ON AUTO-HAEMAGGLUTINATION:

A CONTRIBUTION TO THE PHYSIOLOGY AND PATHOLOGY OF THE BLOOD.

BY

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PART I.—EXPERIMENTAL.

THE phenomena of haemagglutination employed by Landsteiner, Mosso, Hirschfeld, and others as a means of differentiating human individuals into different "blood groups" have hitherto been thought to possess only a biological or evolutionary significance.

It is the object of this communication to show that the facts concerning haemagglutination so far studied really form links in a chain of events in the life-history of the red corpuscle, and that they depend on certain biochemical and physical changes which take place in these cells in health and disease. Evidence will also be brought forward to show that the blood serum and the red cells contain haemagglutinins and haemagglutinogens respectively, which, although they resemble in some respects the specific haemagglutinins and haemagglutinogens already known, differ from these in being non specific in character.

The Capacity of all Red Cells to form Haemagglutinogens.

Human red blood corpuscles and those of the different species of animals so far examined all seem capable of elaborating haemagglutinogen under certain conditions.

An examination of the red blood cells of more than fifty sheep shows that no auto-agglutination (that is, agglutination of the fresh red cells by the native serum) occurs in the blood of healthy animals examined soon after death. If, however, defibrinated sheep's blood be centrifuged or allowed to sediment, then after standing four to seven days at room temperature the red cells become agglutinable by their own serum, although they do not show mass agglutination while standing in the tube. This auto-agglutinability can be shown in the usual way by placing a drop of the clear supernatant serum on a slide and mixing with it a drop of the sedimented red cells. Under ordinary conditions well marked agglutination of the red cells takes place in a few minutes or within half an hour. Agglutination also takes place in red cells which have been previously washed in normal saline. Certain conditions favour this development of auto-agglutinability. Thus, if different samples of washed red cells from recently shed defibrinated sheep's blood be added to (1) normal saline containing 0.25 per cent. of gelatine, (2) normal saline, and (3) native sheep's serum which has been filtered through a porcelain filter candle, it will be found that the red cells in the gelatine solution develop auto-agglutinability after standing four or five days, and those in the saline solution in two or three days, whereas the cells which have stood in the native filtered serum develop well marked agglutinability with native serum in forty eight hours, while red cells which have stood in unfiltered serum only become agglutinable after six or seven days. The cells must be tested in each case by native serum previously pipetted off the defibrinated blood shortly after bleeding. In this way error due to the use of samples of serum of different agglutinin content is avoided. Cold delays the change. Thus defibrinated and sedimented sheep's blood, if kept in an ice chest, only shows commencing auto-agglutinability of the red cells after an interval of eight or nine days, though the change rapidly develops as soon as the blood is restored to room temperature.

These observations show, first, that washed red cells can develop agglutinability when standing in normal saline in the absence of blood serum; second, that the elaboration of agglutinogen is hastened by standing in filtered serum. Now, serum which has passed through a porcelain earth filter is deprived of all haemagglutinins. This fact will be considered later when discussing the specificity of the auto-agglutination reaction. The absence of agglutinins in the filtered serum culture medium in which the red cells stand has probably some bearing on the rapid development of agglutinability by those cells.

The Nature of the Auto-agglutination Reaction.

The question arises whether the agglutinability which occurs—slowly in washed red cells which have stood in native serum and more rapidly in cells which have stood in filtered serum—is a specific character; that is to say, whether the reaction which it produces resembles that which occurs when the freshly drawn red cells of one human individual are mixed with the serum of another individual belonging to a different "blood group."

Experiment shows that the increased agglutinability which sheep's red cells develop after standing some days in native serum is non-specific in character. Such red cells are not only agglutinated by native serum, they also agglutinate with the serum of other species of animals with which in the freshly drawn condition they give no reaction, thus: As the result of testing the mutual reaction of the blood of fifty individual sheep of different breeds with the blood of fifty individual guinea-pigs, it was found that, while the blood serum of the full-grown healthy sheep always agglutinates the red cells of the adult guinea-pig, the blood serum of the healthy adult guinea pig never agglutinates the fresh red cells of the adult healthy sheep. We find, however, that sheep's "old" red cells—that is, red cells which have stood for some days in native serum or in normal saline—are agglutinated by a guinea-pig's blood serum. They are also agglutinated by the serum of other individual sheep which in the freshly drawn state have no agglutinative effect upon them. They also agglutinate with the blood serum of certain human individuals with which when freshly drawn they are inactive. This means that the agglutinogen formed* by "old" sheep's red cells differs from the agglutinogen formed by the same red cells while fresh or immediately after withdrawal, inasmuch as the latter only responds to certain agglutinins while the former responds to all.

There is, however, a close association between the non-specific and the specific type of reaction, for the capacity of the red cells to undergo *specific* agglutination is also increased by age. Thus cells which in the fresh state give no reaction, or only a partial reaction, with certain human and animal blood serums, when allowed to stand in native serum for some days give a reaction, or a more complete reaction, in proportion to their agglutinophil affinity for those particular blood serums in the fresh condition. This may be due to the fact that a non-specific auto-agglutinability has been superadded to the specific agglutinability previously possessed by these cells.

This increase in agglutinability in old cells has a practical application. It emphasizes the desirability for using freshly drawn red cells in testing for blood groups, and it has a bearing on the length of time for which red cells can be stored with safety for transfusion purposes.

The *serum saturation method* provides another way in which we can demonstrate the fact that the non-specific differs from the specific reaction.

If washed red cells which have stood for some days in native serum, and have thereby formed agglutinogen, are mixed with a small volume of native serum, saturation of the non-specific agglutinin occurs which renders the serum incapable of agglutinating a second dose of the same red cells. Serum so saturated, however, retains unimpaired its power of agglutinating fresh red cells from other individuals or from other species of animals with which it has specific affinities. This can be shown either by saturating the serum *en masse* by the appropriate red cells in a tube, or, more strikingly, by using a few drops of the serum on a slide. If a suspension of old washed sheep's red cells is mixed with a drop of this native serum the non-specific auto-agglutinin in the serum is saturated. After complete agglutination has taken place a drop of the clear saturated serum is pipetted off and placed on another part of the slide; if this no longer agglutinates a second dose of the same red cells saturation is complete. A second drop of the same saturated serum is now mixed with fresh human red cells or those of an animal

* In thus speaking of haemagglutination as an interaction between some substance *formed by* the red cells and a constituent in the serum, it may be that we are prejudging the real nature of the reaction. It is quite possible, as Dr. Dale has suggested to me, that the acquirement of agglutinability by the red cells may be really due to the *loss of* some constituent by those cells. The red cells by losing some protective substance may more readily absorb and become more easily acted on by the haemagglutinin present in the blood serum.

known to be specifically agglutinated by the same serum Prompt agglutination occurs; the specific agglutinin present in the serum has not been saturated by the non-specific agglutinogen present in the old native sheep's red cells used to saturate the serum.

With regard to the reverse condition—namely, the neutralization of non-specific agglutinin by saturation with specific agglutinogen—the results obtained are not quite so harmonious.

If sheep's blood serum is saturated with red cells of certain human individuals known to be agglutinable by sheep's serum, serum so treated, although no longer capable of agglutinating a second dose of the same human red cells, retains its capacity to agglutinate washed old sheep's red cells.

On the other hand, if a human serum known to have developed auto-agglutinative capacity as the result of disease be saturated with specific agglutinogen from the washed red cells from another human individual or by guinea-pig's red cells the auto-agglutinative power of the saturated serum seems to be impaired or destroyed.

In human blood apparently the neutralization of the specific agglutinins present in the serum also brings about the neutralization of the weaker and more evanescent auto-agglutinin. It would seem, therefore, that there is some difference in potency or neutralizability between human and sheep's auto-agglutinin, and further investigation is necessary to clear up this point. It seems to be true, however, speaking generally, that while saturation of the stronger specific agglutinins by the specific agglutinogens saturates the weaker non-specific agglutinin, saturation on the other hand of the weaker non-specific agglutinin by non-specific agglutinogen does not always neutralize the specific agglutinins normally present in the serum.

These observations seem to prove that the auto-agglutinogen formed by red cells which have stood in native serum is not identical with the specific agglutinogens which the same red cells contain in the freshly drawn state, and the same thing apparently applies also to the relationship between the non-specific auto-agglutinin and the specific hetero-agglutinins present in the serum.

Haemagglutinogens (like haemagglutinins) apparently form a graded series. Starting with the basal non-specific agglutinogen A, which combines with the non-specific agglutinin A present in every serum, human and animal so far tested, we pass to the specific agglutinogens B, C, D, etc., each different in composition and each capable of combining with its corresponding agglutinin.

The non-specific auto-agglutinogen A, unlike the specific agglutinogens, is not found in the freshly drawn healthy red cells. This is shown by the fact that both intact and fragmented freshly drawn red cells are not agglutinated by their own native serum.

The bruising of the stroma and the rupture of the enveloping membrane of the fresh cells does not cause the liberation of any preformed agglutinogen. The lapse of time and certain degenerative changes in the corpuscles seem to be necessary before this agglutinogen is formed by the cell, and before it can permeate the outer membrane and appear on the surface. If red cells, however, which have stood in native serum be ruptured by grinding with a pestle and mortar, then the contained agglutinogen is liberated more quickly than by the intact cells and the auto-agglutinative reaction is considerably hastened. On the other hand, non-specific auto-agglutinin does exist ready formed in the fresh serums. All old red cells which have become agglutinable by standing and fresh red cells which have developed auto-agglutinability as the result of disease are agglutinated by the non-specific agglutinin present in all varieties of freshly drawn serum so far examined. This grading of specific haemagglutinability and the fact that specific agglutinogens are connected with non-specific agglutinogens is important in relation to recently established knowledge concerning the relation of specific to non-specific immunity reactions in general. It would seem that while the specific agglutinogens can permeate the stroma envelope of the red cell immediately after the blood is shed, the non-specific agglutinogens now described only reach the surface of the corpuscle after it has stood for some days in blood serum or other medium and after its envelope has become permeable to them. The specific agglutinogens of biological interest seem to be capable of rapid mobilization, while the non-specific agglutinogens which are produced during the reaction of the organism to disease are mobilized more slowly. Whether any agglutinogens or agglutinins are present in the blood in the circulation during life and before the blood plasma has become changed into blood serum by coagulation is a question of fundamental importance which will be discussed later in Part II.

On the way in which Agglutinogens are formed by the Red Cells.

Experiments have been carried out to ascertain whether agglutinogen is formed on the surface only, or whether it is present also in the interior of the red cells. If sheep's red cells are washed in saline, then ground with a pestle and mortar, and the fragmented cells again washed several times, the haemoglobin constituent is removed, and on centrifuging the broken cells form a yellowish-white sticky sediment. A suspension in saline of these washed cell fragments agglutinates when mixed with native serum. This shows that the agglutinogen constituent is not removed with the haemoglobin by washing. It also shows that the cell fragments themselves contain agglutinogen, and that this substance is not merely held in solution within the corpuscle, like water in a bag, and liberated when the enveloping membrane becomes permeable by it, or when it is ruptured by injury.

Attempts have also been made to obtain haemagglutinogen in the free state. Saline solution previously used for washing agglutinable red cells was tested for the presence of agglutinogen in two ways: (*a*) Fresh red cells not yet agglutinable were suspended in the fluid, and these were tested later with an agglutinating serum. (*b*) The saline fluid supposed to contain agglutinogen was added in equal volume to an agglutinating serum in which any reduction of agglutinative capacity would indicate some neutralization of free agglutinin in the serum so treated.

A considerable number of trials were made by both methods with various varieties of red cells, human and animal. No constant or marked evidence of the presence in such saline solution of free agglutinogen capable of rendering red cells agglutinable or of saturating agglutinin in serum has been obtained. In two or three cases some neutralization of the agglutinin in the serum occurred; the inhibitory results, however, were not constant and may have been due to the presence of haemolysins which destroyed the red cells before the agglutination reaction could take place.

Further evidence that agglutinogen can only exist in combination with the stroma of the red cell is found in the fact that agglutinable red cells can be washed six or eight times in normal saline without losing their surface agglutinogen. Moreover, as we have seen, fragmentation of the corpuscles followed by washing fails to remove it.

While, however, agglutinogen cannot be obtained in the free state, the linkage between agglutinogen and agglutinin can be separated by repeated washing. Red cells which have been auto-agglutinated by native serum can be separated by washing and enabled to reagglutinate with a fresh dose of the same serum. This also applies, though to a less extent, to the specific reaction.

Certain Conditions which affect Agglutinogen Formation.

We have already found that filtered serum forms a better culture medium for the elaboration of agglutinogen than normal serum.

If this filtered serum is slightly diluted with normal saline fluid still better results are obtained. The addition of gelatine 0.25 per cent. to the saline solution delays agglutinogen formation. In defibrinated sheep's blood the red cells from the lower portion of the tube show more agglutinability when mixed with native serum than cells taken from the upper layer. This may depend on the fact that the agglutinogen in the cells of the upper layer is partly neutralized by the agglutinin of the serum with which they come in freer contact, or it may be due to a readier access to the oxygen of the air in this situation.

The Effect of Oxygenation on Agglutinogen Formation.

Two samples of defibrinated sheep's blood were taken. Sample (*a*) was sealed in a tube; sample (*b*) was placed in an open tube and repeatedly oxygenated either by agitation or by bubbling air through the blood. After several

days both samples were sedimented and examined for auto-agglutinability. No great difference was detected in the two samples. In general the red cells from the oxygenated blood became agglutinable a day or two before those in the sealed tube.

The Effect of Heat.

The non-specific and the specific agglutinins resemble each other in being thermostable. If sheep's serum capable of auto-agglutinating native washed red cells and of specifically agglutinating human red cells be subjected to a temperature of 56° C. for half an hour it retains its capacity to agglutinate both kinds of red cells.

The Removal of Agglutinins from Blood Serum by Filtration through Porcelain.

We have already seen that while agglutinogens are only found in combination with the red cells, agglutinins, on the other hand, exist in the serum in the free state. If freshly drawn sheep's serum be filtered through a porcelain earth filter candle under diminished atmospheric pressure, a pale clear fluid of low specific gravity is obtained free from specific and non-specific agglutinins. Such filtered serum has no agglutinative action on fresh red cells which a control sample rapidly agglutinates, or on old native red cells in which the control sample produces auto-agglutination. It is a point of interest that while filter candles of a certain fineness of porosity block the passage of all haem-agglutinins, those of a slightly more porous character let some through. The size of the agglutinin aggregate would seem to correspond, therefore, to the diameter of the pores of a moderately fine filter candle. An attempt was made by using candles of different degrees of porosity to ascertain whether any difference in filtrability—that is, in the size of the aggregrate—occurred between the non-specific and the specific form. As far as can be ascertained, however, both seem to pass with equal readiness through candles of the same fineness of pore.

It may be that the excellence of filtered native serum as a medium for the formation of agglutinogen by red cells depends on the fact that filtered serum contains no haem-agglutinin, consequently the agglutinogen is not neutralized as soon as formed.

Conclusion.

The subject of auto-haemagglutination in disease will be considered in Part II. It is, however, desirable to sum up the conclusion established in regard to this (*in vitro*) part of the inquiry.

In the first place the phenomena of haemagglutination have been shown to possess a physiological as well as a biological significance. All red blood corpuscles are capable of forming haemagglutinogen under certain conditions.

Agglutinogen, like agglutinin, exists in the non-specific form, agglutinogen A, and in a graded series of specific forms, agglutinogen B, C, D, etc. The specific and the non-specific agglutinogens (like the corresponding agglutinins) although allied, are not identical in composition or character.

Agglutinogen has so far only been found in combination with or attached to the surface or stroma of the red cells. It apparently does not exist in the serum in the free or uncombined state. The specific and non-specific agglutinins, on the other hand, are present in the serum in the free state, and both kinds are removed by filtering the serum through a porcelain filter.

THE Ministry of Pensions announces that officers and nurses who have completed a course of sanatorium treatment and are certified as likely to benefit by a further course of treatment combined with training, will, if they decide to undergo such course, be granted retired pay at the rate appropriate to 100 per cent. disablement during such curative training and for a period of six months after its termination. Further, the retired pay will be continued at a rate not less than that for 50 per cent. disablement for two years thereafter. These special extensions will be conditional on the officers presenting themselves for examination by the local tuberculosis officer from time to time as directed, and complying with any instructions considered necessary for their treatment.

SOME POINTS IN MIDWIFERY PRACTICE.

A PAPER READ BEFORE THE CAMBRIDGE MEDICAL SOCIETY.

BY

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THE doctor probably more than any man, and perhaps the accoucheur most of all, desires that the generation which succeeds him shall emulate his virtues, improve on his methods and avoid his errors, a sentiment expressed in the well-worn tag of the Sophoclean Ajax,

ὦ παῖ, γένοιο πατρὸς εὐγενέστερος.

When we have become old enough to look back, when we have grown candid enough to admit, even to ourselves, our defects and our failures, we can still desire perfection; hence it seemed to me, since in this important branch of our profession so much can be done for the good of the patient, that a profitable discussion might well result from a paper on some points in midwifery practice.

During Pregnancy.

Taking the first of the three great natural divisions of our subject, we will look at a few matters in relation to pregnancy concerning which we are often called on to give advice—what to do and to avoid, and so forth. My custom is to tell the woman to lead a normal active life in all respects, avoiding three things—shock, strain, and fatigue. I advise her also to turn a deaf ear to the counsels of old women of both sexes, and of certain books whose chief characteristics seem to be sticky sentiment and sloppy moralizing.

In the absence of signs or symptoms, it is probably needless to trouble the prospective mother till the expiry of seven months, when external pelvimetry should be done and abdominal examination made to determine if there is likely to be plenty of room for the head. If there seems any ground for fear, the examination should be repeated every week and labour induced if and when indicated. By this means we can avoid what may be fairly regarded as a discredit to our art—the bad forceps case with head at or above the brim. I fear that perhaps too many of us can look back on such cases with mingled feelings. The woman should also be instructed to report any departure from her normal health, and systematic repeated examinations of the urine should be made if there is previous history of renal or toxaemic trouble, or any sign that all is not well.

In preparation for the confinement, I think that the routine administration of quinine, gr. 1 twice a day for a month before the event, with or without digitalis, acts as a good uterine tonic. I shall refer to this again.

I have always opposed, and I hope always to oppose, the practice of midwifery by midwives, sanctioned though it is by the hoariest antiquity, supported by widespread prejudice and ignorance, and backed by the Legislature. When we consider how much the medical man can do—and, indeed, ought to do—to take his patient through her confinement expeditiously, safely, with a minimum of pain, distress of mind, discomfort, and injury, we cannot view with indifference the denial of these advantages to so large a proportion of mothers. True, one sometimes hears a doctor say that he hates this branch of his work, and we all know how very awkward its incidence can be, but such things are not good arguments for the employment of midwives. What is wanted is doctors who love their work.

A word on the thorny question of the induction of labour. My view is that this *dernier ressort* should not be postponed too long. If the patient is wasted and wasting, if vomiting is severe and persistent in spite of treatment, if albuminuria and toxaemia are present, or even an obscure condition of marasmus, we ought not to wait on and on in the hope for a living child when every day diminishes the mother's chances. My mind goes back to such a case in which induction was postponed; the woman had a spontaneous labour at seven months, the child dying in thirty-six hours, and the mother some hours later. Frankly, I am not in love with the dictum of Pinard concerning the right of the fetus to its life. Hydrops amnii