men prior to their receiving treatment. Urine tests when required should be carried out beforehand.

Some such system of peripatetic clinics might well be utilized by the public health authorities when they deal with the control of syphilis among the civilian population, although in this country there are probably few places not within reach of one of the larger towns, where it is presumed treatment clinics already exist.

REFERENCE.

¹ *Syphilis and the Army*. London, 1917. University of London Press. (6s. net.)

ON THE AGGLUTINATIVE AND OPSONIC ACTION

OF CERTAIN CHEMICAL SUBSTANCES ON THE RED BLOOD CORPUSCLES, THE RELATION BETWEEN THIS ARTIFICIAL AGGLUTINATION AND AGGLUTINATION BY SERUM, AND THE BEARING OF THESE OBSERVATIONS ON THE AGGLUTINATION AND PHAGOCYTOSIS OF PATHOGENIC ORGANISMS.

BY

C. J. BOND, C.M.G., F.R.C.S., HON. COLONEL A.M.S.,
HONORARY CONSULTING SURGEON, NORTHERN COMMAND; HONORARY
CONSULTING SURGEON TO THE LEICESTER ROYAL INFIRMARY;
MEMBER OF THE MEDICAL RESEARCH COMMITTEE.

IN THE BRITISH MEDICAL JOURNAL of December 8th, 1917, I drew attention to the association that exists between the haemagglutinative and the opsonic actions of a foreign serum on washed red blood cells in animals and in the human subject. Although the agglutination of the individual red cells, *inter se*, seems frequently to be a preliminary step in the ingestion of these cells by leucocytes, a mutual adhesiveness between red cells and leucocytes is also necessary before actual ingestion can occur. The process of haemagglutination is thus one phase in a larger erythro-leucocyte reaction by which the phagocytosis of red blood cells is brought about.

Not only does the opsonization (or preparation of red cells for ingestion by leucocytes) depend on preliminary action by a serum agglutinative to the particular red cells to be ingested, not only does the number of red cells ingested vary quantitatively with the haemagglutinative capacity of the serum used, but actual ingestion does not occur unless a mutual adhesiveness between phagocytes and red cells is also present.

A careful analysis of this process of so-called haemagglutination under controlled conditions will probably throw further light on an allied problem—namely, the conditions under which pathogenic organisms are prepared for ingestion by phagocytes, and to what extent, if any, previous agglutination by humoral agency is a necessary step in the phagocytosis of bacteria and other organisms. It is on these grounds that the haemagglutinin reaction becomes so important.

The Leucotoxicity of Certain Serums.

IN THE BRITISH MEDICAL JOURNAL of December 8th, 1917, I referred to the fact that a serum may be haemagglutinative to the red cells and yet be toxic to the leucocytes in any given specimen of blood. Under these conditions ingestion does not occur.

This leucotoxic action can be demonstrated by incubating a drop of the whole blood in a closed cell with the foreign serum. If the serum be toxic the leucocytes do not emigrate from the clot on to the slide, or any cells that do leave the clot fail to adhere to the slide and undergo cytolysis. No ingestion of red cells takes place. Thus, in order to obtain successful phagocytosis of red cells the erythro-leucocyte-serum combination must be favourable.

The mechanism by which the successful phagocytosis of bacteria is brought about very probably depends on a right combination of analogous factors.

The Erythro-leucocyte-serum Reaction is not Wholly a Specific Reaction.

HAVING thus seen that the phagocytosis of red cells by leucocytes depends on a delicately balanced humoral cellular reaction which affects serum, red cells, and leucocytes, we now proceed to inquire whether this erythro-leucocyte-serum reaction is specific—that is to say,

whether, in order to bring about the ingestion of the red cells in any given case, the serum which induces the preliminary haemagglutination must be native to the leucocytes, or whether any foreign serum which agglutinates red cells will have an equal opsonic effect. In other words, to what extent can we vary the different factors concerned in the reaction?

While undoubtedly the best result is obtained by employing a native sero-leucocyte combination acting on foreign red cells, experiment shows that it is not absolutely necessary for ingestion that the serum and the leucocytes should be provided by the same individual. In some cases a serum may produce agglutination of the red cells, but fail to bring about ingestion by foreign leucocytes.

The three factors concerned in erythro-phagocytosis may be present but not in right combination or in right proportion. In other cases the very marked specific nature of the reaction suggests that the haemagglutinins have actually been shed into the serum by the leucocytes.

Provided that the foreign serum is not toxic to the leucocytes, then any serum which agglutinates the red cells may prepare them for ingestion up to a certain point, but for complete ingestion on a rapid and large scale, in which almost every leucocyte contains a number of red cells, a further stage of the erythro-leucocyte reaction, or an agglutination of the red cells to the leucocytes, must also be present. This is shown by the following experiments:

1. C. J. B. washed red cells previously agglutinated by a foreign serum, and subsequently washed free of that serum in normal saline, were then incubated with C. J. B. whole blood. Under these conditions slight ingestion of the red cells so treated by C. J. B. native leucocytes occasionally took place.
2. Sheep's washed red cells which had undergone a preliminary agglutination by a non-toxic foreign serum were ingested by C. J. B. leucocytes in larger numbers than sheep's red cells not so previously agglutinated.

The Condition of the Red Cells Influences Ingestion:

The Leucotoxicity of Broken up Red Cells.

While the sero-leucocyte combination has a great influence on the phagocytosis of red cells, the condition of the red cells themselves is also very important. Erythrocytes seem to be more resistant to the phagocytic action of their own native leucocytes than to leucocytes from another individual.

The leucocytes of some individuals seem to have a greater capacity for ingesting sheep's washed red cells than the leucocytes of other persons. Certain animal erythrocytes—for instance, sheep's red cells—are more easily and rapidly ingested by human leucocytes than foreign human erythrocytes from another individual.

The relative capacity of resistance to ingestion by the red cells of different human individuals also varies under different conditions. Thus my own (C. J. B.) red cells, previously washed with normal saline, were ground up in a mortar, a suspension of these disintegrated red cells was made in normal saline. The centrifuged sediment was found to consist of broken up portions of red cells which still retained their haemoglobin constituent. This corpuscular debris formed a yellowish-grey deposit when centrifuged, and on this bottom layer an upper layer of still intact red cells rested if the trituration had not been too complete.

A drop of this suspension of red cell debris in saline was then incubated in a closed cell with two drops of whole C. J. B. blood. The following striking effect was observed. After incubation the floor of the cell was found coated with the red cell debris. This adhered very closely to the glass and was not removed by a gentle stream of saline. This red cell debris also had a marked toxic effect on the leucocytes. Very little emigration of leucocytes from the clot took place in the areas occupied by the debris, while in other free areas a few leucocytes which did emigrate and adhere to the slide showed disintegration changes and gave no iodophil reaction. Thus while whole red cells are not toxic to native leucocytes, broken up red cells are highly toxic to those cells.

But if, instead of employing native red cells, broken up foreign red cells from another individual are incubated with the same C. J. B. whole blood, then the toxic effect on the leucocytes is much less and considerable emigration may occur. This is the case even if the blood serum of the individual supplying the red cells is agglutinative to the red cells of the person supplying the leucocytes.

Again, broken up sheep's red cells incubated with C. J. B. leucocytes are less toxic than broken up foreign human red cells.

What is the meaning of the leucotoxic effect of disintegrated red cells on native leucocytes when the same red cells in the unbroken condition are quite innocuous?

We shall see later, when dealing with the artificial agglutination of red cells by chemical reagents, that it is possible to reduce this toxicity of the disintegrated cells by treating the red cell debris with certain reagents such as liquid paraffin or gum solution before incubation. It would seem, therefore, that while the intact living red cell has no injurious effect on the leucocytes, it still contains a substance or substances which may be poisonous to these cells if ingested in sufficient quantity. Experiment further shows that this substance is not haemoglobin. It may be that the haemagglutinative process acting on the stroma of the red corpuscle brings about a leakage of this endocorpuscular poison. If this is present in the plasma in moderate amount it stimulates an adhesive reaction on the part of the leucocyte, and promotes phagocytosis, but if it is liberated in larger amounts, as in the case of the broken up red cells, it then poisons the leucocytes.

We have now seen that the mechanism by which the red cells are prepared for ingestion by leucocytes consists in its most effective form of an agglutinative serum in combination with native leucocytes acting on foreign red cells—that is, red cells which contain the agglutinin necessary to react with the haemagglutinin in the foreign serum. We also found that we can replace the native serum by a foreign agglutinating serum (provided this be non-toxic to the leucocytes), and still obtain a certain amount of phagocytosis of the foreign red cells, and even under certain conditions the ingestion of native red cells by their own leucocytes if these red cells have been previously agglutinated.

The Agglutination of Red Cells by Chemical Reagents.

The question now arises whether it is possible to do away with the agglutinating serum altogether, substituting for it some chemical reagent, and still obtain the ingestion of the red cells. I have tested a considerable number of substances in order to clear up this point. The reagents which have so far given the best results have been liquid paraffin and a 5 per cent. solution of gum acacia in normal saline.

We must first describe the effect of these reagents on the leucocytes when incubated with whole blood. The floor and sides of a closed cell are painted over with a thin layer of liquid paraffin. Two or more drops of blood are then allowed to fall directly from the finger into the cell. After incubation the clot is washed away in a gentle stream of saline and a film of living leucocytes obtained on the slide. These show pseudopodial activity, and, when tested with 1 per cent. iodine solution (see BRITISH MEDICAL JOURNAL, February 3rd, 1917), give a very marked iodophil reaction, in which also the mauve-coloured drop of iodophil substance remains attached to the cell, and seems less prone to exude into the surrounding medium than in leucocytes incubated without paraffin. Some of the cells also contain droplets of the paraffin in the cell protoplasm. Thus the coating of paraffin seems to protect the phagocytes from disintegration and stimulates them to increased activity. The well known fact that coating with paraffin the walls of a vessel into which blood has been drawn delays coagulation may thus depend, not only on the smooth surface, but also on the influence of the paraffin in preventing the rapid exudation of ferments from the leucocytes into the surrounding plasma. The fact that the iodophil substance tends to be retained in the cell in the presence of liquid paraffin is of interest in this connexion; 5 per cent. gum solution in normal saline also has the same effect on leucocytes when incubated with them.

Liquid paraffin when used as a dressing for wounds seems to favour phagocytosis and to promote the separation of necrotic tissue. An examination of the pus cells recovered from gauze soaked in 5 per cent. gum solution when used as a dressing for wounds also shows a high percentage of living phagocytes. The test of vitality is the retention by the cells of an irregular instead of a circular outline, a capacity to elaborate iodophil substances as shown by the iodine test, and power to ingest pigment

particles, or under appropriate conditions red blood cells on incubation.

The Agglutinating Action of Gum Solution and Liquid Paraffin on the Red Cells.

If to a large drop of 5 per cent. gum acacia solution in normal saline a smaller drop of a suspension of washed human red cells in normal saline be added on a slide and the two mixed together, the red cells will agglutinate in a few minutes. It is impossible to distinguish under the microscope this artificial agglutination from the clumping produced by an agglutinating serum.

When washed red cells are thoroughly mixed in liquid paraffin, on the other hand, although discrete little masses of cells are formed, this effect seems to be due to the mechanical action of the reagent in enclosing and isolating groups of cells, rather than to the production of an increased mutual stickiness in the cells themselves.

When sheep's washed red cells are mixed with liquid paraffin the cell masses are smaller than in the case of human red cells, and this causes an even greater resemblance to true haemagglutination. Many other chemical substances, of course, when added in certain proportions to a suspension of washed red cells in saline, bring about a clumping which cannot be distinguished under the microscope from the agglutination produced by serum. Among antiseptic reagents, acriflavine, 1 per 1,000 solution in normal saline, gives this result in a striking manner. In the case of most antiseptic substances, however, the cells undergo rapid injury and eventual disintegration. This does not occur with gum solution and liquid paraffin.

The Opsonic Action of Liquid Paraffin and Gum Solution on the Red Cells.

The question now arises whether red cells which have undergone artificial agglutination by gum or paraffin are also opsonized, or prepared for ingestion by leucocytes, in the same way that red cells are prepared when acted on by an agglutinating serum. The evidence suggests that this may be the case under certain conditions.

(a) The effect of agglutination on broken up red cells. If a drop of the red cell debris in normal saline previously described be added to a drop of any agglutinating serum on a slide a true agglutination takes place of the fragments of red cells. The colour of the agglutinated mass is yellowish-grey instead of brick red, and the reaction is slower and less complete than in the case of whole red cells treated in the same manner. Fragmented red cells are thus capable of agglutination like whole cells. If now, instead of using an agglutinating serum to clump the disintegrated cells, we use gum solution and add a drop of the agglutinated and sedimented debris to a drop of blood from the same individual and incubate in a closed cell, we find that the toxic effect previously shown by the unagglutinated red cell debris on the leucocytes is absent. The leucocytes emigrate freely from the clot, and some may ingest the red cell debris. If instead of gum solution the red cell debris is rubbed up in a mortar with liquid paraffin before incubation with the whole blood, the inhibition of the leucotoxic effect is even more marked.

(b) The effect of artificial agglutination on whole red cells. Apart from its action on disintegrated cells, previous agglutination by paraffin or gum solution also opsonizes whole red cells under certain conditions. We have already seen that washed sheep's red cells when present in considerable quantity are more toxic to the leucocytes of certain individuals than to those of others. If, however, these washed sheep's red cells have been previously shaken up with gum solution or liquid paraffin before incubation the leucotoxic effect is largely prevented. A mutual adhesion occurs between the red cells and the phagocytes and a number of red cells are ingested. Thus preliminary treatment by certain chemical reagents brings about a change in red cells (whole and disintegrated) which not only produces agglutination of the cells and the cell fragments *inter se*, but also reduces their leucotoxic influence and prepares them for ingestion. The appearances suggest that these effects are brought about by some change in the stroma of the cell. This change prevents the endotoxin from diffusing rapidly into the surrounding medium and thus poisoning the leucocytes, at the same time it renders the red cells mutually adhesive and also adhesive to the leucocytes with which they come in contact.

The Relationship between Haemagglutination and Opsonic Action.

It does not, however, follow that reagents which agglutinate the red cells always prepare them for ingestion. This latter effect depends on a mutual reaction between the red cells and the phagocytes by which the two become mutually adhesive.

Some reagents, like mercury perchloride, which in dilute solution agglutinate red cells are themselves toxic to the leucocytes. Others, like acriflavine, which in dilute solution are not markedly leucotoxic, do not seem to promote the ingestion of the red cells. It is a suggestive fact that those reagents which do promote the ingestion of red cells by leucocytes—for example, liquid paraffin and dilute gum solution—are mostly inert substances; they both, in different degrees, protect the shed leucocytes from rapid divitalization. They both stimulate the elaboration of iodophil substances by the leucocytes, and they check the rapid diffusion of this substance from the cells into the surrounding medium. They both affect the density of the serum and alter the surface tension of the cell bodies which they envelop. They are not erythrocidal or haemolytic in character. Thus a 5 per cent. gum solution in normal saline agglutinates washed red cells, but cells so agglutinated are not disintegrated, they can be washed free of the agglutinin in normal saline, and can then be reagglutinated by the gum solution. Reagents, on the other hand, which haemolyze and destroy the red cells prevent ingestion, possibly because they bring about a rapid diffusion of the liberated endotoxin in sufficient volume to poison the leucocytes.

Factors Concerned in the Haemagglutination and Phagocytosis of Red Blood Corpuscles.

The foregoing observations suggest that the phenomena of haemagglutination as it affects the cellular elements of the blood should be described in terms of both red and white cells. Three factors are probably involved in the process:

1. An erythrocytic agglutination in which the red cells agglutinate *inter se*. This process does not necessarily bring about ingestion, but in some cases seems to favour it.
2. A leucocytic agglutination in which the leucocytes themselves clump together irrespective of any action on the red cells. This is seen when leucocytes are acted on by certain animal blood serums and will be described in a subsequent paper.
3. An erythro-leucocytic agglutination in which the red cells and the leucocytes become mutually adhesive. This occurs when opsonic blood serums prepare the red cells for ingestion.

General Conclusions.

It may be that in our search for chemical reagents with which to sterilize wounds we have relied too much on bactericidal capacity and have not sufficiently considered the influence exerted by these substances on the natural defensive mechanism in its humoral and cellular aspects. We should probably do well to regard antiseptics (when effective) as co-operating with the physiological defence in bringing about an agglutination and opsonization of the invading organisms and thus rendering them vulnerable to phagocytic attack.

I am not without hope that the foregoing observations on the artificial agglutination and opsonization of red blood cells by chemical means may throw some light on an allied problem—namely, the mechanism by which the agglutination and opsonization of pathogenic organisms is brought about naturally, and the possibility of aiding this process by chemical means. It would, for example, be of considerable advantage to know what chemical reagents, if any, are capable of agglutinating bacteria and cocci, and whether organisms so artificially agglutinated are also opsonized and rendered easier of ingestion and digestion by phagocytes. The mechanism by which organisms and phagocytes are rendered mutually adhesive, before phagocytosis can take place, is also one of great importance.

ADMIRAL WILLIAM C. BRAISTED, Surgeon-General of the United States Navy, reports that the naval casualties of the war, exclusive of the Marine Corps and of deaths from disease, amounted to 1,233. Practically all the deaths were due to submarine attacks.

CANCER OF THE TONGUE.

ABSTRACT OF THE BRADSHAW LECTURE DELIVERED
BEFORE THE ROYAL COLLEGE OF SURGEONS
OF ENGLAND.*

BY

D'ARCY POWER, M.B.Oxon., F.R.C.S.Eng.,
SURGEON TO AND LECTURER ON SURGERY AT ST. BARTHOLOMEW'S
HOSPITAL.

CANCER of the tongue is remarkable in the fact that it is almost entirely a human disease; it is always of one type; it is unknown in children; it is common in men, rare in women; it is not associated with any inherited predisposition to carcinoma.

Cancer of the tongue did not become important surgically until the seventeenth century. The Greek, Latin, and Arabian writers on surgery hardly mention it, and, so far as can be ascertained at present, it was unknown to the Anglo-Saxons.

The first definite notice of cancer of the tongue is the case of Ralph Freeman, who died on March 16th, 1634, whilst serving the office of Lord Mayor of London. He suffered from secondary haemorrhage, and it was the opinion of the physicians and surgeons who were in attendance upon him that a mercurial course might have been of use at an earlier period of the disease. The second recorded case occurred in Germany and was looked upon as a miraculous punishment for cursing the clergy. The story runs that "lately a certain baron spoke abusively to all and sundry, but kept his most venomous shafts for the clergy and for those who devoted themselves to God's service. One day a holy brother of good repute, whom he had just lashed, said: 'Your foul tongue has overlong deserved that punishment from an offended God which it will shortly receive.' The baron rode off undismayed, but a few days later a small swelling began to grow at the side of his tongue. Little by little it increased in size until it became an inoperable cancer, and the baron, penitent and confessed, died miserably afflicted." From the middle of the seventeenth century onwards cancer of the tongue became so frequent that it was no longer necessary to invoke a miracle to account for its production.

The zoological distribution of lingual carcinoma was considered in the light of the experience of Sir John McFadyean, principal of the Royal Veterinary College, of Dr. J. A. Murray, Director of the Imperial Cancer Research Fund, and of Dr. Anton Sticker. At the present time cancer of the tongue is known to have occurred in one horse, three cats, and one dog. All these animals were aged, and in each the cancer was of the squamous-celled variety.

It appears fair to assume, therefore, that lingual carcinoma has always occurred in men and domesticated animals; that originally in man it was no more frequent than it now is in animals, but that from the seventeenth century onwards it has increased out of all proportion in man whilst the incidence has remained stationary in animals.

The rate at which cancer of the tongue has increased in man is well shown in the returns of the Registrar-General. Dr. Stevenson, superintendent of statistics, wrote in 1909: "The increase amongst males of deaths from cancer of the jaw, and especially of the tongue, is remarkable, and can scarcely be explained by improved diagnosis. Although cancer of the tongue presents little difficulty of diagnosis in its later stages, the recorded mortality has increased amongst males by no less than 228 per cent. in forty-one years. The increase, moreover, is entirely confined to the male sex."

The possible factors causing this increase in cancer of the tongue were considered. Irritation has long been looked upon as an important factor in causing cancer, and the state of the teeth was therefore passed in review to determine whether pyorrhoea and caries have lately become more common. The virulence, but not the amount, of pyorrhoea seemed to have increased, whilst caries does not appear to be more frequent now than it was in some parts of England during the prehistoric period. It was noted, too, that the Roman skulls found in England had nearly as many carious teeth as there are in the skulls

* The text of the lecture is published in the *British Journal of Surgery* for January, 1919.