and oxygen shows mauve staining of the eosinophil granules.

(Page 34.)

Fig. 27.

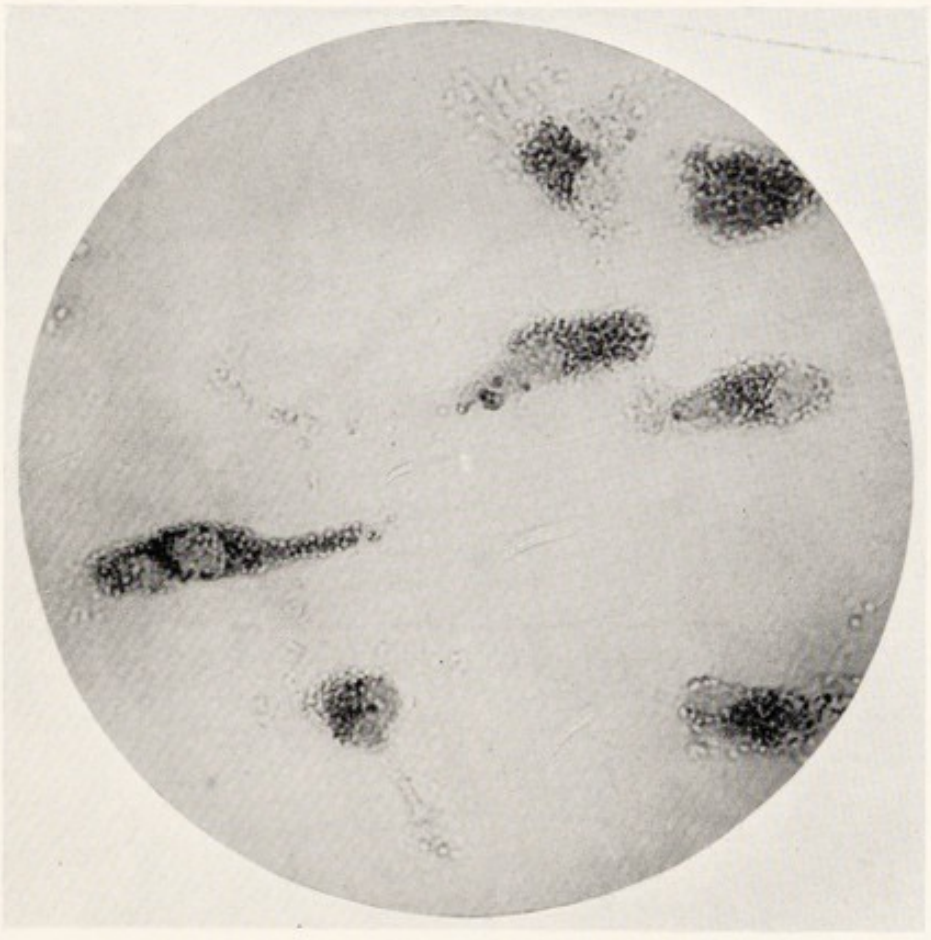


Fig. 28.

FIG. 29.—HUMAN BLOOD INCUBATED WITH SHEEP TOXIC RED CELLS AFTER TREATMENT WITH ISOTONIC SOLUTION OF LITHIUM CHLORIDE AND REWASHING IN NORMAL SALINE: IODINE GUM SALINE. (×1,060.)

The leucocyte film shows the protrusion of dendrites and the ingestion of sheep red cells by human leucocytes.

(Page 39.)

FIG. 30.—INCUBATED BLOOD FROM A CASE OF ACUTE PNEUMONIA: IODINE SALINE. (×780.)

The washed red clot was reincubated with sheep toxic washed red cells. The leucocyte film shows the remains of digested sheep red cells "ghost cells" in the leucocytes.

(Page 40.)

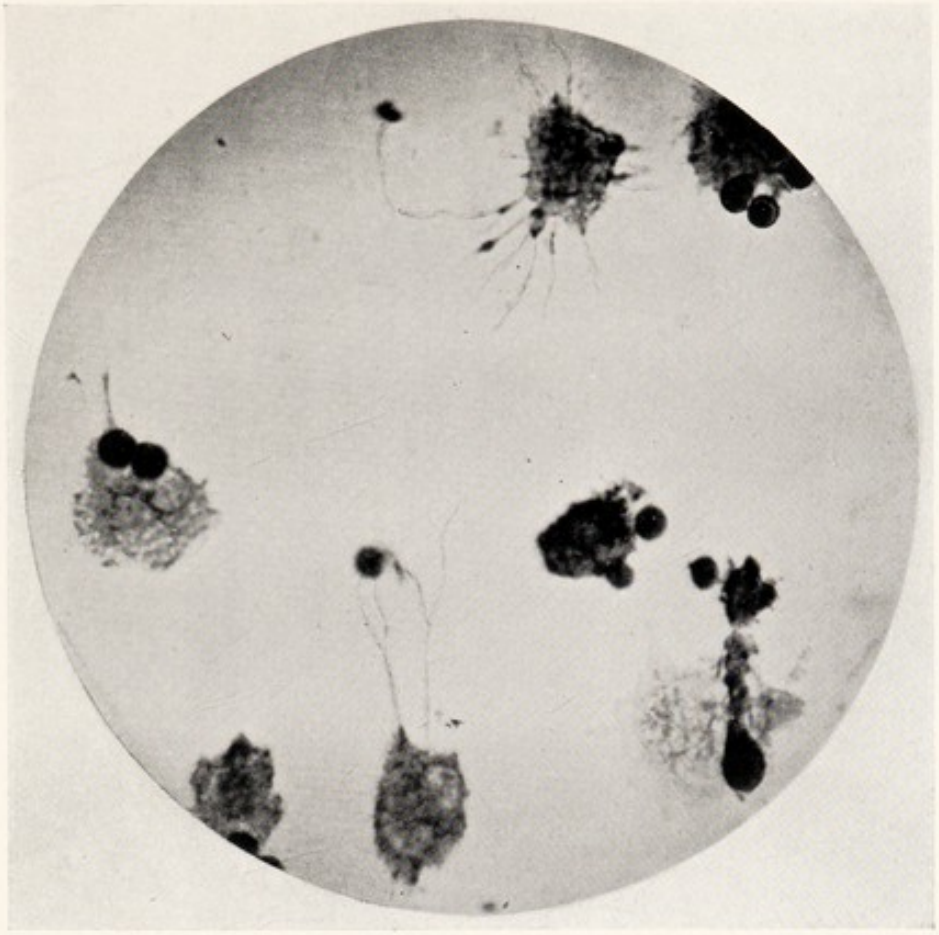


Fig. 29.

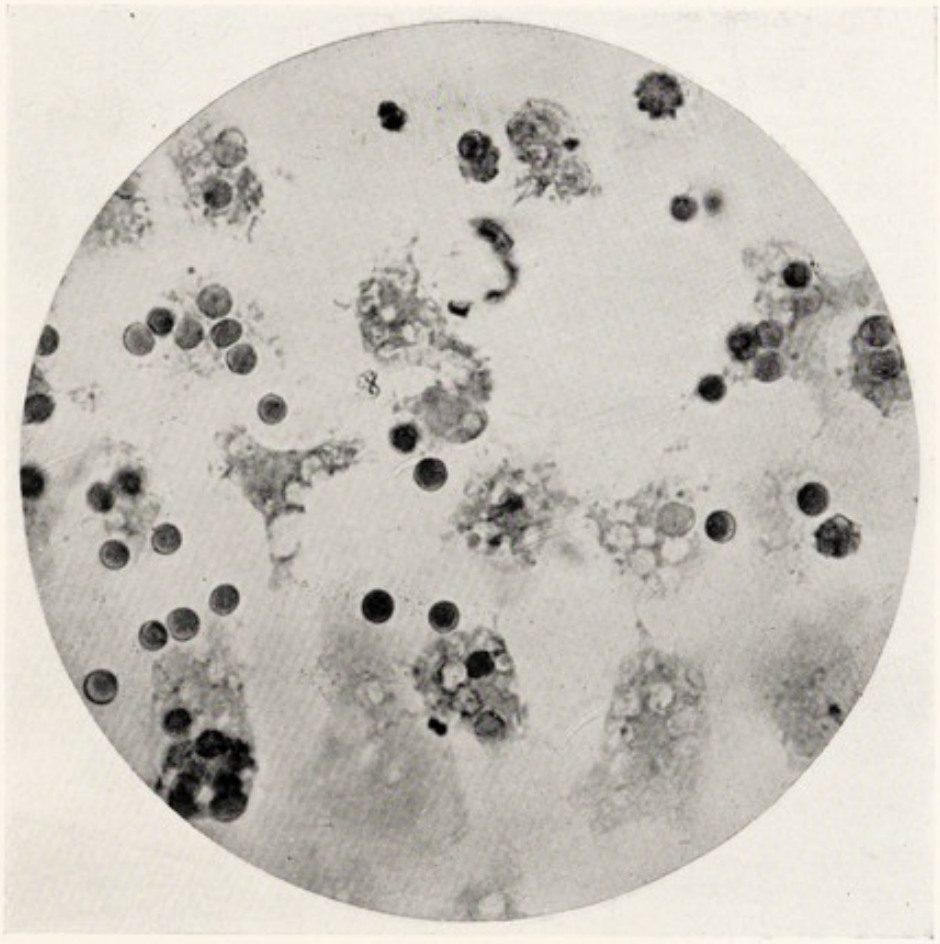


Fig. 30.

FIG. 31.—HUMAN BLOOD INCUBATED WITH SHEEP WASHED RED CELLS: LEUCOCYTE FILM DRIED IN AIR, STAINED WITH LEISHMAN. (×900.)

Shows two dehæmoglobinised, unstained, and ingested sheep red cells, A, and one larger non-decolourised, non-ingested human red cell, B. The lower cell has been approximated to the others in order to include it in the illustration.

(Page 40.)

FIG. 32.—HUMAN DEFIBRINATED BLOOD INCUBATED AFTER STANDING SIX HOURS AT ROOM TEMPERATURE: IODINE SALINE. (×700.)

The leucocyte film was fixed in boiling normal saline and treated with benzidine base 0·05 solution in normal saline, then washed and treated with iodine 1 per cent. solution in normal saline. Shows black "Diffusion" substance granules and masses in the leucocytes.

(Page 44.)

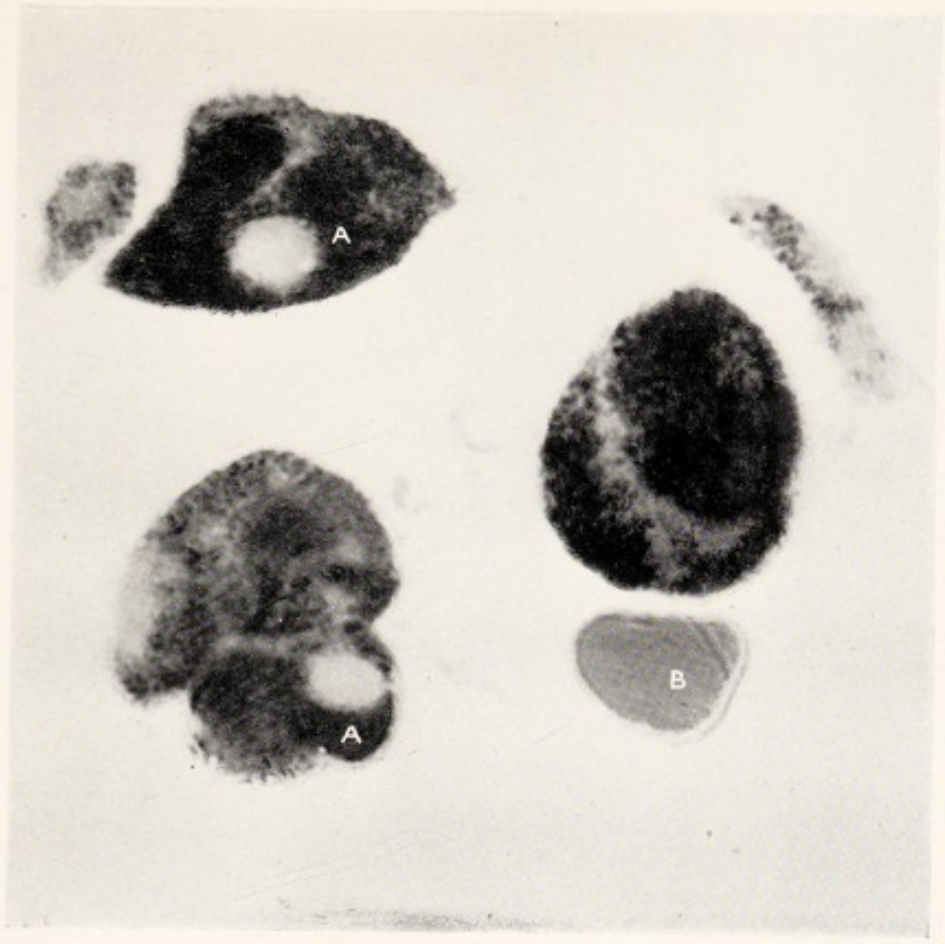


FIG. 31.

FIG. 32.

FIG. 33.—BLOOD INCUBATED FROM A CASE OF PNEUMONIA: IODINE SALINE. (×960.)

The leucocyte film was fixed and treated with benzidine and iodine. Shows extra-cellular black "Diffusion" substance around the disintegrating leucocytes.

(Page 44.)

FIG. 34.—HUMAN BLOOD INCUBATED: NORMAL SALINE. (×900.)

The leucocyte film was fixed in boiling saline and treated with benzidine, iodine, and erythrosin. Shows the liberation of the "Diffusion" substance at the extremities of the pseudopods and dendrites.

(Page 44.)

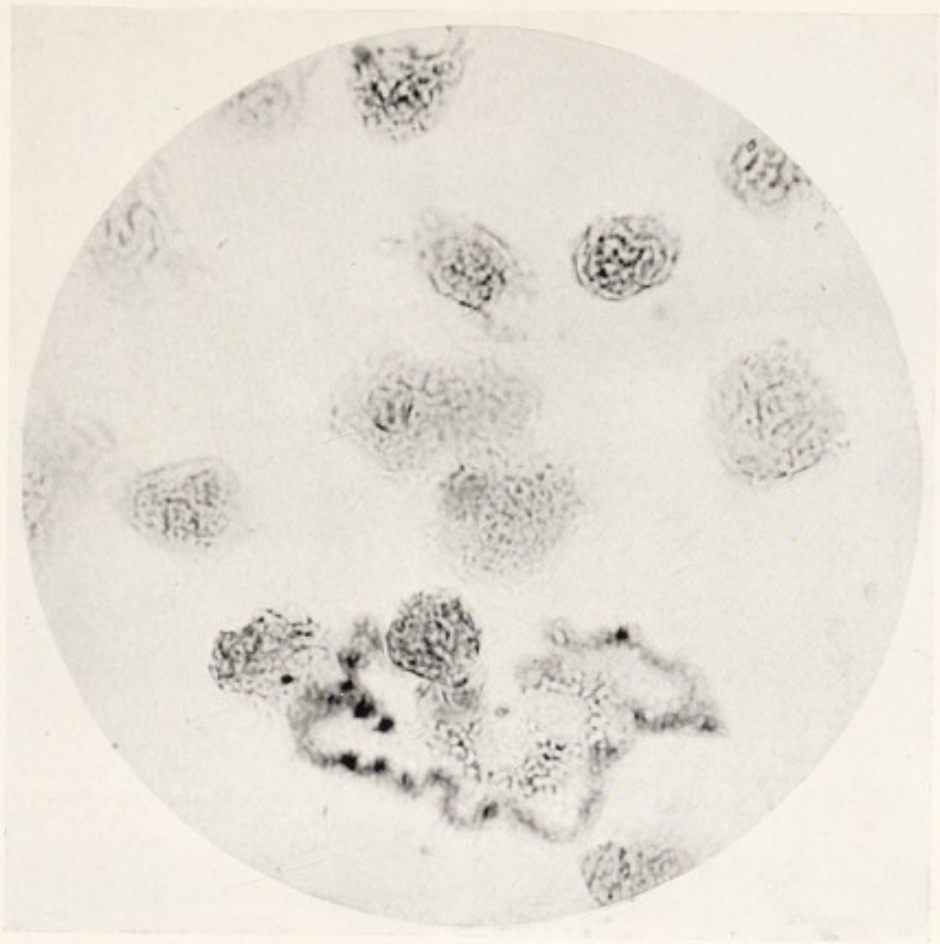


Fig. 33.

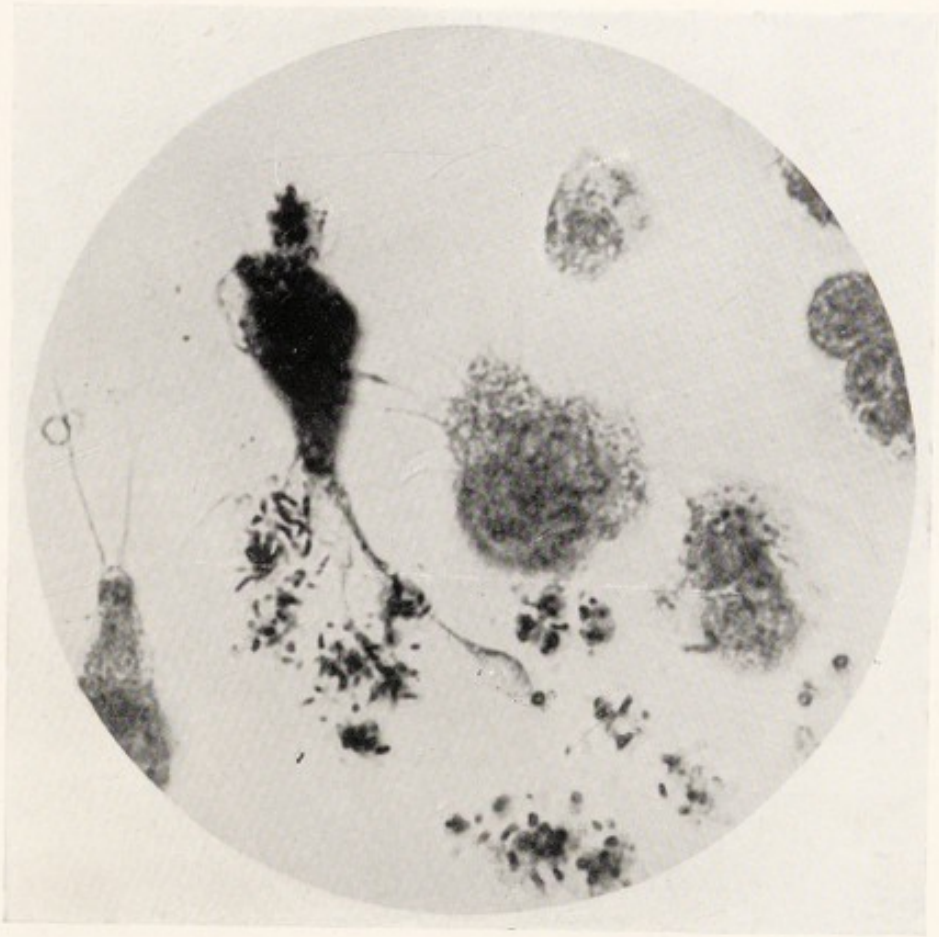


Fig. 34.

FIG. 35.—HUMAN BLOOD INCUBATED: NORMAL SALINE. (×500.)

The leucocyte film was fixed in boiling saline, then treated with benzidine, iodine, and erythrosin. Shows mass liberation of "Diffusion" substance by two exploding leucocytes.

(Page 44.)

FIG. 36.—EPITHELIAL CELLS FROM A URETHRAL SMEAR DRIED IN AIR STAINED WITH IODINE VAPOUR, AND MOUNTED IN CEDAR OIL. (×640.)

Shows "Iodophil" substance in the epithelial cells, X.

(Page 49.)

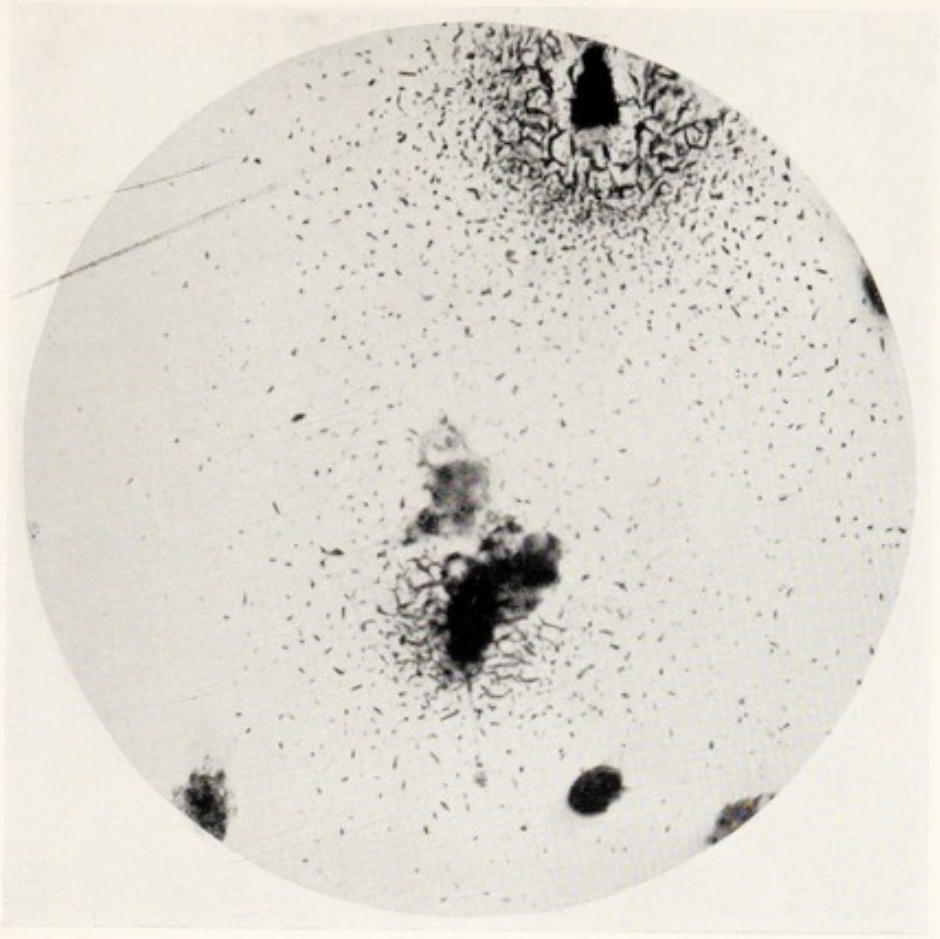


Fig. 35.

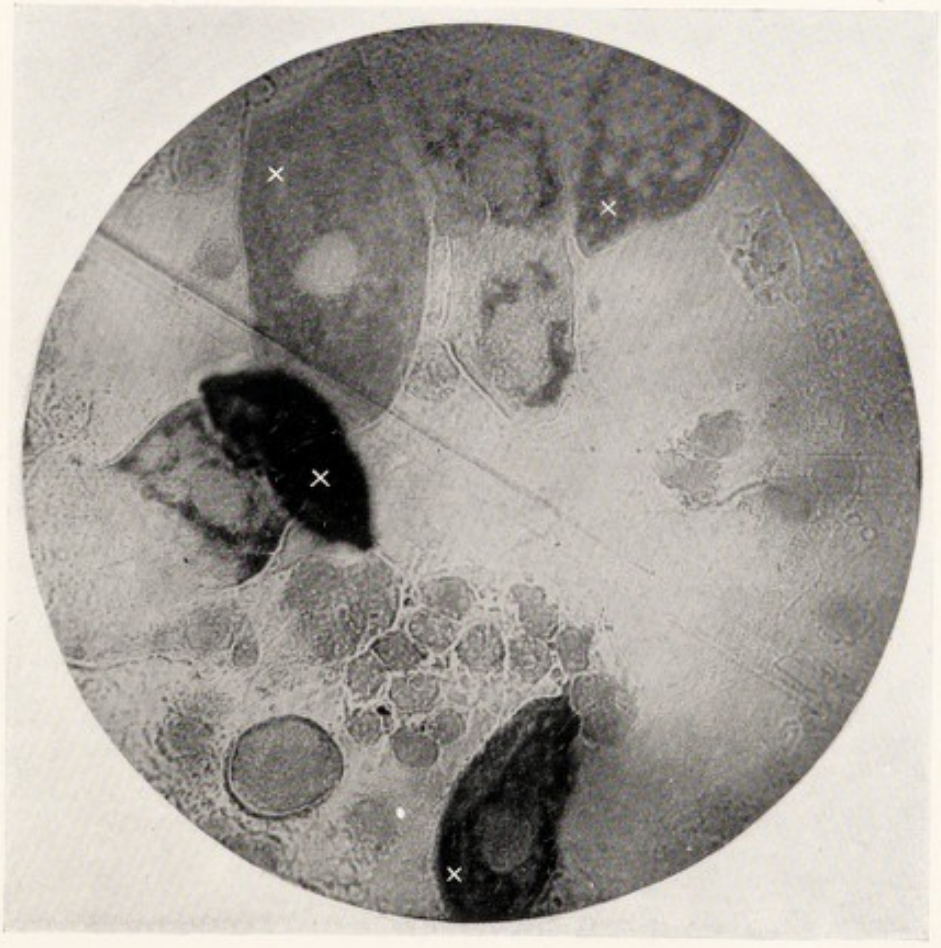


Fig. 36.

FIG. 37.—ALCOHOL PARAFFIN SECTION FROM A CASE OF EPITHELIOMA OF THE SKIN OF THE HAND MOUNTED IN IODINE GUM SALINE. (×680.)

Shows masses of "Iodophil" substance in the epithelial cancer cells, X.

(Page 50.)

FIG. 38.—FROZEN SECTION FROM A MYELOMA OF THE FIBULA MOUNTED IN IODINE GUM. (×680.)

Shows "Iodophil" substance in the large multinucleated myeloma cells; this is absent in the cells of the stroma.

(Page 50.)

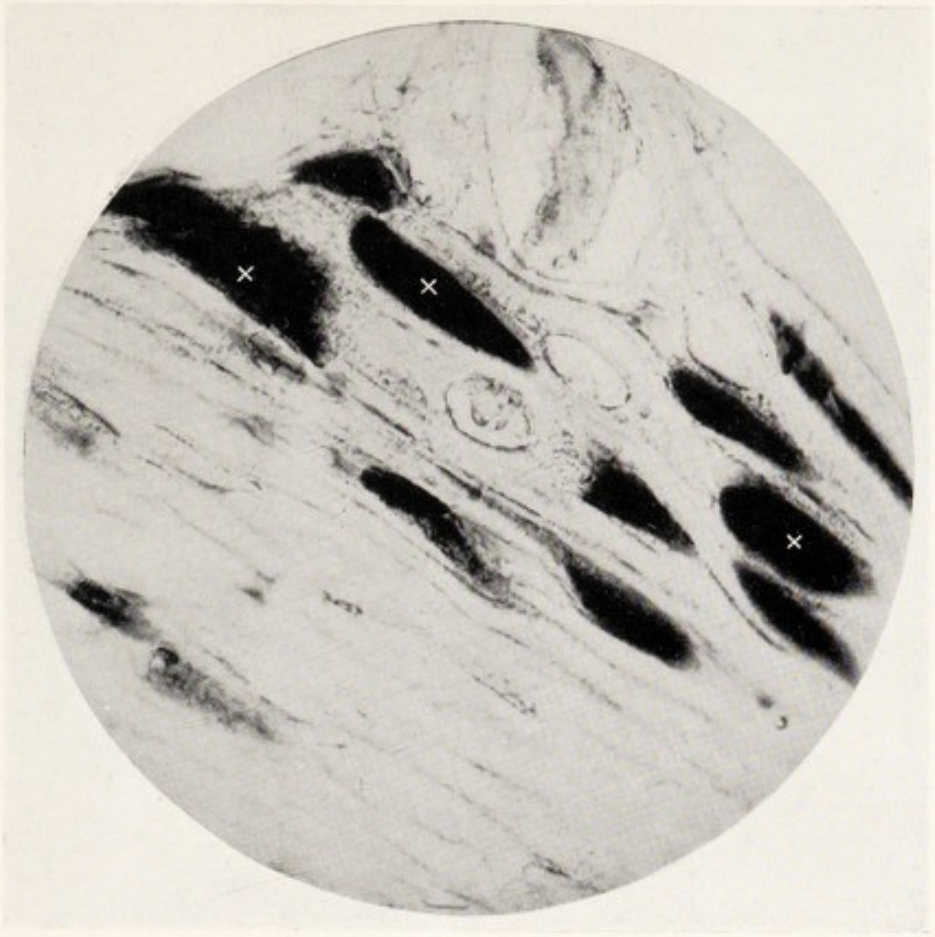


FIG. 37.

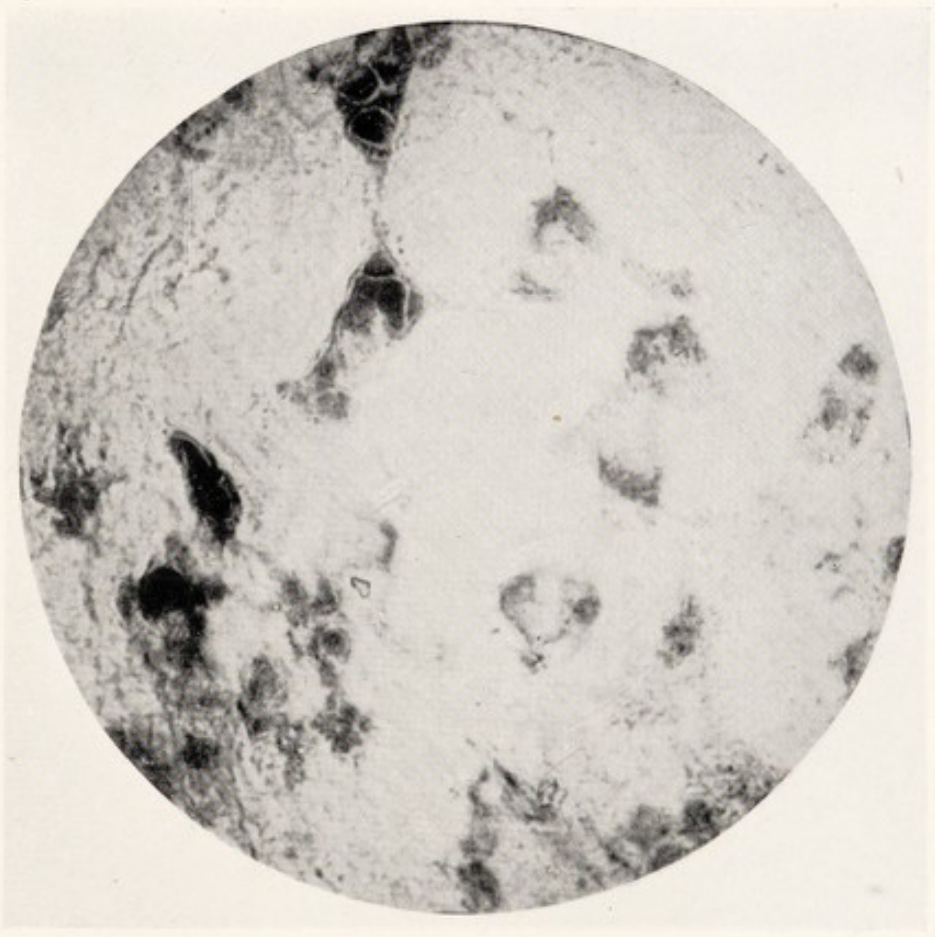


FIG. 38.

FIG. 39.—EPITHELIAL CANCER CELLS FROM A LYMPH GLAND IN A CASE OF EPITHELIOMA OF THE VULVA MOUNTED IN IODINE GUM. (×640.)

Shows "Iodophil" substance in the cancer cells in the lymph gland.

(Page 50.)

FIG. 40.—CANCER CELLS FROM A LYMPH GLAND FROM A CASE OF EPITHELIOMA OF THE FLOOR OF THE MOUTH: SCRAPING FROM GLAND MOUNTED IN IODINE GUM. (×640.)

Shows black "Diffusion" substance granules in a cancer cell.

(Page 51.)

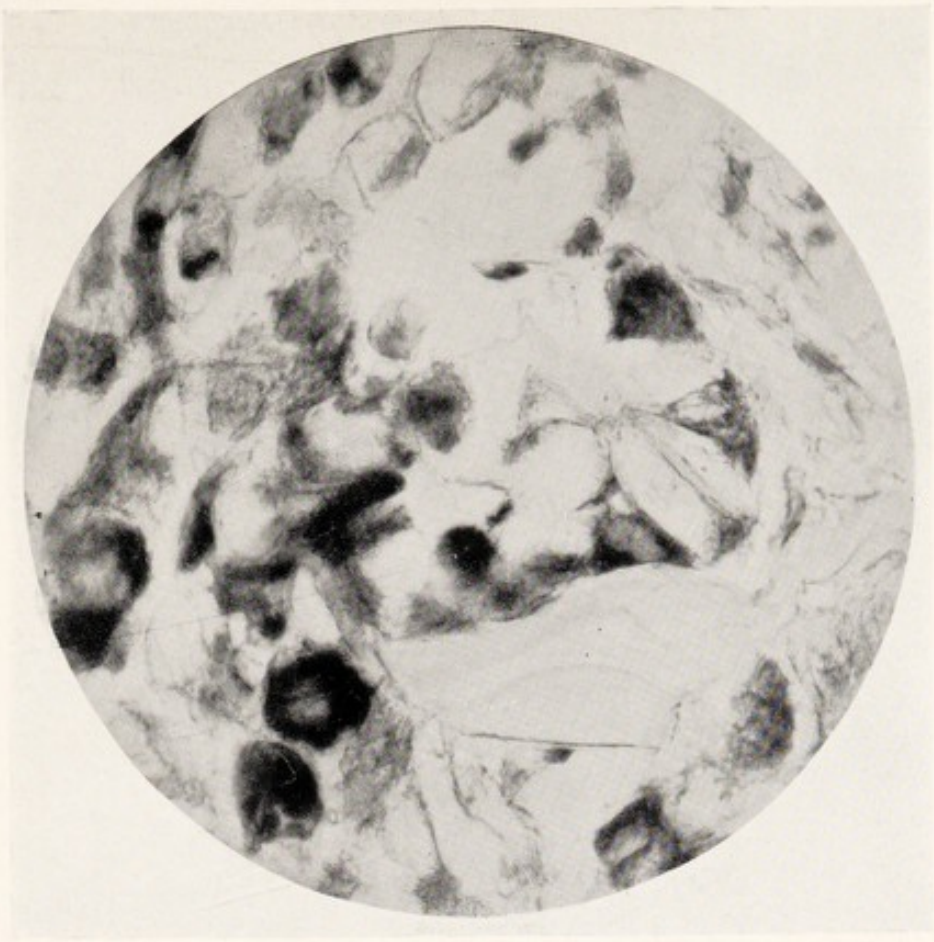


FIG. 39.

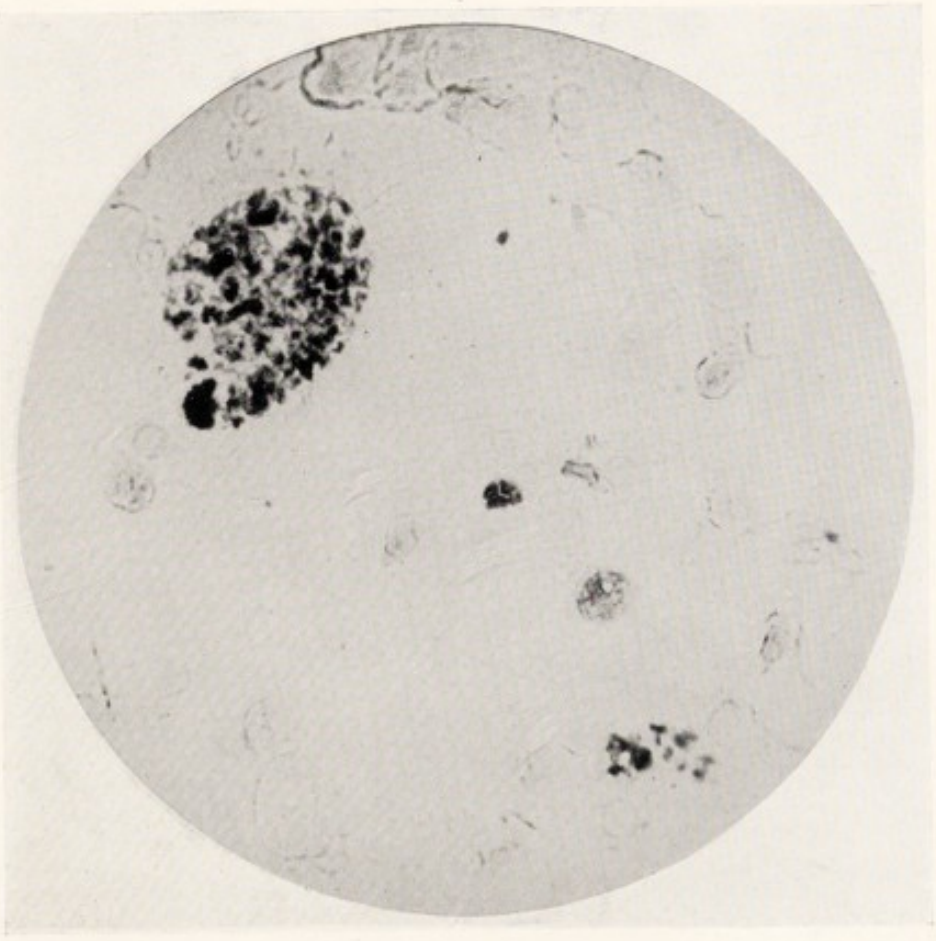


FIG 40.

FIG. 41.—HUMAN BLOOD INCUBATED WITH NATIVE RED CELLS PREVIOUSLY TREATED WITH SHEEP BLOOD-SERUM : IODINE SALINE. (×960.)

Shows stimulation of the leucocytes and phagocytosis of the opsonised native red cells.

(Page 59.)

FIG. 42.—HUMAN (C.J.B.) BLOOD, THREE DROPS, INCUBATED WITH ONE DROP ACETONE EXTRACT OF C.J.B. RED CELLS, EXTRACT EVAPORATED TO REMOVE THE ACETONE AND REDISSOLVED IN NORMAL SALINE: IODINE SALINE. (×450.)

The film shows agglutination and ingestion of native red cells by C.J.B. leucocytes.

(Page 65.)

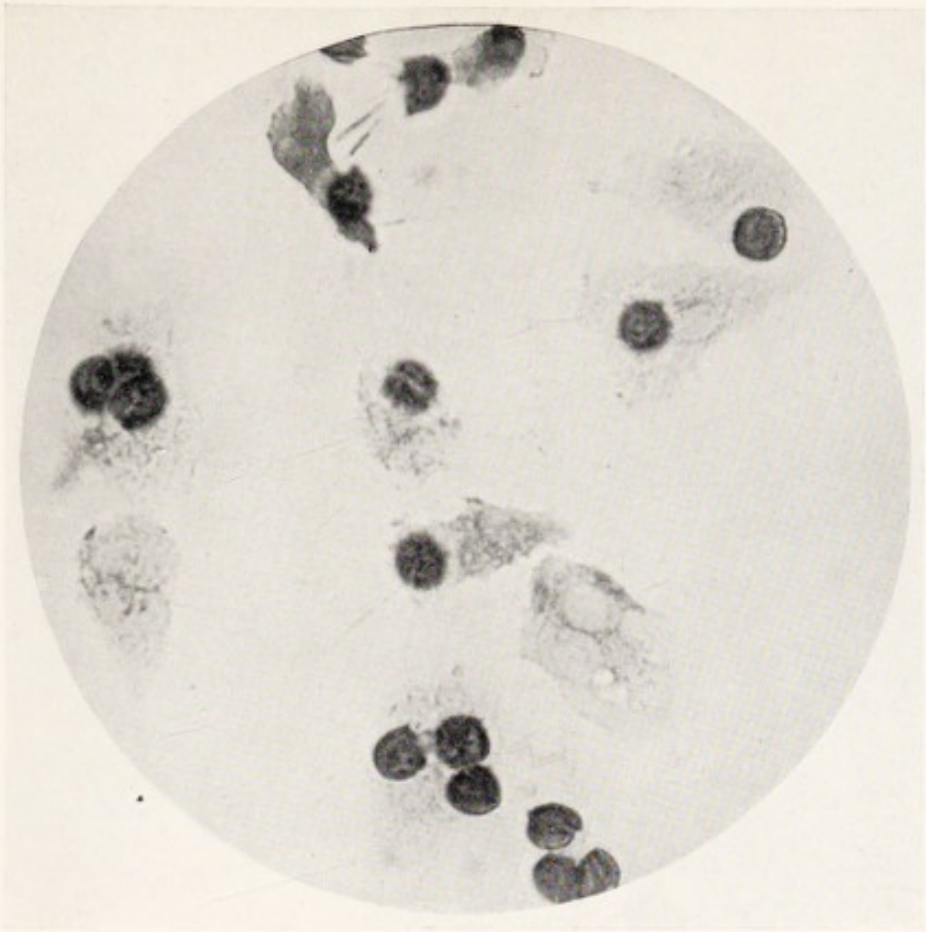


FIG. 41.

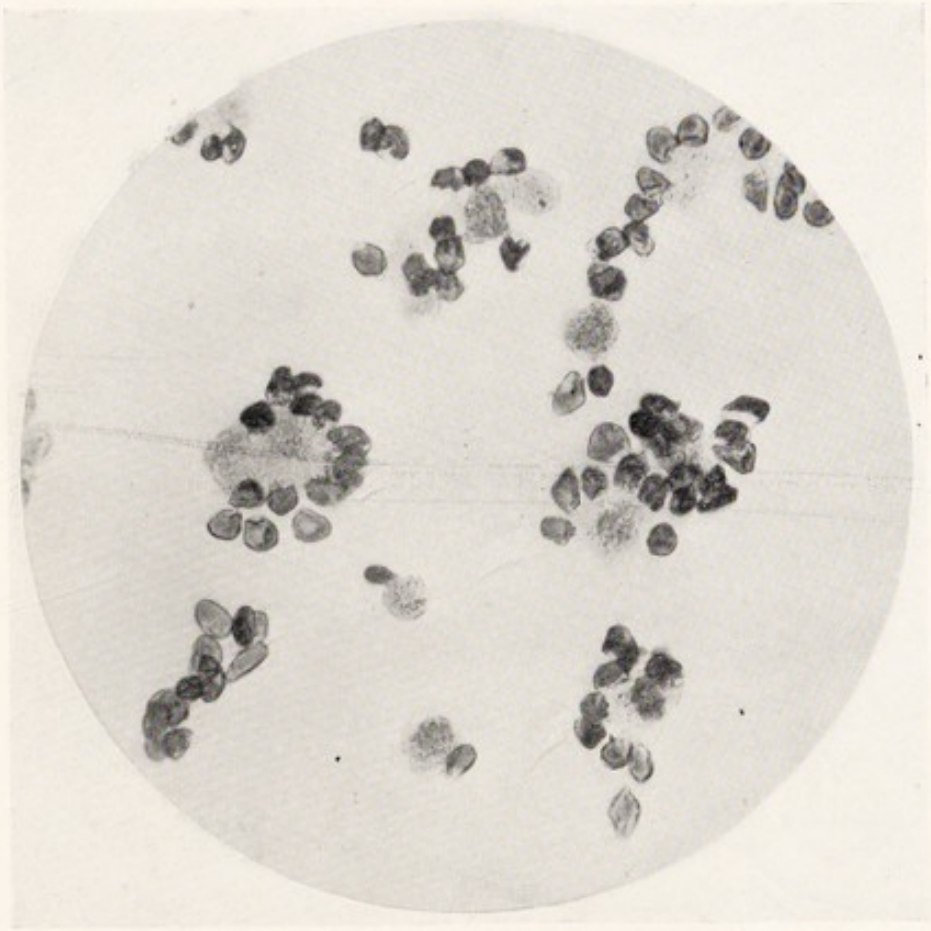


FIG. 42.

FIG. 43.—BLOOD FROM A CASE OF ACUTE LOBAR PNEUMONIA INCUBATED: IODINE SALINE. (×700.)

The washed red clot was reincubated with washed native red cells. The film shows ingestion of the native red cells by the emigrating leucocytes.

(Page 68.)

FIG. 44.—ACUTE LOBAR PNEUMONIA BLOOD INCUBATED: IODINE SALINE. (×680.)

The washed red clot was reincubated with washed native red cells. The film shows the agglutination and ingestion of the native red cells by the pneumonia blood leucocytes.

(Page 68.)

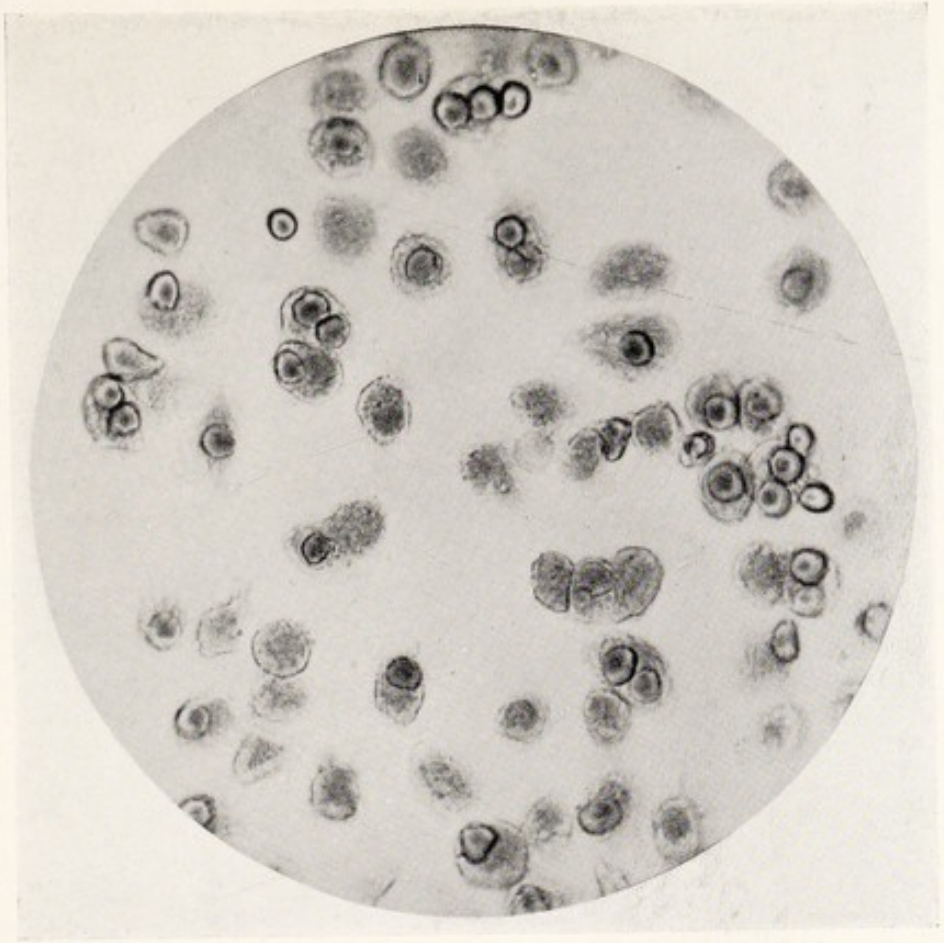


Fig. 43.

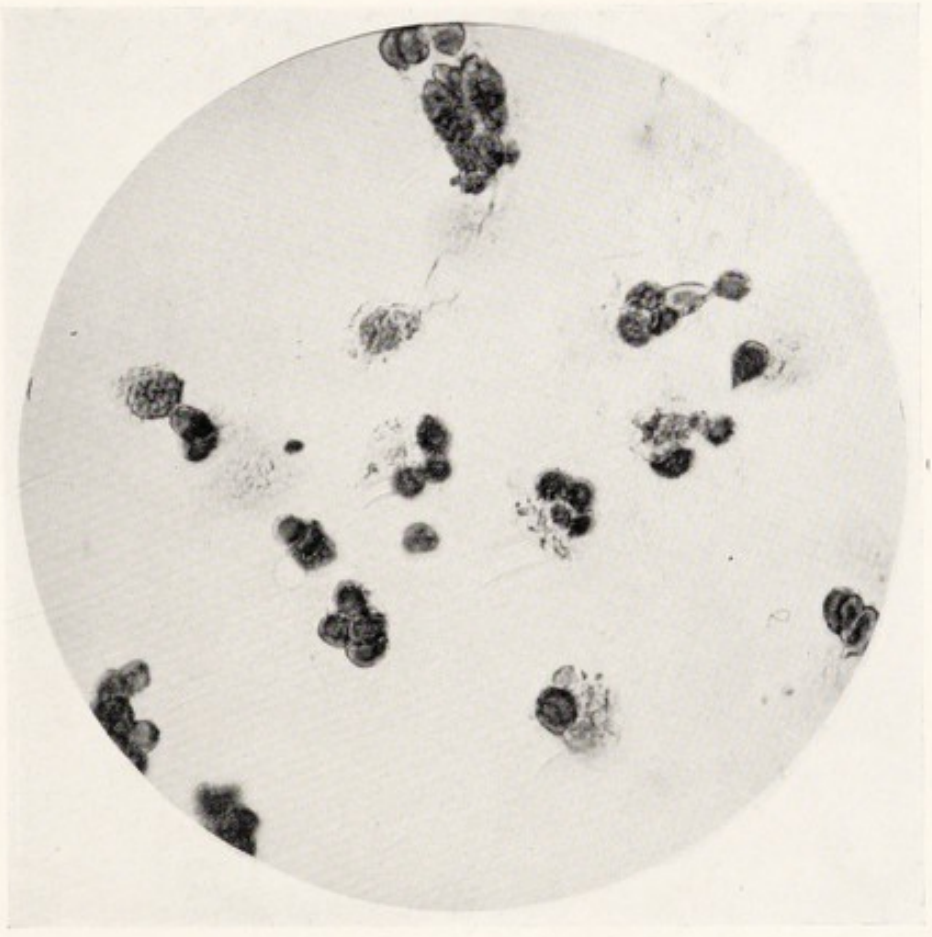


Fig 44.

FIG. 45.—HUMAN (C.J.B.) BLOOD INCUBATED WITH NATIVE (C.J.B.) RED CELLS PREVIOUSLY TREATED WITH THE BLOOD-SERUM FROM A CASE OF ACUTE PNEUMONIA: IODINE SALINE. (×960.)

The red cells were rewashed before incubation. The film shows the ingestion of the treated native red cells by C.J.B. leucocytes.

(Page 72.)

FIG. 46.—HUMAN (C.J.B.) BLOOD INCUBATED WITH NATIVE RED CELLS PREVIOUSLY TREATED WITH THE BLOOD-SERUM FROM A CASE OF PNEUMONIA IN THE APYREXIAL STAGE: IODINE SALINE. (×580.)

The film shows the agglutination and ingestion of native red cells by C.J.B. leucocytes.

(Page 72.)

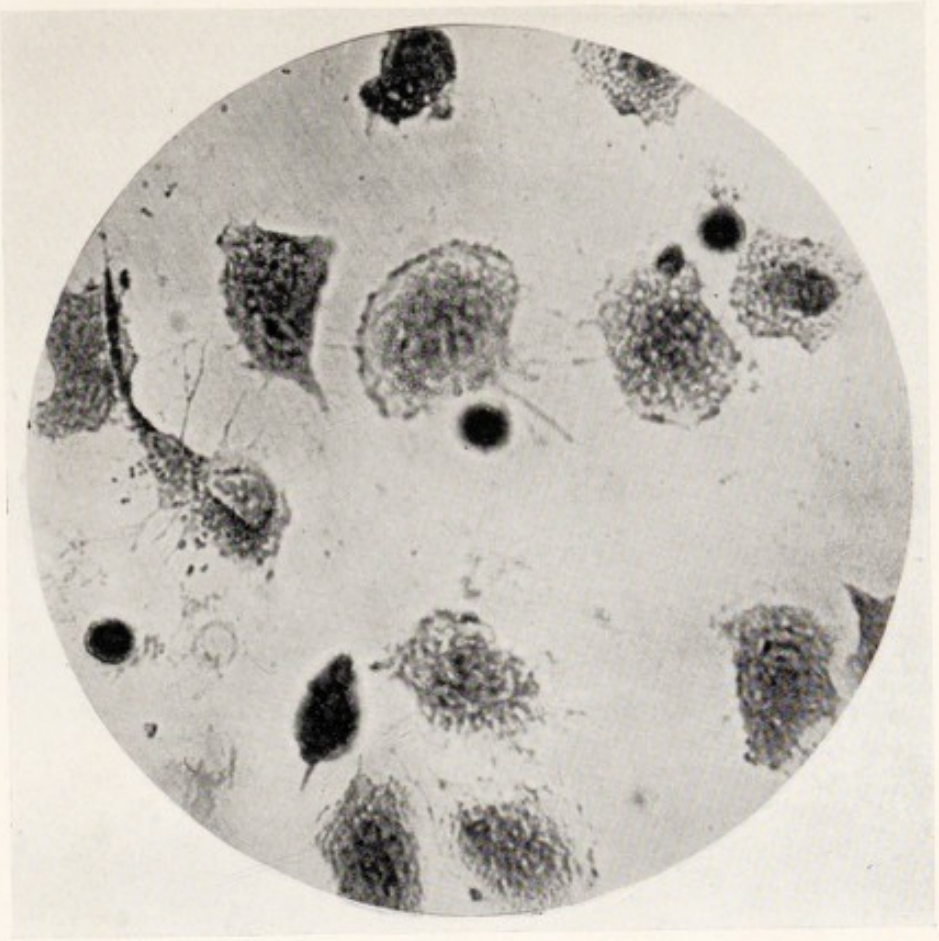


FIG. 45.

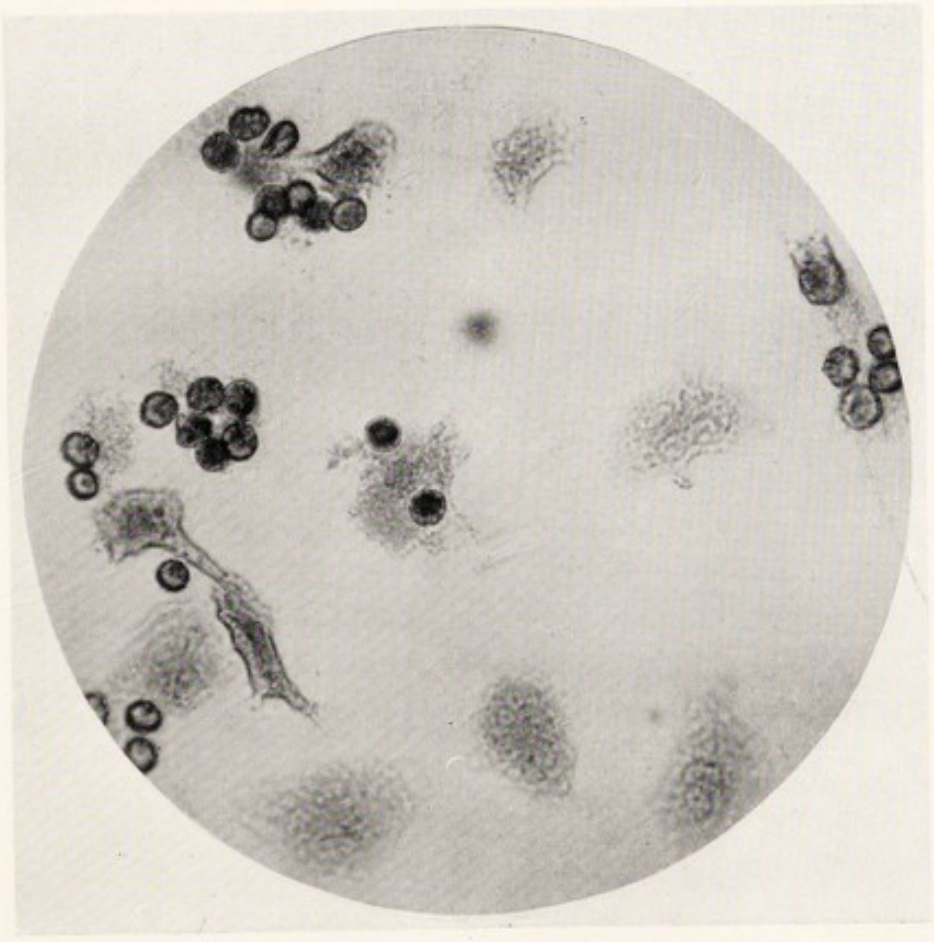


FIG. 46.

FIG. 47.—HUMAN (C.J.B.) BLOOD INCUBATED WITH BLOOD-SERUM FROM THE SPLENIC VEIN DURING THE OPERATION OF SPLENECTOMY FOR AN ENLARGED SPLEEN: IODINE SALINE. (D.G.×780.)

The leucocyte film shows marked stimulation of the leucocytes with migration of the nuclear lobes in the elongated cells.

(Page 75.)

FIG. 48.—HUMAN (C.J.B.) BLOOD INCUBATED WITH THE BLOOD-SERUM FROM THE GENERAL CIRCULATION (FINGER) OF THE SAME PATIENT: IODINE SALINE. (D.G.×780.)

Shows a less degree of leucocytic stimulation and less dispersal of the nuclear lobes.

(Page 75.)

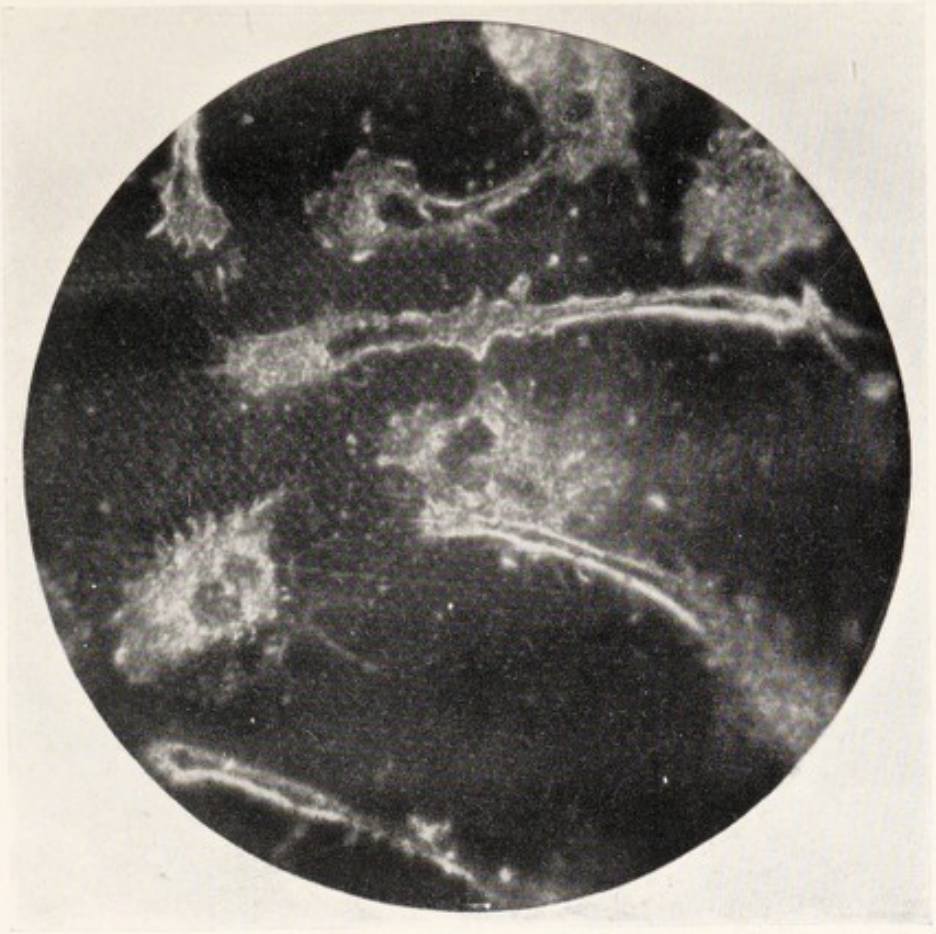


FIG. 47.

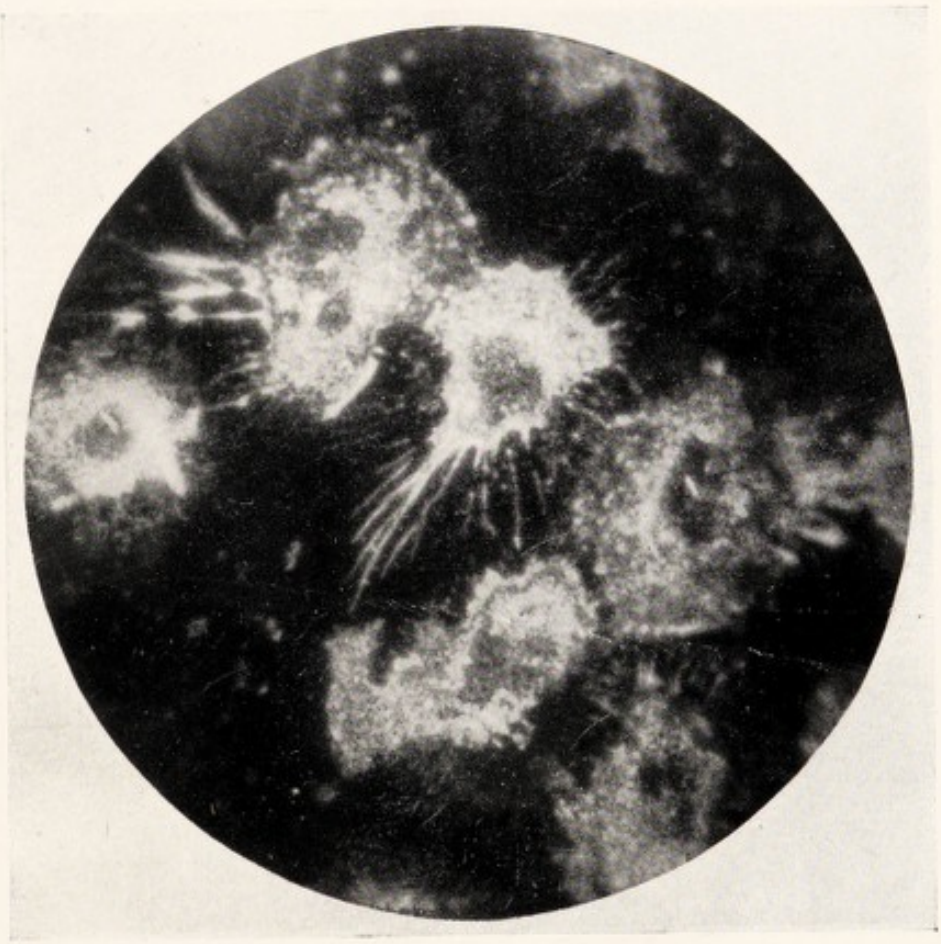


FIG. 48