OBSERVATIONS ON THE SIGNIFICANCE OF THE HAEMOSOZIC VALUE OF THE BLOOD SERUM

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The term haemosozic value of the serum has been made use of to indicate those constituents present in the serum which preserve the red blood corpuscles from solution.² It is well known that on a gradual dilution of the plasma of the blood with distilled water we arrive at a point when the red blood corpuscles are broken up and the haemoglobin goes into solution—the blood is then spoken of as ‘laked blood.’ Based on this reaction Wright and Kilner³ and later Wright and Ross,⁴ described a new method of testing the blood and urine, and shewed that by means of the relationship existing between the haemosozic value of the serum and the haemosozic value of the urine we had a means of discriminating between physiological albuminuria and the albuminuria of renal disease.

Working on the lines laid down by the above-mentioned authors, I found⁵ that the excretory quotient test, *i.e.*—

$$\frac{\text{the haemosozic value of the urine}}{\text{the haemosozic value of serum}}$$
gave values in the different forms of anaemia, and particularly in the anaemia of ankylostomiasis, very similar to those found to be the case in organic disease of the kidneys; viz., the value is always below that observed in health.

1. Dated Calcutta, December 5th, 1907.
2. Armand Ruffer, *British Medical Journal*, 1903, 1904.
3. Wright and Kilner, *Lancet*, April, 1904.
4. Wright and Ross, *Lancet*, October, 1905.
5. McCay, *Lancet*, June, 1907.

Reference to the literature of the subject will show that in healthy Europeans the excretory quotient is always represented by the figure 2 or over, and in Bengalis by 1 or over; while in nephritis or anaemia with oedema, as the case may be, it falls much below 2 in Europeans, and usually well under 1 in Bengalis.

A full description of the technique of the method of carrying out the test will be found in the numbers of the *Lancet* referred to, and need not detain us.

The points with which the present paper will deal are :—

- (i) *On what does haemolysis caused by dilution really depend.*
- (ii) *What is measured by an estimation of the haemosozic value—*
 - (1) *In health,*
 - (2) *In disease.*
- (iii) *The significance of the modification of the haemosozic value of the serum by drugs and the bearing of a lowering on the incidence of blackwater fever.*

I.—THE CAUSE OF THE SOLUTION OF THE RED BLOOD CORPUSCLES ON DILUTION OF THE PLASMA

The blood may be looked upon, so far as the erythrocytes and plasma are concerned, as a mass of impermeable or slightly permeable globules floating in a fluid medium of the same density. Very careful measurements by Krönig and v. Fürth¹ have shewn that the freezing-point of the blood-plasma, or of the blood serum and blood-corpuscle pulp is the same; and the same osmotic equilibrium has been shewn to exist between the various body fluids, with the exception of the urine.

It may, therefore, be accepted that the osmotic tension of the plasma and of the corpuscles is identical; any modification of the density of the plasma, as, for instance, its dilution by distilled water, will upset the osmotic equilibrium existing between the corpuscles and the plasma, and by osmosis water will tend to pass into the red blood corpuscles and the salts to pass out.

1. *Monatschr. für Geburtsb. u. Gynäk.*, 1901.

In practice it has been found that a dilution of the plasma by a weaker and weaker saline solution (two volumes of the diluted saline being added to one volume of blood) gives a very definite point when haemolysis takes place—the haemoglobin going into solution in the plasma. This point may be termed the haemolytic point.

To make the reaction clear it might be expressed in the form of an equation :—

If x equals the total salt concentration of the plasma of the blood examined, and it is found that—

$$1 \text{ vol. of blood} + 2 \text{ vols. of a } \frac{N}{45} \text{ NaCl} = \text{haemolysis,}$$

$$\text{normally } x = \text{about } 0.85 \text{ per cent.,}$$

$$\text{and } \frac{N}{45} = 0.130 \text{ per cent.,}$$

substituting these values in the equation—

$$\begin{aligned} &= 1 \text{ vol. of } 0.85 \text{ per cent. solution} + 2 \text{ vols. of } 0.130 \text{ per cent. solution,} \\ &= \frac{0.85 \text{ per cent.} + 2 (0.130 \text{ per cent.})}{3} = 0.370 \text{ per cent.}^1 \end{aligned}$$

that is, the salt concentration of the plasma in the example at which haemolysis takes place is a concentration of 0.370 per cent.¹; in other words, the plasma of the blood will bear dilution until it becomes reduced from a 0.85 per cent. solution to a 0.37 per cent. solution before any general breaking up of the red blood corpuscles takes place. The fact that haemolysis does take place on a dilution of the plasma shows that the envelope of the red blood cells is impermeable to contained salts and haemoglobin; if this were not the case, these would wander out until the osmotic tension of the plasma and inside the corpuscles reached equilibrium and no haemolysis would occur. Instead of this, the salts and haemoglobin of the corpuscles not being able to pass out, water finds its way in by endosmosis, the corpuscles swell up and, at the degree of dilution corresponding to the haemolytic point, sudden and complete disruption of the erythrocytes takes place and the blood is laked.

1. I have purposely taken the haemolytic point very low. In the blood of man haemoglobin is extruded from the red cells at a concentration of a sodium chloride solution of 0.47 per cent.

We have, therefore, in this dilution method a very definite means by which the degree of dilution of the plasma or serum necessary to cause haemolysis can be estimated, and by using diluted normal saline solutions, as introduced by Wright, its value can be expressed in terms of NaCl.

So far as our theoretical knowledge of the physical chemistry of the blood goes, it would appear that the solution of the red blood corpuscles brought about by a dilution of the plasma with distilled water or a weak saline solution, as the case may be, depends on a difference of osmotic tension inside and outside the red blood corpuscles—haemolysis taking place when the osmotic pressure within the corpuscles is able to overcome the resisting power of the corpuscles to disruption.

II.—WHAT IS MEASURED IN AN ESTIMATION OF THE HAEMOSIZIC VALUE OF THE SERUM

I. *In health*.—As indicated above the haemosizic value of the plasma or serum should vary distinctly with its osmotic pressure, which in its turn depends on the total number of molecules in solution.

Neglecting the large proteid molecules, which have very little effect on the osmotic pressure, it would appear that the haemosizic value is really a measure of the total salt concentration or total salinity of the plasma or serum, *i.e.*, the total number of inorganic molecules in solution.

The evidence that this is the case :—

(i) The specific electrical conductivity of fluids tested by Dr. Waller was found to be roughly proportional to the salt content as estimated by this dilution method.¹

(ii) Working mainly on Bengalis over a series of eighty-four observations, I have found² the salt content as estimated by the dilution method to be 1.054 per cent. expressed in terms of NaCl. This is a slightly higher figure than that given for the percentage of salts in the serum in Europeans but, by actual quantitative analyses, I found

1. *Lancet*, quoted in Wright and Kilner's paper.

2. *Scientific Memoirs*; 'Metabolism of Bengalis'; in press.

that in healthy Bengalis the average percentage of total salts present in the blood was 1·06 per cent.—a figure practically identical with the average salt concentration obtained by the dilution method.

While it may be admitted that the electrical conductivity is a more accurate measure of the number of inorganic molecules in the serum than the amount of ash it yields, still quantitative estimations give a fairly close approximation to the total salts present.

It may, therefore, be accepted that for all practical purposes in estimating the haemosozic value of the serum in healthy individuals, what is actually measured is the total salt concentration or total salinity.

2. *In disease.*—In the observations recorded by Wright and Kilner, and by myself, on the salt concentration of the serum in anaemic conditions, it will be seen that the serum is modified in the same characteristic manner as found in estimations of the haemosozic value in nephritis with oedema—the serum giving in these cases a very high figure for its haemosozic value.

Some examples of the results are shewn in Table I.

TABLE I

Reference	Authority	Disease	Haemosozic value of serum in terms of NaCl	Remarks
<i>Lancet</i> , 1904	Wright and Kilner	Pernicious anaemia	2·4%	1,700,000 Red blood corpuscles
Do.	do.	Chlorosis	1·56%	
Do.	do.	do.	3·00%	Oedema of legs
Do.	do.	Anaemia	2·40%	Gastric ulcer
Do.	do.	do.	2·44%	Thrombosis of veins of legs
<i>Lancet</i> , 1907	McCay	Anaemia	2·12%	Ankylostomiasis ; oedema and ascites
Do.	do.	do.	6·09%	Do. Death
Do.	do.	do.	3·95%	Oedema and ascites
Do.	do.	do.	2·32%	Ankylostomiasis, marked oedema
Do.	do.	do.	1·72%	

From these results and from other evidence brought forward it appeared probable that in anaemia and in oedema from whatever cause there was an increase in the total salts of the blood, and particularly an actual retention of chlorides by the tissues and blood plasma.

While not denying that in oedema and nephritis there is retention of chlorides so that the system and blood contains an *absolutely* larger amount of total salts, it appeared most improbable that the blood plasma should possess such a *relatively* high percentage as that obtained for the haemosozic value in Table I.

In order to clear up the mystery of these high percentages of salt of the serum—in one instance as high as 6.0 per cent.—a further investigation was undertaken, several different lines of research being resorted to. As the results obtained would appear to be of some importance, I make no excuse for giving them in detail.

A.—INVESTIGATIONS TO EXCLUDE THE POSSIBILITY OF A COMBINATION OF SALTS WITH COLLOID MATERIAL

It seemed absurd to suppose that in the series of cases shewn in Table I there could be anything like the large percentage of *free* salt present in the serum which the estimation of the haemosozic value indicated; further, it appeared probable that some of the salt might be combined with the colloids of the serum and that, on manifold dilution with distilled water—as actually takes place in the estimation of the haemosozic value—the combined salt gradually becomes dissociated, and coming off *pari passu* with the degree of dilution, maintains for some time the isotonicity of the diluted serum and the red blood corpuscles. Eventually, however, as the serum becomes more and more diluted its salt concentration falls below the point necessary for the maintenance of the integrity of the red blood corpuscles, and haemolysis takes place.

To put this view to the test a number of cases, in which the haemosozic value was high, were examined with regard to the percentage of chlorides in the serum before and after a twenty-fold dilution with distilled water. The results obtained are shewn in Table II.

TABLE II

Case	Date	Haemosozic value of serum in terms of NaCl	Percentage of chlorides of serum before dilution	Percentage of chlorides of serum after dilution
1	14/7/07	1·64%	1·05%	1·06%
2	17/7/07	3·24%	0·70%	0·75%
3	29/7/07	1·358%	0·724%	0·73%
4	28/8/07	5·44%	0·692%	0·692%
5	14/9/07	3·18%	0·668%	0·688%
6	19/11/07	3·46%	0·719%	0·734%
7	21/11/07	2·26%	0·485% ¹	0·498%
8	22/11/07	0·97%	0·691%	0·691%

The table shews no definitely large increase in the percentage of chlorides of the serum either before or after a twenty-fold dilution with distilled water. We may, therefore, safely conclude that whatever is the cause of the high percentage of the haemosozic value of the serum in anaemia and oedema it cannot be due to a retention of the chlorides in combination with a colloid as Förster and Marie are inclined to believe.

The percentage of NaCl in the serum is, of course, no guide in determining to what extent retention of chlorides has taken place, for the hydraemic condition of the serum may conceal a great increase in NaCl and, further, the tissues may take up a large quantity of NaCl.

This is probably the case in oedema.

B.—INVESTIGATIONS UNDERTAKEN WITH THE VIEW OF DETERMINING WHEREIN LAY THE ACTUAL CAUSE OF HIGH HAEMOSOZIC VALUE

So far in these investigations I had firmly believed that the high haemosozic values were due to something—most probably inorganic salts—present in the serum in much greater proportion than in health and, therefore, requiring a larger number of dilutions to lower the osmotic pressure of the serum sufficiently to cause disruption of

1. Oedema disappearing may explain the low percentage of chlorides.

the red blood corpuscles. Having failed to find any marked increase in the chlorides of the serum, I began to suspect some change in the resisting power of the erythrocytes as a likely explanation.

In order to test this view, the following method was devised and carried out in a series of cases presenting a high haemosozic value :—

The haemosozic value of the patient's serum was estimated in the usual way—his own red blood corpuscles serving as the indicator of haemolysis. Having obtained the figure representing the haemosozic value of the serum in terms of NaCl, a sample of the same serum was now tested for its haemosozic value, not against his own red blood corpuscles, but using the red blood corpuscles of a normal individual as the indicator of haemolysis. The results obtained in this way are very striking and throw considerable light on the question at issue ; they are shewn in Table III.

TABLE III

Case	Date	Haemosozic value of serum <i>versus</i> own red blood corpuscles	Haemosozic value of serum <i>versus</i> normal red blood corpuscles	Remarks
1	17/7/07	3·24%	0·984%	Anaemia and oedema
2	29/7/07	1·358%	0·89%	Bright's disease, etc.
3	30/7/07	1·69%	1·06%	Do.
4	16/8/07	5·44%	1·06%	Ankylostomiasis anaemia and oedema
5	19/11/07	3·46%	1·02%	Do.

It is evident from the results obtained in this way that in using the patient's own red blood corpuscles as an indicator a far larger number of dilutions with distilled water of his serum are necessary to effect haemolysis than when the red blood corpuscles from a healthy individual are acting as the indicator. In both cases it is the serum from the same patient that is diluted, the indicator—red blood cells—alone changing, yet it has been found that the serum may require up to a thirty- or forty- fold dilution to effect haemolysis of his own erythrocytes, whereas a six- or eight- fold dilution will be sufficient to cause disruption of normal red blood cells. These

figures would, therefore, appear to point to a greatly increased resisting power of the erythrocytes in those conditions in which the haemosozic value of the serum is high, and not to a change in the total salinity of the serum whereby its osmotic pressure is greatly enhanced. We may, therefore, conclude that the increased resisting power of the red blood corpuscles to haemolysis in the several conditions examined does not depend on an increased salt concentration of the plasma—as the results obtained in healthy individuals would lead us to believe—and it cannot be explained by physical reasons alone.

Some other factor is present which in some way is able to increase the resisting power of the red blood cells to disruption. What that factor is, it is impossible to say at present. It may be that in some way the permeability of the erythrocytes is greatly increased—as for instance by the presence of ammonium salts—thus allowing of a free exchange of inorganic matter between the corpuscles and the diluted plasma, or, what would appear to be more likely, a new substance is present which is able to prevent a breaking down of the red blood corpuscles in the diseased conditions in which a high haemosozic value of the serum is found. The evidence that something capable of doing this does exist will be discussed below.

C.—INVESTIGATIONS UNDERTAKEN TO SHEW THAT IN CERTAIN DISEASED CONDITIONS THE HIGH HAEMOSOZIC VALUE IS NO CRITERION OF THE ACTUAL QUANTITY OF SALTS PRESENT IN THE BLOOD

Having failed to find evidence of any great increase in the chlorides or of the salt concentration when measured against normal red blood cells, I next turned to actual estimations—quantitatively—of the percentage of salts in the blood of individuals shewing a high haemosozic value.

It will be fairly obvious that, if the blood in the particular conditions shewing such high values for the haemosozic power does really contain a higher—much higher—percentage of salts; and if, due to this, the haemosozic value is greatly increased, then by quantitative analysis this increased salinity should be demonstrated.

The following table—Table IV—gives the facts obtained by analysis :—

TABLE IV

Case	Date	Disease	Haemosozic value of serum in terms of NaCl	Total salts of blood	Total solids of the blood
1	28/8/07	Marked anaemia and oedema general	5·444%	1·20%	10·579%
2	14/9/07	Anaemia and oedema	3·18%	1·103%	16·176%
3	19/11/07	Do.	3·46%	0·987%	12·862%
4	21/11/07	Do.	2·26%	0·988%	15·563%
5	Do.	Heart oedema	0·848%	0·926%	14·646%

These results would appear to be absolute proof that while the haemosozic value of the serum in health corresponds very closely with its total salts, in certain diseased conditions characterised by a high haemosozic value—notably nephritis, anaemia and oedema generally—the haemosozic value is no measure whatsoever and in no way corresponds to the actual percentage of salts in the blood or serum. Again, by this method of investigation the conclusion is forced on us that some unknown factor, other than an increase in the percentage of salts in the blood, is present able to increase the resisting power of the red blood corpuscles.

Further, from the investigations carried out in cases of marked oedema it would appear that there is very little increase in the percentage of salt present—the blood being able in some way or other to maintain its chemical composition at a fairly uniform level, at least so far as its inorganic salts are concerned.

III.—THE SIGNIFICANCE OF THE MODIFICATION OF THE HAEMOSOZIC VALUE OF THE SERUM BY DRUGS, AND THE BEARING OF A LOWERING ON THE INCIDENCE OF BLACKWATER FEVER

As it was evident from the results recorded above that the haemosozic value of the serum or, more accurately, perhaps, the resisting power of the red blood corpuscles varied within very wide limits in disease, it appeared to be a matter of some importance to determine the action of certain commonly used drugs, and more particularly what effect ‘quinine’ had on the resistance of the erythrocytes.

In blackwater fever there is undoubtedly a great and wide-spread breaking down of the red cells of the blood ; it was, therefore, evident that any drug, the absorption of which caused a lowering of the resisting power of the red cell, would, other things being equal, tend to bring those cells within the danger zone and precipitate an attack of blackwater fever.

In blackwater fever it is generally accepted that there is a virulent malarial infection primarily—the infection probably being repeatedly effected daily—so that many of the red cells have been already broken up, many others on the point of breaking up, and a large number of those remaining injured by the malarial parasite ; if now, for any reason the salt concentration of the plasma becomes seriously lowered the effect will be to bring the red corpuscles—and particularly the innumerable injured corpuscles—nearer and nearer to their haemolytic point according to the greater and greater lowering of the total salinity of the plasma. That it is those corpuscles, injured by the presence of the malarial parasite, that do break up in blackwater fever is evident from the fact that no parasites can be found, once the attack is precipitated, the corpuscles hitherto containing them having disappeared on disruption.

In order to obtain information of the effects of malaria and its treatment by 'quinine' on the salt concentration of the blood, a series of observations was begun. However, as malaria during the period was rare in Calcutta, I was forced to carry out most of the investigation on healthy individuals, but the results in the few cases of malaria treated with quinine sulphate shew exactly the same modification of the plasma as met with in health on administration of the drug.

The following table makes clear this modification due to the particular drugs administered. In order to check the results, a chemical estimation of the chlorides of the blood serum was made before and after the drug was given, and, as will be seen, the results are in harmony with the changes observed in the salt concentration.

TABLE V

Case No.	Date	Disease	Haemosozic value of serum in terms of NaCl (before)	Drug and dose	Haemosozic value of serum in terms of NaCl (after)	Chlorides of serum before drug	Chlorides of serum after drug
1	20/7/07	Healthy	0.984%	Quinine Sulph. gr. 30	0.754%	—	—
2	23/7/07	Do.	0.85%	„ gr. 20	0.808%	—	—
2	25/7/07	Do.	0.85%	„ gr. 30	0.758%	—	—
2	26/7/07	Do.	0.85%	„ gr. 20	0.63%	—	—
2	27/7/07	Do.	0.85%	„ gr. 20	0.806%	—	—
3	29/7/07	Do.	0.888%	„ gr. 30	0.742%	—	—
3	31/7/07	Do.	0.888%	„ gr. 30	0.707%	—	—
3	1/8/07	Do.	0.888%	„ gr. 30	0.707%	—	—
4	3/8/07	Do.	0.965%	„ gr. 20	0.762%	—	—
4	5/8/07	Do.	0.965%	„ gr. 10	0.736%	—	—
5	7/8/07	Do.	1.07%	„ gr. 5	0.965%	0.753%	0.724%
5	9/8/07	Do.	1.07%	„ gr. 5	0.914%	0.753%	0.723%
5	10/8/07	Do.	1.07%	„ gr. 5	0.863%	0.753%	0.705%
5	12/8/07	Do.	1.07%	„ gr. 10	0.701%	0.753%	0.612%
5	13/8/07	Do.	1.07%	„ gr. 10	0.876%	0.753%	0.720%
5	14/8/07	Do.	1.07%	„ gr. 10	1.016%	0.753%	0.758%
5	15/8/07	Do.	1.07%	„ gr. 20	1.04%	0.753%	0.754%
6	23/9/07	Do.	0.928%	(Qui. Sulph. gr. XV, Magnes. Sulph. gr. 30, Ac. Sulph. dil. Mi. 15)	0.779%	0.68%	0.676%
6	25/9/07	Do.	0.928%	Do.	0.861%	—	—
7	23/9/07	Do.	1.168%	Magnes. Sulph. gr. 30	0.928%	0.701%	0.684%
7	25/9/07	Do.	1.168%	Do.	0.812%	—	0.679%
7	26/9/07	Do.	1.168%	Do.	0.719%	—	0.675%
7	27/9/07	Do.	1.168%	Do.	0.719%	—	—
7	28/9/07	Do.	1.168%	Do.	1.04% (watery stools)	—	0.695%

The results shewn in above table are of very great interest ; in every instance in which a sulphate was given by the mouth there was a well-marked fall in the haemosozic value of the serum measured in terms of NaCl by the dilution method. Further, it will be seen that the rapidity of the fall depended more or less on the strength of the dose.

Another important point, brought out in Nos. 2, 5, 6 and 7, is the recovery or rise to the normal of the haemosozic power when daily doses are administered on consecutive days. We might look on the early effects of sulphates on the haemosozic value as a negative phase which gradually passes off on regular administration of the drug.

The following table shews the results obtained in cases of malaria treated by quinine sulphate.

TABLE VI

No.	Date	Disease	Haemosozic value of serum in terms of NaCl <i>versus</i> normal red cells	Drug and dose	Haemosozic value of serum in terms of NaCl <i>versus</i> normal red cells	Remarks
1	22/7/07	Benign Tertian	0.928%	Quin. Sulph. gr. 20	0.776%	Blood examined on 4th day, <i>i.e.</i> , after Quin. Sulph. gr. 80 had been given
2	1/8/07	Malig. Tertian	1.004%	Quin. Sulph. gr. 30	0.97%	
	3/8/07	Do.	Do.	Do.	0.848%	—
3	24/8/07	Malig. Tertian	1.027%	Quin. Sulph. gr. 10	0.976%	10 grains given for two days
	29/8/07	Do.	Do.	Quin. Sulph. gr. 5	0.812%	5 grains for 5 days
4	26/8/07	Malig. Tertian	0.928%	Quin. Sulph. gr. 30	0.849%	—
5	26/8/07	Malig. Tertian	0.928%	Quin. Sulph. daily 45 grs.	0.742%	45 grains daily for two days

The same fall in the haemosozic value is seen as occurs in health when sulphates are administered. These cases were all examined by using the red blood corpuscles of a healthy individual as the indicator—the patients were in hospital, and a few drops of blood were drawn off and sent to the laboratory in exactly the same way as for Widal's test. It is important to note that by using normal red corpuscles as the indicator I eliminated any change in the resisting power of the patient's red cells due to causes other than changes in the total salt concentration of the blood.

THE SIGNIFICANCE OF THE MODIFICATION OF THE HAEMOSOZIC
VALUE EFFECTED BY SULPHATES AS SHOWN IN TABLES V AND VI

In an earlier part of this paper I brought forward evidence to shew that the haemosozic value of the serum in health expressed in terms of NaCl gave results almost identical with those obtained by a quantitative estimation of the total salts of the blood.

In the few cases of malaria examined—using normal red blood corpuscles for an indicator—it may be accepted that the percentages obtained for the haemosozic value are an actual measure of the total salinity of the blood, both before and after the administration of sulphate of quinine.

Therefore, both in healthy individuals (Table V) and in malaria (Table VI) the lowering of the haemosozic value of the serum is really due to an actual diminution of the total salts of the blood, and not to some other cause such as a lessening of the resisting power of the red blood corpuscles. That this is the case we have a certain amount of corroborative evidence in the accompanying depression of the chlorides of the serum (*vide* Table V, Nos. 5, 6 and 7).

It is, therefore, evident that—other things being equal—this depression of the haemosozic value of the serum or, what would appear to be the same thing—so far, at least, as healthy individuals or those suffering from malaria are concerned—the lessened salt concentration of the blood, produced by the administration of sulphates, does upset the osmotic equilibrium existing between the red blood corpuscles and the plasma in which they float. The diminution in the number of inorganic molecules present in the plasma lessens its osmotic pressure, and the osmotic pressure inside the red blood cells remaining as before, the effect will be to tend to burst the corpuscles open. This effect will be the greater the more the inorganic molecules of the plasma are diminished in number; and disruption from this cause will, of course, be the more easily effected the more the corpuscle is injured by the presence of the malarial parasite.

As already stated, the haemolytic point of normal blood is about

0·37 per cent.,¹ *i.e.*, the red blood corpuscles will bear a dilution of the plasma until it becomes about equal to a 0·37 per cent.¹ solution before actual general haemolysis takes place. This fact can be easily verified by the simple experiment of mixing an equal volume of blood and distilled water together when, it will be seen, no general laking of the blood has occurred; but, on the further addition of a fraction of a volume of distilled water quite suddenly, on the proper dilution being attained, general haemolysis takes place. It might be argued from this that if the erythrocytes will bear a dilution of the plasma until its density is reduced from about a 0·85 per cent. solution to one of about 0·37 per cent.,¹ the lowering produced by sulphates—such as quinine sulphate, magnesium sulphate or acid sulph. dil.—would be quite insufficient to cause haemolysis. (The greatest reduction observed after the administration of sulphates in any of the persons examined only lowered the density of the plasma from a 1·07 per cent. solution to a 0·701 per cent. solution.) This is quite true so long as we are dealing with normal blood or even in conditions of ordinary malarial infection, otherwise haemolysis and blackwater fever would follow after the administration of sulphates in every case of malaria.

In order, therefore, to explain the onset of an attack of blackwater fever, it is necessary to bridge the interval between 0·63 per cent., the lowest salt concentration above recorded, and 0·37 per cent.,¹ the lowest depression of the density of the plasma *normal* corpuscles will bear.

A purely physical explanation might be advanced which would cover much of the ground. In blackwater fever we are dealing with a condition in which many of the red blood cells are invaded by the malarial parasite, resulting in injury to the cohesive power of the stroma and haemoglobin so that the resisting power of the cells is greatly lessened; it is, therefore, quite probable that a sudden lowering of the osmotic tension of the plasma due to a decrease in the number of inorganic molecules contained—brought about by the administration of sulphates—becomes the determining factor

1. Probably 0·47 per cent. NaCl is more correct.

in the breaking up of those injured cells. In other words, a difference of osmotic tension between the red cells and the plasma, which in health would have no haemolytic effect, in blackwater fever becomes a very important factor in the causation of the attack.

The question therefore arises :—*What other factor is there in blackwater fever the presence of which permits of haemolysis taking place under circumstances that would not occur in health or in ordinary malaria ?*

Two explanations are evident from the results above recorded. The first of these is the actual injury done to the red corpuscles by the presence of the malarial parasite ; that this in itself is not sufficient would appear probable from the fact that even severe malarial fever treated with heroic doses of quinine sulphate will not develop into blackwater fever, except in certain well-defined areas. The second explanation would rest on the probable formation within the system of an haemolysin or haemolytic ferment by the action of which the resisting power of the red cells becomes diminished so that, in extreme cases, solution takes place without any great change in the composition of the plasma ; but, in the majority of recorded cases, the power of resistance of the erythrocytes being diminished by the haemolysin, the lowering of the osmotic pressure of the plasma—due to the action of sulphates—becomes the determining factor, and sets up an attack. The presence of a substance such as this would completely explain the onset of haemolysis long before any such dilution of the plasma was attained as has been found necessary in health. The degree of dilution necessary for haemolysis would form a most interesting and important subject for research in patients just before the onset and during an attack of blackwater fever.

Once recovery has begun to set in, a condition such as found in ankylostomiasis would be expected, viz., a very greatly increased resisting power of the remaining red blood cells due, in all probability, to the formation within the system of an antihæmolysin.

That something in the nature of an haemolysin is present and is the cause of the great anaemia of ankylostomiasis seems now to be generally accepted. The anaemia is not due to a mere sucking of

blood by the ankylostoma—in fact, it is very doubtful if they suck blood at all.

The great increase in the haemosozic value, which I have found to hold true in these conditions, and particularly when oedema is well marked, would appear from the evidence brought forward above, to depend on a greatly enhanced resisting power of the patient's red blood corpuscles. This high haemosozic value is made up of two factors: one, the ordinary haemosozic value, depending on the number of inorganic molecules present in the plasma; the other, something most probably of the nature of an antihaemolysin, by virtue of which the erythrocytes of patients—suffering from ankylostomiasis for some time, or recovering therefrom—are endowed with a very great power of resistance compared with normal red corpuscles. A similar explanation must hold also for certain other forms of anaemia and for the anaemia of nephritis, as, even in renal disease, I have found a high haemosozic value is no measure of the actual total salts of the blood.

A body possessing haemolytic properties has been isolated from the tissues of the ankylostoma, and the formation of an antibody within the system would be expected; indeed, the presence of such a substance forms the only reasonable explanation of the high resisting power of the erythrocytes in ankylostomiasis, at least in its later stages, the only stages I have had a chance of investigating.

GENERAL SUMMARY OF CAUSATION OF ONSET OF BLACKWATER FEVER

Practically everyone agrees that the malarial parasite is the real cause underlying the condition, and that by prevention of malaria blackwater fever would become non-existent. The great majority of clinical observers consider 'quinine' the actual exciting cause precipitating an attack. This action of quinine is so widely believed in by those of great experience in the disease as to appear worthy of general acceptance. The word 'quinine' has been used very loosely in connection with malaria; in probably 99 per cent. of instances of administration of the drug by the mouth, 'quinine'

means the sulphate. From the investigations and results recorded in an earlier part of this paper on the action of sulphates on the inorganic salts of the blood, it seems very probable that the lowering of the total salt concentration from the administration of sulphates, such as quinine sulphate, magnesium sulphate, etc., in a patient saturated with malaria, is quite sufficient to precipitate an attack. In all probability, besides the actual weakening of the erythrocytes from malarial infection, another factor, causing a lessened power of resistance of the red cells, comes into force, and there is reason to believe this is of the nature of a haemolytic toxin or haemolysin.

How this haemolysin is formed ; the conditions necessary for its formation ; why its formation is limited to malaria occurring within certain well-defined areas, etc., are questions which, for the present, cannot be answered. It is not improbable that the relative virulency of the parasites under different climatic conditions may be found to explain the mystery. For the present, it would appear, from the effects of sulphates on the salts of the blood, that the administration of quinine sulphate even in small doses may just make all the difference between a malarial fever of a special type and a malarial fever that develops into blackwater fever.

HOW THE HAEMOSAZIC VALUE OF THE BLOOD MAY BE INCREASED—
A PROBABLE RATIONAL INDICATION FOR THE TREATMENT
AND PROPHYLAXIS OF BLACKWATER FEVER

As it was possible, by the administration of sulphates—probably also by giving potassium salts, alkaline carbonates and compounds of alkalis with vegetable acids—to decrease the total number of inorganic molecules in the blood plasma, and thus, in malaria, tend to produce haemolysis of the red blood corpuscles and perhaps precipitate an attack of blackwater fever, it seemed a rational indication to discover some means of increasing the total number of inorganic molecules of the plasma ; perhaps, in that way, lessening the tendency of the red blood corpuscles to haemolysis and thus attain a position of being able to prevent the development of blackwater fever entirely.

The SO_4 of sulphates, probably on absorption, combines with the Na, Ca, etc., of the blood plasma, forming sulphates which are eliminated at once on arrival at the kidneys, thus decreasing the number of inorganic molecules in the plasma; it, therefore, seemed possible by giving a salt that need not necessarily be eliminated at once—*i.e.*, one not absolutely foreign to the system—to be able to increase the total salts of the blood and thus raise the haemosozic value of the plasma. The salts most likely to cause this increase were the chlorides. I therefore began a series of observations on the effects of the administration of chlorides by the mouth on normal individuals. As it was very necessary, in view of the malarial origin of blackwater fever, that quinine should be given, the form of chloride administered was quinine hydrochloride.

The results obtained are shewn in Table VII.

TABLE VII

Case No.	Date	Haemosozic value of serum in terms of NaCl (before)	Drug given		Haemosozic value of serum in terms of NaCl (after)	Chlorides of serum before drug	Chlorides of serum after drug
1	17/7/07	0.903%	Quinine Hydrochloride	gr. 25	1.044%	—	—
2	16/8/07	1.09%	Do.	do.	20 1.09%	0.712%	0.723%
2	18/8/07	1.09%	Do.	do.	20 1.09%	—	0.732%
2	19/8/07	1.09%	Do.	do.	20 1.09%	—	0.719%
2	20/8/07	1.09%	Do.	do.	20 1.170%	—	0.745%
2	21/8/07	1.09%	Nil.		0.899%	—	0.734%
3	21/8/07	0.865%	Quinine Hydrochloride	gr. 10	1.04%	0.73%	—
3	23/8/07	0.865%	Do.	do.	10 0.865%	—	0.719%
3	24/8/07	0.865%	Nil.		0.865%	—	—
3	25/8/07	0.865%	Nil.		0.959%	—	—
3	26/8/07	0.865%	Quinine Hydrochloride		1.392%	—	0.877%
				gr. XXX			
3	27/8/07	0.865%	Do.	do. gr. XXX	1.866%	—	0.740%
3	28/8/07	0.865%	Do.	do. gr. XXX	1.856%	—	0.718%
4	21/11/07	0.865%	Quin. Hydrochlor.	gr. XV,	1.076%	0.723%	0.736%
			Sod. Chlor. gr. 90,, Ac.				
			Hydrochlor. dil. Mi. XV				
4	23/11/07	0.865%	Do.	do.	1.157%	—	—
4	24/11/07	0.865%	Do.	do.	1.43%	—	0.964—
6	5/11/07	0.814%	Quin Hydrochlor.	gr. X, Ac.	1.016%	—	—
			Hydrochlor. Mi. X				

These results present a very different picture to that obtained from an administration of the sulphate of quinine or other sulphates. No lowering of the haemosozic value of the serum was observed in any one instance, and, in the majority of cases, the value was increased. That this increase is in part, at least, due to an increase in the number of inorganic molecules of the serum would appear probable from the accompanying increase in the chlorides of the serum. The increase is very marked when full doses of quinine hydrochloride are given, and this is further enhanced by an addition of sodium chloride and hydrochloric acid to the prescription.

The importance of the bearing of these results on the incidence of blackwater fever is obvious; the raising of the haemosozic value of the plasma in the treatment of malaria, especially in districts where blackwater fever is prevalent, should, other things being equal, be of great service in lessening the liability to its onset.

With the knowledge of the action of sulphates on the salinity of the blood gained from the above-recorded investigations, and what the significance of that action really is, it appears to me quite time that the treatment of malaria by quinine sulphate should cease, and that a fair trial should be given to its substitute, quinine hydrochloride or the acid hydrochloride. If this were done I think there is a reasonable hope that the number of cases of blackwater fever following almost immediately on the ingestion of sulphates in some form—and this covers the very large majority of the cases—would soon shew a rapid diminution, with a saving of many valuable lives yearly.

In connection with the views here put forward it would be interesting to obtain reliable information with regard to the relative liability to blackwater fever of those infected with the *Ankylostoma Duodenale*, and those—mostly Europeans—who are non-infected.

From the arguments made use of above I should expect that the high resisting power of the red blood corpuscles, characteristic of those suffering from *Ankylostomiasis*, would probably prevent altogether the occurrence of blackwater fever in such cases or, at least, lessen the likelihood of its onset.

CONCLUSIONS

1. The haemosozic value of the serum expressed in terms of NaCl corresponds with the total salt concentration.
2. In conditions of an increase in the haemosozic value above the normal, the haemosozic value is a measure of
 - (1) The total salt concentration.
 - (2) A factor other than the salt concentration increasing the resisting power of the red blood corpuscles; probably an antihæmolysin.
3. The lowering of the haemosozic power of the serum by the administration of certain drugs—sulphates, potassium salts, etc.—would appear to be a most important factor, under certain circumstances, in the precipitation of an attack of blackwater fever.
4. The raising of the haemosozic value of the serum by the administration of chlorides, such as quinine hydrochloride, sodium chloride, etc., would appear to be a rational indication for the prophylaxis and treatment of blackwater fever.

In conclusion, I wish to express my thanks to those who assisted me in the investigation by providing the material for examination. I am specially indebted to Dr. Upendra Nath Brahmachari, Campbell Medical School, Calcutta, for the great majority of the pathological cases investigated.

I desire also to thank Lieut.-Col. Drury, I.M.S., Major Chatterton, I.M.S., and Captain Mackelvie, I.M.S., physicians of the Medical College Hospital, Calcutta, for permission to examine the patients under their care.

Since writing the above paper I have had the opportunity, due to the kindness of Major L. Rogers, I.M.S., Professor of Pathology, Medical College, Calcutta—of putting the views advanced with regard to the causation of blackwater fever to some extent to the test.

The patient was recovering from a typical attack when the blood for examination was taken. The hæmoglobinuria had disappeared, and the ordinary symptoms of the disease were rapidly subsiding.

At this period it was evident that the active cause of the haemolysis had disappeared and that in all probability the erythrocytes that had escaped disruption would be found highly resistant. Such, indeed proved to be the case.

The blood at our disposal was very small in amount and had been mixed with a small amount of citrate of sodium to prevent coagulation.

From the examination of this citrated blood it was found that one volume of the blood required dilution with two volumes of, at least, a $\frac{N}{250}$ NaCl solution before haemolysis took place. This is a very remarkable result, and would most distinctly point to the presence of some substance conferring a very high degree of immunity to haemolysis on the red blood corpuscles of a patient recovering from blackwater fever. (Normal red blood corpuscles break up when one volume of blood is mixed with two volumes of about a $\frac{N}{60}$ to $\frac{N}{40}$ NaCl solution). This substance must be of the nature of an antihæmolysin, the presence of which would certainly mean the earlier presence of a hæmolysin.