THE PHYSICAL STATE OF THE BLOOD SERUM IN RELATION TO ITS AGGLUTININ AND ANTIBODY CONTENT:

THE EFFECT OF FRICTION AND PRESSURE.

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.

Two years ago I noticed that if two separate drops of a blood serum containing haemagglutinin were allowed to dry on a slide and if one of the drops was dissolved in normal saline solution without friction, while the other was rubbed with a glass rod, the drop which had undergone solution without friction retained its full haemagglutinative capacity, but the drop which had been rubbed failed to agglutinate the red cells or did so very feebly.

Starting from this point I next investigated the effect of combined friction and pressure on certain blood serums of known haemagglutinative capacity. If a serum is ground in a mortar and the turbid liquid then centrifuged a white sediment falls, leaving a more or less clear supernatant fluid. This clear fluid is then pipetted off and again subjected to the grinding process. A second precipitate is thus obtained on centrifuging the liquid, often more rapidly and in larger volume than on the first grinding. The process of grinding, centrifuging, and pipetting off the clear fluid can be repeated a number of times with a gradual loss of volume of fluid partly due to froth formation. This frothing is more noticeable in some serums than in others. It frequently occurs at the second or third grinding with horse, ox, sheep, and some guinea-pigs' serums.

By this fractional mechanical process the constitution of the serum can be profoundly modified. A remarkable change is brought about not only in the physical condition, but also in the haemagglutinins and other antibodies. It will be convenient to describe these changes as they affect the supernatant fluid, which we may designate liquor 1, liquor 2, liquor 3, etc., according to the number of times the grinding process has been applied, and the sediment, which may be designated 1, 2, and 3, etc., in the same way.

The Liquor.

Fig. 1 illustrates the effect of subjecting horse serum which powerfully agglutinates human (C. J. B.) washed red cells and sheep's red cells to three successive fractional grinding processes. The circles represent a drop of the serum, or liquor, or sediment to which the red cells are added. The + and - signs in each circle indicate the presence or absence of agglutinating capacity, while the numbers 1, 2, 3 indicate the number of times the fluid has been ground in the mortar

and the precipitate separated by centrifuging.

In the case of horse's serum free haemagglutinin is found in the clear liquor after three fractional grindings, and it is absent from the sediment after this has been washed in normal saline solution and reprecipitated by centrifuging. This result with horse serum is also characteristic of human and some other animal blood serums which I have examined. In some cases the liquor may even show a higher haemagglutinin content than the blood serum from which it has been obtained; thus Fig. 2 represents a human serum (No. 13) which only contained one (+) degree of haemagglutinating capacity for (C. J. B.) red cells, whereas liquor 1 contained two (++) degrees, and liquor 2 three (+++) degrees. If the original serum contains no trace of agglutinating capacity for given red cells, then the liquors obtained by grinding it are also devoid of haemagglutinin for the same red cells. It is, however, necessary to point out that some serums if examined

hastily may be set down as negative, whereas if a drop of such a serum on a slide be mixed with a measured volume of a suspension of washed red cells and gently agitated, and then allowed to stand for some time, a trace of agglutinative action will be seen on gentle re-agitation, and such a serum must not be recorded as negative.

This raises the important question of the specificity of the haemagglutinin reaction.

Specificity of the Haemagglutinin Reaction in Blood Serums and Liquors after Grinding.

The clumping of appropriate red cells by normal untreated blood serum is, of course, a highly specific reaction, and the specificity has a practical application in the selection of donors and recipients belonging to appropriate groups in the operation of blood transfusion. It is important to find, therefore, that, in liquors obtained by subjecting such serums to the fractional grinding process, the haemagglutinin reaction retains its specific character. This is true as a general statement; it is possible, however, that as the result of standing, or more rapidly by mechanical treatment, the specificity may be reduced and a serum or liquor may become agglutinative to certain red cells to which it formerly only showed a trace of agglutinative action. This specific character is shown by the non-agglutinating serum 2 and the powerfully agglutinative serum 11 (see Figs. 3 and 4). In serum 2

C. J. B. E. S. B. SHEEP RED CELLS.			C. J. B. E. S. B. SHEEP		
SERUM 2.....			SERUM 11.....		
—	—	—	++	—	+
LIQUOR {	1. —	—	1. ++	—	tr
	2. —	—	2. ++	—	+
	3. —	—	3. ++	—	+
SEDIMENT {	1. —	—	1. —	—	—
	2. —	—	2. —	—	—
	3. —	—	3. —	—	—

FIG. 3.

FIG. 4.—Serum 11.

the — agglutinating quality appears in both liquors and sediments in relation to human (C. J. B. and E. S. B.) and to sheep's red cells. Serum 11, on the other hand (see Fig. 4), agglutinates C. J. B. red cells two (++) degrees, sheep's red cells one (+) degree, and E. S. B. red cells — degree.

Here the specific action of the haemagglutinin reaction is shown in the liquor in differential relation to these three varieties of red cells.

Increase of Haemagglutinating Capacity on Standing.

It will be observed that in serum 11 (Fig. 4) the haemagglutinin content increases in the liquor as the fractional grinding process proceeds. Liquor 3 shows a higher agglutinating capacity to C. J. B. red cells than the untreated serum. This heightening of agglutinin content in a serum by mechanical treatment was also previously recorded in Fig. 2 (serum 13), and it is on a par with what occurs in some serums as the result of standing. Thus serum 17, marked as negative (but which may have contained a trace of agglutinin to C. J. B. red cells), was pipetted off the blood clot and allowed to stand at room temperature for seven days. When retested it gave a (+) reaction with the same red cells. The same kind of change which tends to occur slowly in a serum as the result of standing is brought about more rapidly by the mechanical effect of friction and pressure. The grinding process seems to set free a certain amount of locked-up haemagglutinin and at the same time separates out a solid residue in the form of a sediment which is non-agglutinative.

The Sediment.

In the case of nearly all blood serums so far submitted to the fractional mechanical process, the sediments obtained on centrifuging the grinded serum up to the third and even the sixth term have failed to give any haemagglutinin reaction with appropriate red cells.

The various sediments have been washed one or more times in normal saline solution, in which they are very sparingly, if at all, soluble, and a drop of the suspension mixed with the washed red cells on a slide.

This presence of free haemagglutinin in the liquor and its absence in the sediment is in striking contrast to what we find in the case of transudates, exudates, and other body fluids.

Chemical Composition of the Sediment.

The chemical composition of the sediment obtained by grinding the blood serum requires a more elaborate chemical investigation than I have yet been able to give to it. It gives a blue, not violet, colour with the xantho-proteic test, and only a faint trace of black coloration (lead sulphide) with Molisch's test. There is no reduction with Fehling's solution, and the sediment is not appreciably soluble in ether or chloroform, nor does it stain black with osmic acid.

Transudates, Exudates, Secretions, and Excretions.

As a general statement it is true to say that peritoneal and pleural effusions, cerebro-spinal fluid, and lymph frequently fail to give any free haemagglutinin even when obtained from patients whose blood serum shows

FIG. 5.—Pleural effusion A.

FIG. 6.—Pleural effusion B.

two or more degrees (++) of agglutinative capacity. This is true of pleural and peritoneal effusions of low specific gravity, free from any considerable cellular element, and secreted without marked inflammatory reaction. The following examination of a serous fluid removed by aspiration from the chest of the same patient at an interval of six weeks illustrates this point.

Effusion (a) was a pale watery fluid of low specific gravity, removed from a patient, A. W., whose blood serum was (++) to C. J. B. red cells. The fluid itself gave a (-) reaction with the same red cells. When ground in a mortar, liquors 1, 2, and 3 contained no free haemagglutinin, while the corresponding sediments 1, 2, and 3 showed (+) degrees with the same red cells (see Fig. 5). Six weeks later a second aspiration withdrew a yellow fluid of higher specific gravity closely resembling blood serum. This gave a different result on grinding (see Fig. 6). This second effusion agglu-

FIG. 7.—E. J. ascitic fluid.
C. J. B. red cells. FIG. 8.—E. J. blood serum.
C. J. B. blood cells.

tinated (++) C. J. B. red cells, and the liquors 1, 2, and 3 obtained from it were also agglutinative, while the corresponding sediments gave no reaction. Thus the haemagglutinin existed in these two serous fluids in quite different conditions. The first, which resembled a transudate, contained no free haemagglutinin, but haemagglutinin was

recovered from the sediment. In the second fluid, which resembled a blood serum, haemagglutinin was present in the free state in the untreated fluid, and was recovered from the liquor but not from the sediment. I think this is a suggestive fact and one which bears on the problem of the changes undergone by blood plasma when it passes out of the capillaries and comes in contact with the tissue cells. The haemagglutinin ceases to exist in the free state in this altered fluid though it remains in some (at present unknown) combined form, and can be recovered again in the free state by subjecting the fluid to a mechanical process which includes friction and pressure. As a general rule haemagglutinin cannot be recovered from the liquor or sediment of fluids obtained from individuals whose blood serum contains no haemagglutinin. A sample of ascitic fluid from a patient whose blood serum was negative to C. J. B. red cells was itself negative, and Figs. 7 and 8 show the result obtained respectively from the blood serum and the ascitic fluid in this case. On the other hand, a peritoneal effusion poured out during an acute reaction to an infective process in another patient gave a (++) result in the liquor and a (-) result in the sediment. (See Fig. 9.)

FIG. 9.—B. D. ascitic fluid.
C. J. B. red cells.*The Cerebro-spinal Fluid.*

If we regard the cerebro-spinal fluid as the lymph of the brain and as blood plasma altered by the action of the endothelial cells of the choroid plexuses and the serous meninges, it is interesting to find that when subjected to the fractionating grinding process this fluid obeys the rule which we have found to apply to other exudates. I have investigated by this method the cerebro-

FIG. 10.—Blood serum and cerebro-spinal fluid, same patient.
C. J. B. red cells.

spinal fluid and the blood serum from the same individual. The blood serum was positive (8 doses) and the cerebro-spinal fluid negative to the Wassermann test. The blood serum agglutinated (+) C. J. B. red cells while the cerebro-spinal fluid gave a (-) result. (See Fig. 10.) The blood serum of this patient when treated by the grinding process resembled normal blood serum and gave a (+) in the liquor and a (-) in the sediment. The cerebro-spinal fluid in the same individual, on the other hand, which was (-) to C. J. B. red cells in the untreated condition, gave a (+) sign with the same red cells in the sediment and a (-) sign in the liquor.

Non-specificity of the Haemagglutinin Reaction in Exudates and Secretions.

We have already seen that when a blood serum is submitted to the grinding process the haemagglutinin which

FIG. 11.—B. W. ascitic fluid. Reaction with C. J. B. and E. S. B. red cells.

is recovered from the liquor preserves (for the most part) its specific character. That is to say, it only agglutinates such varieties of red cells as are agglutinated by the corresponding untreated serum. This specificity of reaction frequently disappears when transudates, exudates, and other body fluids are submitted to the grinding

process. For instance, a sample of ascitic fluid obtained from a patient with heart disease was examined by this method. It was found to be (++) to C. J. B. red cells and (-) to E. S. B. red cells. The blood serum of this patient showed (+++) to C. J. B. red cells and a trace only to E. S. B. red cells. When this ascitic fluid was submitted to three fractional grindings the haemagglutinin recovered from the sediment showed the same degree of agglutinative capacity to both kinds of red cells. Fig. 11 illustrates this result.

The Saliva.

A haemagglutinin has been recovered from the sediment obtained by submitting salivary fluid to the grinding process. This recovered haemagglutinin agglutinated certain varieties of red cells which were not agglutinated by the blood serum of the same individual. There was, however, some evidence of a partial specificity in the reaction because the agglutination was delayed and less complete with these incompatible red cells than with other red cells which were agglutinable by the blood serum of the patient.

Mucus.

It is of interest to find that haemagglutinin can be obtained from the sediments when nasal mucus is submitted to the grinding process. This bears on the important problem of the agglutinin and antibody content of mucus and of the part played by the mucous secretions in combating invasion by pathogenic organisms.

Milk.

I have submitted samples of human, sheep's and cow's milk to the fractional grinding process. The results are of considerable interest and will, I hope, be dealt with more fully in a later communication. A sample of human milk was obtained from a patient whose blood serum was known to be (++) agglutinative to human (C. J. B.) and sheep's red cells. The milk was first centrifuged and the supernatant cream removed, the milk *minus* the cream then showed (+) agglutination to the same red cells. Some of the slightly turbid yellow fluid was then submitted to the grinding process and three successive liquors

FIG. 12.—Human milk. Reaction with C. J. B. red cells.

and sediments were obtained. Fig. 12 shows (+) haemagglutinin in liquor 1, a trace only in liquor 2, and (-) in liquor 3. The corresponding sediments show (-) haemagglutinin in sediments 1 and 2, and (+) in sediment 3. This corresponding descent and ascent of haemagglutinin content in liquor and sediment is important, and suggests that a passage of haemagglutinin from the combined to the free state takes place as the mechanical treatment proceeds. Speaking generally, we may say that human milk, as a secretion, stands midway between blood serum on the one hand and an exudate like lymph on the other, from the haemagglutinin point of view. One point of considerable practical importance came out in the course of this investigation. Boiling destroys all the haemagglutinin in human milk, whereas pasteurization—that is, the subjecting of the milk to a temperature of 60° C. for forty minutes—does not affect the haemagglutinin content as far as the reaction to human (C. J. B.) red cells is concerned, although it reduces the power of clumping sheep's red cells.

It is interesting to note also that the haemagglutinins do not disappear from human milk when preserved for a considerable time, provided the cream has been removed by separation. A sample of centrifuged separated human milk which had remained in a plugged sterile test tube for six months at room temperature gave when tested (++) of haemagglutinative capacity to human and sheep's red cells.

It is interesting to compare sheep's milk and cow's milk

with human milk from the haemagglutinative point of view. So far, samples of centrifuged separated milk from these animals have given (-) results with human (C. J. B. and E. S. B.) red cells. When the milk was ground, traces only appeared in sediments 2 and 3 in cow's milk, but we must not forget the factor of individual difference. Some sheep's blood serums are (+) and some (-) to the same red cells, and the same may be true of the blood serums, and possibly the milk of cows.

If later investigation should confirm the fact that, on the whole, cow's milk contains less haemagglutinin capable of acting on human red cells than human milk, a further contribution will be added to the comparative study of human and cow's milk. The destruction of the haemagglutinins by boiling but not by pasteurization has not, as far as I can ascertain, been recorded previously. I do not find it mentioned in Dr. Lane-Claydon's book on *Milk in its Hygienic Relations*.

Excretions: the Urine.

Urine may be regarded as the type of a body fluid in which excretory largely replace secretory characters.

Urine probably contains (in a degraded form) waste products of the metabolic activities of body cells of all kinds. Hence it is a matter of interest to find that the haemagglutinin in sediments obtained by submitting urine to the grinding process lose their specific character. That is to say, they no longer only agglutinate such red cells as are agglutinated by the corresponding blood serum. Fig. 13

	C. J. B.	E. S. B.	SHEEP.
BLOOD SERUM.....	(-)	(-)	(-)
LIQUOR {	1. (-)	1. (-)	1. (tr)
	2. (-)	2. (-)	2. (tr)
SEDIMENT {	1. (+)	1. (+)	1. (+)
	2. (+)	2. (+)	2. (+)

FIG. 13.—Normal urine. Reaction with C. J. B., E. S. B., and sheep's red cells. From an individual with blood serum negative to same red cells.

illustrates the result in a normal urine from an individual whose blood serum was (-) to C. J. B., E. S. B., and sheep's red cells. In this urine sediments 1 and 2 show (+) to all these varieties of red cells; the corresponding liquors, on the other hand, are (-), though they give a trace of reaction to sheep's red cells. We may now compare with this healthy urine an albuminous urine from a patient with acute nephritis (see Fig. 14). The blood serum of this patient contained a trace only of agglutinin to C. J. B. red cells and was (+) to sheep's and (-) to E. S. B. red cells. Liquor 3 gave a (-) result with C. J. B. and E. S. B. red cells, but (+) result with sheep's red cells, to which the blood serum of the patient was also (+). Sediment 3 gave (++) with C. J. B., (++) with sheep's, and (+) with E. S. B. red cells.

	C. J. B.	E. S. B.	SHEEP.	L. M.
BLOOD SERUM.....	(+)	(-)	(+)	(-)
LIQUOR 3.....	(-)	(-)	(+)	(-)
SEDIMENT 3.....	(++)	(+)	(++)	(+)

FIG. 14.—Albuminous urine (L. M.); acute nephritis.

In the BRITISH MEDICAL JOURNAL of March 2nd, 1918, I suggested that the diminution of haemagglutinin content observed in the blood serums of some cases of trench nephritis might be due to a leakage of the haemagglutinin with the globulins and serum albumins escaping in the urine.* It is of interest, therefore, to find some slight indication of specificity of action in the haemagglutinin obtained by grinding the albuminous urine from a patient with nephritis. Thus liquor 1 resembles the blood serum and is (+) to sheep's red cells, and although the haemagglutinin from sediment 3 is (+) instead of (-) to E. S. B. red cells, the reaction is less rapid and complete than with C. J. B. or with sheep's cells, to both of which the blood serum of the patient is more positive.

The fact that the sediment obtained by grinding both a normal and an albuminous urine is capable of

* Since this was written free haemagglutinin, specific in character, has been demonstrated in specimens of albuminous urines from two cases of nephritis.

agglutinating the red cells of the individual secreting the urine shows the extent to which the specificity of the reaction has been lost. (See Fig. 14.) At first sight this seems to prove too much, and suggests that the factor, whatever it be, in the sediment which clumps the native red cells is merely an accidental product of the grinding process, and that it has no real relation to haemagglutinins pre-existing in the blood serum, inasmuch as it occurs in the urines of individuals with non-agglutinating as well as with agglutinating blood serums. There are, however, certain facts which suggest that the haemagglutinins recovered from these urines by grinding do bear some relation to haemagglutinins pre-existing in the blood serum of these individuals either in the free or a locked-up state. The appearance of haemagglutinins in blood serums on standing and the increased volume in the liquor of a ground serum suggest that the difference between a non-agglutinating and an agglutinating serum is not due to the entire absence of haemagglutinin in the former but to the fact that it exists in a combined or locked-up form in which it cannot exercise any effect on the red cells until liberated by certain chemical changes which take place on standing or by the grinding process.

Further, if the haemagglutinins which appear in the liquor or sediment when a blood serum or exudate is ground are only the accidental result of the grinding process then they should appear equally in all liquors and sediments. But they are absent from the liquors and sediments obtained by grinding blood serums and exudates from individuals whose blood serums show no trace of any haemagglutinative action on any red cells. There is evidence to show that the blood serum of every individual contains haemagglutinins of one kind or another. Thus my own blood serum has failed to agglutinate the red cells of some hundreds of individuals with whom it has been tested. It belongs to group 4. At the same time my blood serum strongly agglutinates the washed red cells of the pigeon. Hence we are not justified in assuming that the haemagglutinins which appear in the sediments of a ground urine are entirely new products which bear no relationship to haemagglutinins existing in the blood serum of the same individual in a combined form. It is true haemagglutinins of a non-specific kind appeared in sediment 3 of a normal as well as an albuminous urine, but in these cases also the first and second sediments contained no haemagglutinin. On the whole, therefore, I am inclined to think that the non-specific haemagglutinin recovered from urine mechanically treated does bear some relation to a haemagglutinin previously existing in the blood serum in a combined and inactive form. If, on the other hand, it should eventually prove to be possible to produce *de novo* a substance having non-specific haemagglutinative activity in a fluid like the urine by submitting it to friction with pressure, an important point will have been ascertained and one which may be useful in differentiating certain abnormal from normal urines. A word is necessary at this stage concerning the part taken by the sediment in the haemagglutinin reaction. Everything points to the fact that the sediment itself contains no haemagglutinin, but that these substances are locked up in, and are carried down with, the precipitate when the ground liquid is centrifuged. On standing for some time in normal saline solution a sediment may give off haemagglutinin, the clear solution when pipetted off being actively haemagglutinative. Thus the normal saline solution in which sediment 3 from the albuminous urine had stood for four days was (+) to sheep, (+) to E. S. B., and (trace) to C. J. B. red cells. The sediment obtained by grinding a blood serum or an exudate also probably acts as a carrier only of haemagglutinin.

The Effect of Grinding on the Complement Content in Guinea-pig's Serum.

Dr. Mackarell, pathologist to the Leicester Royal Infirmary, has kindly tested with a suitable haemolytic system several samples of serum from different guinea-pigs for complement content after treatment by the grinding process. The following are the details of one such experiment. A sample of guinea-pig's serum was divided into two volumes, A and B. Volume A was submitted to three fractional grindings, and liquor 3 and sediment 3 were each tested for complement content, with the following result:

Liquor 3 (diluted 1 in 3):

0.005 c.cm.	...	No haemolysis
0.01 "	...	Partially haemolyzed
0.015 "	...	Wholly haemolyzed
0.02 "	...	Wholly haemolyzed

Sediment 3 in normal saline solution:

0.1 c.cm.	...	No haemolysis
0.2 "	...	No haemolysis
0.3 "	...	No haemolysis
0.4 "	...	No haemolysis

Volume B, the untreated control serum (diluted 1 in 3), gave:

0.005 c.cm.	...	Almost complete haemolysis
0.01 "	...	Complete haemolysis
0.015 "	...	Complete haemolysis
0.02 "	...	Complete haemolysis

This result, confirmed by other tests, shows (1) that a slight diminution of complement occurs in a guinea-pig's serum when submitted to the mechanical effect of friction and pressure; it also shows (2) that the complement (like the haemagglutinin) passes over in the liquor and is not attached to or precipitated with the sediment.

The Effect of the Grinding Process on Syphilitic Blood Serums.

Dr. Mackarell has also kindly investigated the effect on the complement deviating factor of submitting positive serums to the fractional grinding process. In a number of serums positive (over ten doses) as tested by the Wassermann test, the liquor up to the third term retained its complement deviating power, while the corresponding sediment was negative. Evidently the substance or substances, whatever these may be, in the syphilitic serum which have to do with complement deviation are not thrown down with the precipitate when such a serum is ground in a mortar; they pass over with the liquor. One sample of negative serum was submitted to the grinding process in order to ascertain whether any complement deviating substance was produced; the result, however, was negative.

The Effect of Grinding on the Bacterio-agglutinin Content of a Blood Serum.

Captain Heath, R.A.M.C., pathologist to the 5th Northern Hospital, has kindly tested a number of blood serums which had previously been submitted to the grinding process to ascertain: (1) Whether any effect had been produced in the agglutinating titre of the serum so

DILUTION OF SERUM	100	200	400	600	800	1000	2000	4000	6000	8000	10000	20000	30000	50000	100000
CONTROL SERUM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RUBBED SERUM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CONTROL SERUM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RUBBED SERUM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CONTROL SERUM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RUBBED SERUM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

FIG. 15.—Shows increase in agglutination titre in polyvalent Flexner Y serum, produced by grinding.

treated, and (2) whether the bacterio-agglutinins present in the serum still retained their specific character. Fig. 15 represents in tabular form the result obtained in a polyvalent Flexner Y serum, R.A.M. College, dilution 1 in 100,

DILUTION OF SERUM	1:40	1:100	1:400	1:600	1:800	1:1000	1:2000	1:4000	1:6000	1:8000	1:10000	1:20000
CONTROL SERUM	+	+	+	+	+	+	+	+	+	+	+	+
RUBBED SERUM	+	+	+	+	+	+	+	+	+	+	+	+

FIG. 16.—Stock paratyphoid B serum.

titre 1 in 4,000. Fig. 16 gives the result in a paratyphoid B serum. From these and other examples it seems clear that by submitting a serum to a mechanical process like grinding it is possible to cause (1) a marked increase in agglutinating capacity to certain organisms. This increase may amount in some cases to 100 per cent. when compared with the untreated serum. (2) This increase in agglutinative capacity is specific in character. The agglutinating titre of the treated serum to other species of organisms is not raised above that of the control serum. (3) The agglutinating substances pass into the liquor and are not precipitated with the sediment. In the experiment recorded the serums were submitted to three fractional

grindings and liquor 3 and sediment 3 tested for agglutinin content against the untreated or control serum. In each case the titre of the liquor was markedly increased, while that of the sediment and the normal saline solution in which the sediment was suspended was considerably below that of the control serum.

GENERAL CONCLUSIONS.

The above observations seem to show that by submitting blood serum and other body fluids to a mechanical process, which includes friction combined with pressure, physical changes can be brought about in these fluids which throw some light on the condition in which haemagglutinins, bacterial agglutinins, complement, complement deviating substances, and antibodies exist in the blood serum. They suggest that the blood serum and the body fluids, the secretions and excretions, form a graded series with the blood serum at one end, and the excretions, such as the urine, at the other. The haemagglutinins seem to be present for the most part in a free state in the blood serum, and in a more or less combined form in the transudates and exudates, while in excretions, like the urine, the haemagglutinins when liberated and recovered in the free state are found to have lost their specific character. This is shown by testing the agglutinative capacity of the fractional series of liquors and sediments to the same varieties of washed red cells. Not only do the haemagglutinins show marked differences of composition in the different liquors and sediments, but they also vary according to the extent to which the fluid has been submitted to the mechanical fractional process. This is also true of the bacterial agglutinins contained in blood serum, and it has been found possible by these means to considerably increase the agglutinating titre of a blood serum to a given organism while retaining at the same time the specificity of the reaction. This suggests that it may be possible to raise the agglutinating capacity of a serum by a mechanical process *in vitro* for therapeutic purposes. These observations also show that the complement deviating factor in positive syphilitic serums passes over along with the haemagglutinins and bacterial agglutinins into the liquor, and is not thrown down with the sediment.

A word must be added about the relative leuco-toxicity of the liquors and sediments obtained by the fractional grinding treatment of blood serums and body fluids. In the BRITISH MEDICAL JOURNAL, December 8th, 1917, and January 11th, 1919, I drew attention to the fact that some blood serums are far more toxic to the leucocytes of some individuals than to those of other persons, my own (C. J. B.) leucocytes being taken as a standard. Thus, if a drop of the serum to be tested is incubated in a closed cell with a drop of whole (C. J. B.) blood, the effect of the foreign serum on the vitality of the leucocytes can be ascertained. Emigration from the clot, capacity to elaborate iodophil substances and phagocytic activity are taken as the standard of vitality. I find that out of 80 different blood serums from different individuals 13 were very toxic, 25 were slightly toxic, and 42 were non-toxic to my own leucocytes. In practically all cases the sediment is markedly toxic, while the liquor may or may not be toxic to the same leucocytes. In a few cases the liquor obtained by grinding a toxic serum has become non-toxic to the same cells. This problem of the leuco-toxicity of different blood serums is of considerable practical importance in regard to the operation of blood transfusion and vaccine and serum therapy. From some recent observations I am inclined to regard horse serum as markedly toxic to the leucocytes of certain individuals, and this fact may have some reference to the varying liability to serum sickness shown by different individuals.

Finally, while I am fully aware of the incomplete character of these observations, I think that they show the great importance of certain physical factors—for instance, friction and pressure—in relation to the antibody content of the blood serum and other body fluids. I am hopeful that the treatment of these fluids by a mechanical process which combines friction and pressure may open up a road for the further investigation of the biochemistry of the blood serum.

A FULLY equipped hospital, to be maintained by the French Government, has been opened at Athens by the French Minister.

A REPORT ON TWO CASES OF ENCEPHALITIS LETHARGICA.

BY

MAJOR C. W. J. BRASHER, CAPTAIN J. R. CALDWELL,
R.A.M.C.(T.F.), R.A.M.C.(S.R.),

AND

CAPTAIN E. J. COOMBE, R.A.M.C.(S.R.).

THE disease which has been termed "encephalitis lethargica" appears to have been first observed in Vienna during the winter of 1916-17, and thirteen cases have been described by C. von Economo.¹ It does not appear that any connexion between these cases and an epidemic of influenza was observed, but the two cases described below are of interest on account of their occurrence during the epidemic of influenza in October, 1918, and also because the micro-organism found in the cerebro-spinal fluid in both cases was not the diplo-streptococcus found by von Eisner in an ape infected by material from some of these cases in the Vienna epidemic.

The micro-organism found by one of us (J. R. C.) appeared to be morphologically identical with that which was found in the blood, sputum, pleural fluid, and cerebro-spinal fluid in cases of influenza, trench fever, and nephritis among British troops in France during the autumn of 1917 and the spring and summer of 1918, described by Major-General Sir John Rose Bradford, A.M.S., Captain E. F. Bashford, R.A.M.C., and Captain J. A. Wilson, R.A.M.C., in their "preliminary report on a 'filter-passing' virus to the Director-General Medical Services, British Armies in France."² The filter-passing Gram-positive organism described by these observers as having been found in cases of encephalitis lethargica is regarded by them as allied to that which has been isolated in cases of polyneuritis, but it does not appear that this organism has been found in cases of typical influenza followed by encephalitis lethargica.

Both of the cases described below were admitted as influenza in which pneumonia supervened prior to the onset of cerebral symptoms.

CASE I.

Pte. W. H. L., aged 22, was admitted to the Military Hospital, Fargo, Salisbury Plain, on October 19th, 1918, from Southern Command Malaria Centre, Larkhill, where he had been undergoing treatment for malaria contracted at Salonica.

He was very ill on admission, with severe headache, pains in limbs, and generalized bronchopneumonia; the temperature was 103°. He was given 5 c.cm. polyvalent vaccine (*B. influenzae*, *Streptococcus longus*, and *Micrococcus catarrhalis*). Some improvement was noted, and the vaccine was repeated at intervals of three or four days, but the headache persisted and the temperature oscillated between 100° and 103°. The spleen was not palpable, and blood films were examined for malarial parasites, as it was thought that the persistent fever might be due to malignant tertian malaria. No malarial parasites were found. The patient became very emaciated, he was somnolent, and lay curled up in bed with head retracted. When awake he complained of persistent severe headache, and it was thought that he might be suffering from acute tuberculosis with meningitis. The pupils were equal, dilated, and reacted sluggishly to light. The optic discs were normal, and no choroidal tubercles could be seen. A differential blood count showed no lymphocytosis, but, on the contrary, there was a distinct leucopenia. The relative proportion of white cells being normal—but the total number was only 12,000 per c.cm. on admission—this was regarded as pointing to chronic malaria, and quinine hydrochlor. gr. xx was given thrice daily, without benefit.

On October 29th the temperature rose to 104°, pulse rate 110, respiration rate 24; rigidity of neck and Kernig's sign were present. The latter symptoms persisted until November 6th, when lumbar puncture was performed, but the cerebro-spinal fluid was normal.

November 16th. The condition had improved since November 6th, but there was persistent pyrexia.

November 20th. Dullness left apex and upper lobe and apex of lower lobe, crepitations and tubular breathing. Curvature of spine in dorsal region.

November 26th. The tubercle bacillus was not found in the sputum, and x-ray examination revealed no sign of tuberculosis in the chest. Blood count:

Reds ...	...	...	...	4,000,000
Whites ...	...	...	...	5,000
Haemoglobin content ...	...	...	...	80 per cent.
Colour index ...	...	...	...	1
Mast cells ...	...	...	...	0.5 per cent.
Polymorphonuclears ...	...	...	...	79
Eosinophils ...	...	...	...	5
Small lymphocytes ...	...	...	...	5
Large lymphocytes ...	...	...	...	15
Transitionals ...	...	...	...	0.5